[Chem. Pharm. Bull. 32(4)1477—1483(1984)]

Inhibitors of Cyclic AMP Phosphodiesterase in *Panax ginseng* C. A. MEYER and *Panax japonicus* C. A. MEYER[1)]

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(Received August 29, 1983)

Ginsenosides and chikusetsusaponins present in the roots of *Panax ginseng* C. A. MEYER and rhizomes of *Panax japonicus* C. A. MEYER were identified as inhibitors of cyclic AMP phosphodiesterase. The structure–activity relationships of 30 saponins, 2 prosapogenins and 3 sapogenins were studied. Saponins which have a 20(*S*)-protopanaxadiol or oleanolic acid moiety as the sapogenin were generally more inhibitory towards cyclic AMP phosphodiesterase than saponins with a 20(*S*)-protopanaxatriol moiety as the sapogenin. The effects of various ginsenosides on corticosterone secretion and cyclic AMP phosphodiesterase activity appeared to be parallel.

Keywords——*Panax ginseng*; *Panax japonicus*; 20(*S*)-protopanaxadiol; 20(*S*)-protopanaxatriol; ginsenoside; chikusetsusaponin; phosphodiesterase inhibitor; corticosterone secretion-inducing activity

We have reported that measurement of cyclic AMP phosphodiesterase inhibition can be used as a screening tool to detect biologically active components contained in medicinal plants. Inhibitors contained in the root of *Anemarrhena asphodeloides* BUNGE, the fruit of *Forsythia suspensa* VAHL. and the root of *Polygala tenuifolia* WILLD. were identified as norlignans,[2)] lignans[3)] and saponins,[4)] respectively. Several polymethoxy flavonoids were isolated as inhibitors from the peel of *Citrus reticulata* BLANCO. and from the rhizome of *Iris florentina* L.[5)] Several alkaloids were isolated from *Picrasma quassioides* BENNET. as inhibitors.[6)] Benzoquinone and several benzaldehyde derivatives were identified as inhibitors from *Phyllostachys nigra* MUNRO var. *henonis* STAPF. and *Phragmites communis* TRINN.[7)] This paper deals with the identification of cyclic AMP phosphodiesterase inhibitors contained in the root of *Panax ginseng* C. A. MEYER and in the rhizome of *Panax japonicus* C. A. MEYER, and also describes the structure–activity relationships of these compounds and analogous compounds which were isolated from *Panax pseudoginseng* WALL. subsp. *himalacius* HARA, *Panax notoginseng* (BURK.) F. H. CHEN, *Panax japonicus* C. A. MEYER var. *major* (BURK.) C. Y. WU *et* K. M. FENG and *Aralia elata* SEEMANN.

Results and Discussion

The methanol extracts of the root of *Panax ginseng* C. A. MEYER and the rhizome of *Panax japonicus* C. A. MEYER were fractionated with chloroform and *n*-butanol. In both

plants, the *n*-butanol soluble fraction (saponin fraction) was found to be more active as an inhibitor of cyclic AMP phosphodiesterase than the other fractions. The main saponins of each plant in the saponin fraction were identified as ginsenoside-Rb_1 and chikusetsusaponin V (=ginsenoside-Ro) by thin layer chromatographic (TLC) comparison with authentic samples. The ginsenosides and chikusetsusaponins have been identified in these plants are listed in Tables I and II. These authentic samples were tested for cyclic AMP phosphodiesterase inhibitory activity.

Among the ginsenoside congeners in Panax main saponins, ginsenoside-Rb_1, -Rb_2 and -Rd whose sapogenin is 20(*S*)-protopanaxadiol were very much more active inhibitors than -Rg_1, -Rg_2 and -Re, whose sapogenin is 20(*S*)-protopanaxatriol. Ginsenoside-Ro (=chikusetsusaponin V), whose sapogenin is oleanolic acid, was the most active among Panax main saponins (Table I). Chikusetsusaponins III, IV and V showed generally high inhibitory activity except for chikusetsusaponin I (ginsenoside-Rg_2), whose sapogenin is 20(*S*)-protopanaxatriol (Table II).

Saponins isolated from other Panax and Aralia genus plants, *Panax pseudoginseng* subsp. *himalacius*, *P. notoginseng*, *P. japonicus* var. *major* and *Aralia elata*, were also tested for cyclic AMP phosphodiesterase inhibitory activity (Table III).

