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Inhibition of Adenosine 3',5'-Cyclic Monophosphate Phosphodiesterase by Flavonoids. II¹⁾

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Thirty-eight flavones obtained from *Scutellaria baicalensis* and ninety six related flavones were tested for inhibitory activity against adenosine 3',5'-cyclic monophosphate phosphodiesterase from beef heart. The most active inhibitor is 8-methoxy-5,7,2'-trihydroxyflavone from *S. baicalensis*. Its IC_{50} value is $0.6(\times 10^{-5} \text{ M/l})$. The structure-activity relationships were investigated. a) O-Methylated flavones generally show lower inhibitory activity than the corresponding hydroxyflavones among the flavones having under four substituents. b) O-Methylated flavones generally show higher inhibitory activity than the corresponding hydroxyflavones in the flavones having over five substituents. (c) C-Glycosides are almost all poor inhibitors. (d) O-Glycosides are generally less potent than the corresponding flavones.

Keywords—*Scutellaria baicalensis*; Labiatae; flavone; cyclicAMP phosphodiesterase; inhibitor; structure-activity relationship

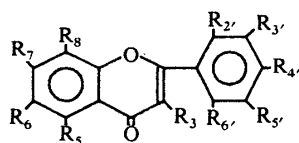
The adenosine 3', 5'-cyclic monophosphate (cAMP) phosphodiesterase inhibition test provides a useful tool for the screening of biologically active compounds contained in medicinal plants. We reported that flavonoids contained in various medicinal plants are cAMP phosphodiesterase inhibitors.¹⁻⁴⁾ The present paper deals with the identification of cAMP phosphodiesterase inhibitors contained in the root of *Scutellaria baicalensis* GEORGI (Japanese name "ohgon," Labiatae), which is one of the most useful medicines in traditional Chinese herbal therapy. "Ohgon" is generally considered to have anti-inflammatory and defervescent activities. It was reported to contain 38 species of flavonoids.⁵⁻²¹⁾ Flavonoids in this plant participate in the cholagogic,²²⁾ purgative,²³⁾ diuretic,²⁴⁾ detoxicating,^{25, 26)} anti-inflammatory²⁷⁻³¹⁾ and anti-allergic³²⁻³⁵⁾ actions. Recently amelioration of lipidosis,³⁶⁻⁴⁰⁾ retardation of thrombopoiesis⁴¹⁻⁴³⁾ and effects on arachidonic acid metabolism⁴⁴⁻⁴⁷⁾ have been reported. Therefore we wish to examine and identify cAMP phosphodiesterase inhibitors contained in *Scutellaria baicalensis*. The structure-activity relationships of flavones isolated from *Scutellaria baicalensis*, and related flavones were investigated.

Results and Discussion

Hot methanol and water extracts of commercial *Scutellariae Radix* were fractionated as shown in Chart 1. The ether-soluble fractions of methanol and water extracts were more active than the corresponding ether-insoluble fractions. Spots of the major flavones baicalein (9) and baicalin (12), were detected in the ether-soluble fraction by thin layer chromatography (TLC).

In order to study the structure-activity relationships, a large number of flavones were

TABLE I. Inhibitory Activity of Flavones on cAMP Phosphodiesterase



No.	R ₃	R ₅	R ₆	R ₇	Substituent					R _{5'}	R _{6'}	IC ₅₀ ($\times 10^{-5}$ M/l)	Source and reference
1	H	H	H	H	H	H	H	H	H	H	H	13.2	Com (T.K.)
2	OH	H	H	H	H	H	H	H	H	H	H	> 500	Com (T.K.)
3	H	OH	H	OH	H	H	H	H	H	H	H	52.4	S.r. ⁴⁷⁾
4	H	OH	H	OMe	H	H	H	H	H	H	H	150	p.p. ¹⁾
5	H	OH	H	OgluA	H	H	H	H	H	H	H	37.3	S.d. ⁴⁸⁾
6	H	OiPr	H	OiPr	H	H	H	H	H	H	H	6.8	Syn. ⁴⁷⁾
7	H	OH	H	OBz	H	H	H	H	H	H	H	21.3	Syn. ⁴⁷⁾
8	H	H	H	OH	H	H	H	OH	H	H	H	5.4	Syn. ⁴⁹⁾
9	H	OH	OH	OH	H	H	H	H	H	H	H	7.6	S.b. ¹⁷⁾
10	H	OH	OMe	OH	H	H	H	H	H	H	H	5.7	S.b. ¹⁷⁾
11	H	OH	OMe	OMe	H	H	H	H	H	H	H	48.7	O.i. ⁵⁰⁾
12	H	OH	OH	OgluA	H	H	H	H	H	H	H	189	S.b. ¹⁷⁾
13	H	OH	OH	OgluAMe	H	H	H	H	H	H	H	12.8	S.b. ¹⁷⁾
14	H	OH	OH	Oglu	H	H	H	H	H	H	H	24.2	S.b. ¹⁹⁾
15	H	OH	OMe	OgluA	H	H	H	H	H	H	H	36.3	S.b. ¹⁷⁾
16	H	OH	OMe	OgluAMe	H	H	H	H	H	H	H	309	S.b. ¹⁷⁾
17	H	OH	OH	Ogent	H	H	H	H	H	H	H	44.4	O.i. ⁵⁰⁾
18	H	OH	H	OH	OH	H	H	H	H	H	H	7.2	S.b. ¹⁸⁾
19	H	OH	H	OH	OMe	H	H	H	H	H	H	15.0	S.b. ¹⁷⁾
20	H	OH	H	OBz	OH	H	H	H	H	H	H	> 500	Syn. ⁵¹⁾
21	H	OH	H	OMe	OMe	H	H	H	H	H	H	5.3	S.b. ¹⁸⁾
22	H	OMe	H	OH	OMe	H	H	H	H	H	H	7.2	S.r. ⁵⁾
23	H	OMe	H	OMe	OMe	H	H	H	H	H	H	76.3	S.r. ⁵⁾
24	H	OH	H	OgluA	OMe	H	H	H	H	H	H	4.2	S.b. ¹⁷⁾
25	H	OH	H	OAc	OMe	H	H	H	H	H	H	16.9	S.r. ⁵⁾
26	H	OAc	H	OAc	OMe	H	H	H	H	H	H	31.0	Syn. ¹⁷⁾
27	H	OMe	H	OAc	OMe	H	H	H	H	H	H	7.8	S.r. ⁵⁾
28	H	OH	H	OH	H	OH	H	H	H	H	H	14.2	S.b. ¹⁹⁾
29	H	OH	H	OH	H	H	H	OH	H	H	H	26.9	S.r. ⁵⁾
30	H	OH	H	OMe	H	H	H	OH	H	H	H	61.6	D.g. ⁵²⁾
31	H	OH	H	OH	H	H	H	OMe	H	H	H	57.4	p.p. ¹⁾
32	H	OMe	H	OH	H	H	H	OMe	H	H	H	> 500	p.p. ¹⁾
33	H	OMe	H	OMe	H	H	H	OMe	H	H	H	> 500	Syn. ⁵⁾
34	H	OH	H	Oglu-api	H	H	H	OH	H	H	H	62.3	Com. [Sch]
35	H	OH	H	Oglu-rha	H	H	H	OH	H	H	H	36.1	E.i. ⁵³⁾
36	H	OH	H	Oglu-rha	H	H	H	OMe	H	H	H	60.4	E.w. unp.
37	H	OH	H	Oallo	H	H	H	Oallo	H	H	H	29.4	T.t. ⁵⁴⁾
38	OH	OH	H	OH	H	H	H	H	H	H	H	444	Syn. ⁵⁵⁾
39	OH	OH	H	OH	H	H	H	OH	H	H	H	22.5	Com (T.K.)
40	OH	OH	H	OMe	H	H	H	OH	H	H	H	> 50	p.p. ¹⁾
41	OMe	OH	H	OH	H	H	H	OMe	H	H	H	33.4	p.p. ¹⁾
42	OMe	OH	H	OMe	H	H	H	OMe	H	H	H	56.4	p.p. ¹⁾
43	OMe	OMe	H	OMe	H	H	H	OMe	H	H	H	26.2	p.p. ¹⁾
44	Oglu-pca	OH	H	OH	H	H	H	OH	H	H	H	4.9	D.g. ⁴⁾
45	OH	OH	H	Orha	H	H	H	OH	H	H	H	18.9	H.m. ⁵⁶⁾

TABLE I. (continued)

No.	Substituent										IC ₅₀ ($\times 10^{-5}$ M/l)	Source and reference
	R ₃	R ₅	R ₆	R ₇	R ₈	R _{2'}	R _{3'}	R _{4'}	R _{5'}	R _{6'}		
46	Ogal- glu	OH	H	OH	H	H	H	OH	H	H	> 500	P.g. ⁵⁷⁾
47	H	OH	OH	OH	H	H	H	OH	H	H	21.2	S.b. ⁵⁸⁾
48	H	OH	OMe	OH	H	H	H	OH	H	H	4.2	S.r. ⁴⁷⁾
49	H	OH	OMe	OMe	H	H	H	OH	H	H	11.8	A.c. ⁵⁹⁾
50	H	OH	OMe	OH	H	H	H	OMe	H	H	> 500	p.p. ¹⁾
51	H	OH	OMe	OMe	H	H	H	OMe	H	H	31.6	S.b. ⁵⁸⁾
52	H	OMe	OMe	OMe	H	H	H	OMe	H	H	43.8	p.p. ¹⁾
53	H	OH	OH	OgluA	H	H	H	OH	H	H	9.8	S.b. ⁵⁸⁾
54	H	OH	H	OH	OMe	H	H	OH	H	H	6.5	S.b. ¹⁷⁾
55	H	OH	OMe	OMe	OH	H	H	H	H	H	8.3	S.b. ¹⁷⁾
56	OH	H	H	OH	H	H	OH	OH	H	H	14.5	Syn. ⁵⁵⁾
57	H	OH	H	OH	H	H	OH	OH	H	H	20.8	D.g. ⁴⁾
58	H	OH	H	OMe	H	H	OH	OH	H	H	34.3	D.g. ⁴⁾
59	H	OH	H	OH	H	H	OMe	OH	H	H	35.3	p.p. ¹⁾
60	H	OMe	H	OH	H	H	OMe	OMe	H	H	50.3	p.p. ¹⁾
61	H	Oglu	H	OH	H	H	OH	OH	H	H	62.5	S.s. ⁶⁰⁾
62	H	OH	H	Oglu	H	H	OH	OH	H	H	32.3	G.j. ⁶¹⁾
63	H	OH	H	OH	H	H	Oglu	OH	H	H	17.3	R.l. ⁶²⁾
64	H	OH	H	OH	H	H	OH	Oglu	H	H	7.9	T.a. ⁶³⁾
65	H	OgluAc	H	OAc	H	H	OAc	OAc	H	H	190	Syn. ⁴¹⁾
66	H	H	OMe	OMe	H	H	OMe	OMe	H	H	1.8	Syn. ⁵⁵⁾
67	H	OH	H	OH	OMe	OH	H	H	H	H	0.6	S.b. ²⁰⁾
68	H	OH	H	OMe	OMe	OH	H	H	H	H	75.0	S.r. ⁵⁾
69	H	OH	H	OH	OMe	OMe	H	H	H	H	6.7	S.r. ⁵⁾
70	H	OH	H	OgluA	OMe	OMe	H	H	H	H	19.3	S.i. ⁶⁴⁾
71	H	OMe	H	OH	OMe	OMe	H	H	H	H	7.2	S.d. ⁶⁵⁾
72	H	OH	H	OMe	OMe	OAc	H	H	H	H	5.5	Syn. ⁵⁾
73	H	OMe	H	OMe	OMe	OMe	H	H	H	H	104.9	Syn. ⁶⁵⁾
74	H	OAc	H	OMe	OMe	OAc	H	H	H	H	> 500	Syn. ⁵⁾
75	H	OH	OH	OMe	H	H	OH	OH	H	H	> 500	p.p. ¹⁾
76	H	OH	OMe	OH	H	H	OH	OH	H	H	5.3	H.a. unsp.
77	H	OH	OMe	OMe	H	H	OMe	OH	H	H	3.2	S.r. ⁴⁷⁾
78	H	OH	H	OH	OMe	OH	H	H	H	OMe	16.9	S.b. ¹⁹⁾
79	H	OH	H	OMe	OMe	OH	H	H	H	OH	6.7	S.b. ²⁰⁾
80	H	OH	H	OH	OMe	OMe	H	H	H	OMe	7.4	S.d. ⁶⁵⁾
81	H	OH	H	OMe	OMe	OH	H	H	H	OMe	6.3	S.i. ⁶⁴⁾
82	H	OH	H	OMe	OMe	OMe	H	H	H	OMe	16.5	Syn. ⁶⁵⁾
83	H	OH	H	OMe	OMe	OMe	H	H	H	OBz	419.6	Syn. ⁶³⁾
84	H	OMe	H	OMe	H	H	OMe	OMe	OMe	H	10.6	M.P. ⁵⁵⁾
85	H	OMe	OMe	OMe	OMe	H	H	OMe	H	H	28.2	C.r. ¹⁾
86	OH	OH	H	OH	H	OH	H	H	H	OH	9.6	S.b. ²⁰⁾
87	OH	OH	H	OH	H	OH	H	OH	H	H	16.3	Com [Sig]
88	OH	OH	H	OH	H	H	OH	OH	H	H	31.7	Com [Sig]
89	OMe	OH	H	OMe	H	H	OMe	OMe	H	H	64.2	Syn.
90	OMe	OMe	H	OMe	H	H	OMe	OMe	H	H	12.9	p.p. ¹⁾
91	Oglu	OH	H	OH	H	H	OH	OH	H	H	448	A.v. ⁶⁶⁾
92	Ogal	OH	H	OH	H	H	OH	OH	H	H	296	A.v. ⁶⁶⁾
93	Orha	OH	H	OH	H	H	OH	OH	H	H	17.6	Com [T.K.]
94	OH	OH	H	Oglu	H	H	OH	OH	H	H	13.9	H.m. ³⁸⁾
95	OH	OH	H	OH	H	H	Oglu	OH	H	H	16.7	A.m. ⁶⁸⁾
96	OH	OH	H	OH	H	H	OH	Oglu	H	H	10.0	A.m. ⁶⁸⁾
97	Ocello	OH	H	OH	H	H	OH	OH	H	H	> 500	A.s. ⁶⁹⁾

TABLE I. (continued)

No.	R ₃	R ₅	R ₆	R ₇	Substituent						IC ₅₀ (× 10 ⁻⁵ M/l)	Source and reference
98	OH	OH	OH	Orha	H	H	H	OH	H	H	3.4	H.m. ⁵⁶⁾
99	H	OH	OH	OH	OH	H	OH	OH	H	H	> 500	Syn. ¹⁾
100	H	OH	OMe	OMe	OMe	H	OMe	OMe	H	H	36.1	C.r. ¹⁾
101	H	OMe	OMe	OMe	OMe	H	OMe	OMe	H	H	3.2	C.r. ¹⁾
102	H	OH	OMe	OMe	OMe	OH	H	H	H	OMe	2.3	S.b. ¹¹⁾
103	H	OH	OMe	OMe	OMe	OMe	H	H	H	OMe	24.9	Syn.
104	H	OH	H	OH	OMe	OH	H	H	OH	OMe	2.5	S.b. ¹⁹⁾
105	H	OMe	H	OMe	OMe	H	OMe	OMe	OMe	H	2.1	M.p. ⁵⁵⁾
106	H	OH	glu	OH	H	H	H	OH	H	H	24.3	S.j. ⁷⁰⁾
107	H	OH	glu	OMe	H	H	H	OH	H	H	> 500	S.j. ⁷²⁾
108	H	OH	glu	OH	H	H	H	OMe	H	H	> 500	Syn. ⁷¹⁾
109	H	OH	glu	Oglu	H	H	H	OH	H	H	> 500	S.o. ⁷³⁾
110	H	OH	glu	OMe	H	H	H	OMe	H	H	> 500	Syn. ⁷⁰⁾
111	H	OMe	glu	OMe	H	H	H	OMe	H	H	> 500	Syn. ⁷⁰⁾
112	H	OAc	gluAc ₄	OMe	H	H	H	OAc	H	H	29.0	Syn. ⁷²⁾
113	H	OH	glu	OMe	H	H	H	Oglu	H	H	23.1	C.c. ⁷⁴⁾
114	H	OH	glu	OH	H	H	OH	OH	H	H	> 500	S.j. ⁷⁵⁾
115	H	OH	glu	OMe	H	H	OH	OH	H	H	> 500	S.j. ⁷⁵⁾
116	H	OH	glu	OMe	H	H	OMe	OMe	H	H	> 500	Syn. ⁷⁰⁾
117	H	OH	H	OH	glu	H	H	OH	H	H	> 500	G.j. ⁶¹⁾
118	H	OH	H	OMe	glu	H	H	OH	H	H	> 500	Syn. ⁷²⁾
119	H	OH	H	OH	glu	H	H	OMe	H	H	> 500	Syn. ⁷¹⁾
120	H	OMe	H	OMe	glu	H	H	OMe	H	H	> 500	Syn. ⁷⁰⁾
121	H	OH	H	OH	glu	H	OH	OH	H	H	> 500	G.j. ⁶¹⁾
122	H	OH	H	OMe	glu	H	OH	OH	H	H	> 500	Syn. ⁷⁵⁾
123	H	OH	H	OH	glu	H	OMe	OH	H	H	> 500	f.W. ⁷⁶⁾
124	H	OH	H	OMe	C ₅ H ₉	H	H	OH	H	H	15.1	D.a. unp.
125	H	OH	H	OMe	C ₅ H ₉	H	H	OAc	H	H	17.8	Syn.
126	H	OAc	H	OMe	C ₅ H ₉	H	H	OAc	H	H	8.0	Syn.
127	OH	OH	H	OH	C ₅ H ₉	H	H	OMe	H	H	62.2	Syn. ⁷⁷⁾
128	Orha	OH	H	Oglu	C ₅ H ₉	H	H	OH	H	H	40.8	E.m. ⁷⁷⁾
129	Orha	OH	H	Oglu	C ₅ H ₉	H	H	OMe	H	H	47.6	E.m. ⁷⁷⁾
130	OH	OH	H	-OC(CH ₃) ₂ (CH ₂) ₂ -	H	H	H	OMe	H	H	60.4	Syn. ⁷⁸⁾
131	H	OMe	H	-OC(CH ₃) ₂ CH=CH-	H	H	H	H	H	H	8.2	T.p. ⁷⁹⁾
132	OMe	H	H	-O-CH=CH-	H	H	H	H	H	H	24.0	D.m. ⁸⁰⁾
133	OMe	H	H	-O-CH=CH-	H	H	H	-OCH ₂ O-	H	H	68.4	D.m. ⁸⁰⁾
134	OMe	OMe	OMe	OMe	OMe	H	OMe	OMe	OMe	H	2.1	M.p. ⁵⁵⁾

Abbreviations: Ac, acetyl; A.c., *Artemisia capillaris*; allo, allose; A.m., *Anaphalis margaritacea*; api, apiose; A.s., *Alnus sieboldiana*; A.v., *Apocynum venetum* var. *basikurumon*; Bz, benzoyl; C.c., *Commelia communis*; cello, cellobiose; C₅H₉, prenyl group; Com [Sch], commercial reagent [Schucherd]; Com [Sig], commercial reagent [Sigma]; Com [T.K.], commercial reagent [Tokyo Kasei]; C.r., *Citrus reticulata*; D.a., *Dolabella auricularia*; D.g., *Daphne genkwa*; E.i., *Evodiapanax innovans*; E.m., *Epimedium macranthum*; E.w., *Exacum walkeri*; f.W., from Dr. H. Wagner; G.j., *Gleditsia japonica*; gent, gentiobiose; glu, glucose; gluA, glucuronic acid; H.a., *Helenium autumnale*; H.m., *Hibiscus mutabilis*; iPr, isopropyl; M.P., *Murraya paniculata*; O.i., *Oroxylum indicum*; pca, p-coumaric acid; P.g., *Panax ginseng*; p.p., previous paper; rha, rhamnose; R.l., *Reseda luteola*; S.b., *Scutellaria baicalensis*; S.d., *Scutellaria discolor*; S.i., *Scutellaria indica*; S.j., *Swertia japonica*; S.o., *Saponaria officinalis*; S.r., *Scutellaria rivularis*; S.s., *Swertia spp.*; Syn, synthesis; T.a., *Trachelospermum asiaticum* var. *intermedium*; T.t., *Thalictrum thunbergii*; unp, unpublished.

screened for inhibitory activity on cAMP phosphodiesterase by measuring the concentration (IC₅₀) of each compound required to give 50% inhibition of the enzyme activity. The compounds studied included 32 species of flavones and 9 species of flavone glycosides isolated from genus *Scutellaria*. The results are presented in Table I.

Many flavones (9, 10, 18, 21, 24, 54, 55, 67, 79, 86, 102 and 104) isolated from *S.*

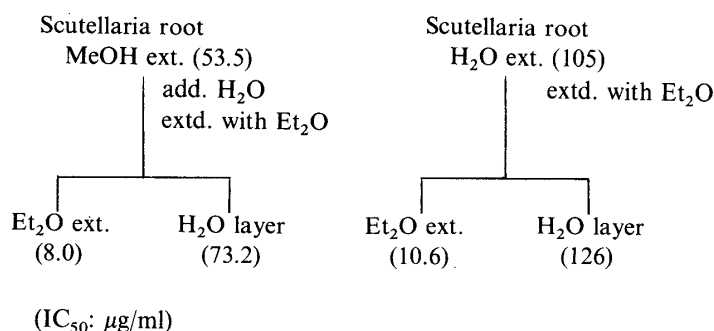


Chart 1

baicalensis are strong inhibitors of cAMP phosphodiesterase. On the other hand, the flavones from other *Scutellaria* genus plants, *S. rivularis*, *S. discolor* and *S. indica*, have lower inhibitory activity. The structure–activity relationships might be summarized as follows. (a) O-Methylated flavones have lower inhibitory activity than the corresponding hydroxyflavones in the derivatives having under four substituents (1–74). However, 10, 21, 48 and 49 did not show lower activities than the corresponding hydroxyflavones, 9, 18 and 47. (b) O-Methylated flavones have higher inhibitory activity than the corresponding hydroxyflavones in the derivatives having over five substituents (75–134), except for 89 (*versus* 88). (c) C-Glycosides (106–123) are almost all poor inhibitors. (d) O-Glycosides are generally less potent than the corresponding flavones. Moreover, four flavonoids (66, 77, 101 and 134) among five in which two methoxyl groups are located at C₆ and C₃, have very high inhibitory activity, equal to that of papaverine used as a positive control. In the series of luteolin glucosides (61–64) and quercetin glycosides (91–97), the 4'-glucosides (64 and 96) were the strongest inhibitors in this series. Major flavones (9, 10, 12, 14, 15, 19, 24 and 102) of *S. baicalensis* showed comparatively strong inhibitory activity. Therefore, it is suggested that the main inhibitors of cAMP phosphodiesterase in this plant are flavones.

Beretz *et al.* reported that in the case of monomeric flavonoids, methoxyl groups apparently lowered the inhibitory effect on cAMP phosphodiesterase.⁸¹⁾ Ferrell *et al.* also reported that quercetin and cyanidin chloride were the most potent inhibitors among 45 flavonoides tested.⁸²⁾ Their results however do not agree with ours.²⁾

We reported the inhibition kinetics (Dixon plots) of some flavonoids, nobiletin (101),²⁾ nor-kurarinon, kurarinol, kuraridine,⁴⁾ kuwanon G and mulberrofuran.³⁾ These showed the same non-competitive inhibition kinetics as papaverine, used as a positive control.

We reported that onjisaponin F⁸³⁾ from *Polygala tenuifolia* and nobiletin²⁾ from *Citrus reticulata* were strong inhibitors of cAMP phosphodiesterase and showed a prolonging effect on hexobarbital sleeping time in mice and an inhibitory effect on barium sulfate transport in the small intestine of mice, respectively. The present results indicate a possible correlation of the pharmacological activity and the inhibitory activity against cAMP phosphodiesterase by flavones in this plant, and give strong support for the effectiveness of this assay as a screening method to detect biologically active compounds contained in medicinal plants.

Experimental

The following instruments were used to obtain the physical data. An Aloka LSC-903 liquid scintillation counter was used. Silica gel (Merck, precoated plate, 0.25 mm) was used for TLC and detection was achieved by illumination with an ultraviolet (UV) lamp or by spraying 5% FeCl₃ or 10% H₂SO₄ followed by heating.

Extraction and Separation—Commercial *Scutellaria* root (100 g), which was purchased from Uchida Pharmacy for Oriental Medicine, was extracted with boiling MeOH and the MeOH extract was concentrated to dryness to give a residue. The residue was suspended in H₂O and extracted with Et₂O, and the Et₂O layer was concentrated to dryness to give a residue (Et₂O-soluble fraction, 0.8 g). The aqueous layer was frozen and dried to

give a powder-like product (Et₂O-insoluble fraction, 19.7 g). Another 100 g of this crude drug was extracted with boiling H₂O and the H₂O extract was fractionated as above to give an Et₂O-soluble fraction (1.3 g) and an insoluble fraction (25.2 g). These fractions were tested for inhibitory effect on cAMP phosphodiesterase (Chart 1).

Detection of Baicalein (9) and Wogonin (19)—Compounds **9** and **19** were detected as the main spots on TLC ($R_f=0.15$ for **9**, $R_f=0.40$ for **19**, solvent C₆H₆:EtOAc:HOAc=25:15:0.05), by comparison with authentic samples, in the Et₂O-soluble fractions of the MeOH and H₂O extracts.

Detection of Baicalin (12) and Oroxylin 7-Glucuronic Acid (15)—Compounds **12** and **15** were detected as main spots on TLC ($R_f=0.21$ for **12**, $R_f=0.26$ for **15**, solvent *n*-BuOH:HOAc:H₂O=4:1:5 upper phase), by comparison with authentic samples, in the Et₂O-insoluble fraction of the MeOH extract.

Detection of Wogonin 7-Glucuronic Acid (24)—Compound **24** was detected as the main spot on TLC ($R_f=0.27$ for **24**, solvent *n*-BuOH:AcOH:H₂O=4:1:5 upper phase), by comparison with an authentic sample, in the Et₂O-insoluble fraction of the H₂O extract.

Methylation of 88 and 102—Compounds **88** and **102** were methylated with CH₃N₂ and afforded **89** and **103**, respectively.

Acetylation of 124—Compound **124** was acetylated with Ac₂O in pyridine and afforded **125** and **126**.

Assay of cAMP Phosphodiesterase—cAMP phosphodiesterase activity was assayed by a modification of the reported method.^{84,85} The standard reaction mixture (500 μ l) contained Tris-HCl (pH 7.5; 0.05 M), MgCl₂ (1 mM), bovine serum albumine (250 μ g), [³H]cAMP (0.01 mM; 1.2×10^6 dpm), beef heart phosphodiesterase (2.25 mU) and a sample (5–100 μ g). The lower solubility of some compounds interfered with the determination of IC₅₀ in the usual concentration range (25–100 μ g). In such cases, a lower concentration range (5–20 μ g) was used in this assay. The reaction was initiated by the addition of [³H]cAMP. The reaction mixture was incubated for 30 min at 37°C and the reaction was stopped by immersing the test tube in boiling water for 3 min. Snake venom nucleotidase (500 μ g) was added to the cooled reaction mixture and the whole was incubated for 30 min at 37°C. Then a 50% suspension of Dowex AGI-X8 resin was added to the reaction mixture. The resin, which adsorbed unchanged [³H]cAMP, was collected by centrifugation for 5 min at 3000 cpm and the radioactivity of an aliquot of the supernatant containing [³H]adenosine was measured with a liquid scintillation counter. Samples insoluble in water were dissolved in dimethylsulfoxide (DMSO) and added to the reaction mixture. The DMSO concentration in the reaction mixture was held constant at 2%. All assays were performed in duplicate. The IC₅₀ value is the concentration of a compound required to give 50% inhibition of cAMP phosphodiesterase activity.

Enzymes Materials and Chemicals—Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase and cAMP were obtained from Sigma, and [³H]cAMP from the Radiochemical Centre. Papaverine, used as a positive control, and DMSO were purchased from Toyko Kasei and Wako, respectively.

Authentic Flavones—The authentic samples which were used for the inhibitory assay on cAMP phosphodiesterase were isolated or prepared during structural studies (Table I).

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References and Notes

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