For studies on structure–activity relationships, a number of saponins, prosapogenins and sapogenins were tested for cyclic AMP phosphodiesterase inhibitory activity. The results are summarized in Table IV (sapogenin: 20(*S*)-protopanaxadiol), Table V (sapogenin: 20(*S*)-protopanaxatriol), Table VI (sapogenin: oleanolic acid) and Table VII (sapogenin: various

Table I. Inhibitory Effect of Ginseng Saponins on Cyclic AMP Phosphodiesterase

Ginsenoside	IC 50 ($\times 10^{-5}$ M)	Sapogenin	Reference
Ro (=Chikusetsusaponin V)	5.8	Oleanolic acid	12
Ra_1	7.6	20(*S*)-Protopanaxadiol	16
Ra_2	35.5	20(*S*)-Protopanaxadiol	16
Rb_1	13.7	20(*S*)-Protopanaxadiol	12
Rb_2	19.9	20(*S*)-Protopanaxadiol	12
Rb_3	8.3	20(*S*)-Protopanaxadiol	15
Rc	26.4	20(*S*)-Protopanaxadiol	12
Rd	8.4	20(*S*)-Protopanaxadiol	12
Re	>100	20(*S*)-Protopanaxatriol	14
Rf	30.0	20(*S*)-Protopanaxatriol	14
Rg_1	43.1	20(*S*)-Protopanaxatriol	13
Rg_2	>100	20(*S*)-Protopanaxatriol	14

Table II. Inhibitory Effect of Chikusetsusaponins on Cyclic AMP Phosphodiesterase

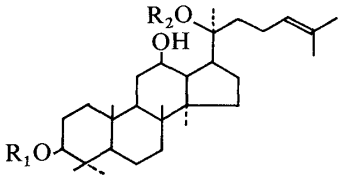
Chikusetsusaponin	IC 50 ($\times 10^{-5}$ M)	Sapogenin	Reference
I (=Ginsenoside-Rg_2)	>100	20(*S*)-Protopanaxatriol	18
Ia	32.5	20(*S*)-Protopanaxadiol	18
III	11.5	20(*S*)-Protopanaxadiol	17
IV	1.2	Oleanolic acid	19
IVa	9.7	Oleanolic acid	18
V (=Ginsenoside-Ro)	5.8	Oleanolic acid	20
LT_5	3.0	Dammar-24-ene-3β, 20*S*-diol	21
LN_4	1.6	Dammar-24-ene-3β, 20*S*-diol	21

TABLE III. Inhibitory Effect of Panax and Aralia Saponins on Cyclic AMP Phosphodiesterase

Saponin	IC 50 ($\times 10^{-5}$ M)	Source	Reference
Pseudoginsenoside-F_{11}[a]	>100	Leaves of *Panax pseudoginseng* subsp. *himalacius*	22
Notoginsenoside-R_1[b]	>100	Roots of *Panax notoginseng*	23
Notoginsenoside-R_3[c]	20.6	Roots of *Panax notoginseng*	24
Notoginsenoside-Fa[c]	21.0	Leaves of *Panax notoginseng*	25
Notoginsenoside-Fc[c]	7.2	Leaves of *Panax notoginseng*	25
Notoginsenoside-Fe[c]	8.1	Leaves of *Panax notoginseng*	25
Gypenoside-IX[c]	0.5	Leaves of *Panax notoginseng*	25
Majonoside-R_1[d]	>100	Rhizomes of *Panax japonicus* var. *major*	26
Tarasaponin VI[e]	1.7	Roots of *Aralia elata*	27
Tarasaponin VII[e]	1.8	Roots of *Aralia elata*	27
Tarasaponin V[e]	1.1	Roots of *Aralia elata*	27

Sapogenin: *a*) 20(*S*)-protopanaxatriol; *b*) 3β, 6α, 12β, 25-tetrahydroxy-(20*S*, 24*R*)-epoxy-dammarane; *c*) 20(*S*)-protopanaxadiol; *d*) 3β, 6α, 12β,25-tetrahydroxy-(20*S*, 24*S*)-epoxy-dammarane; *e*) oleanolic acid.

TABLE IV. Inhibitory Effect of Saponins [20(*S*)-Protopanaxadiol] on Cyclic AMP Phosphodiesterase



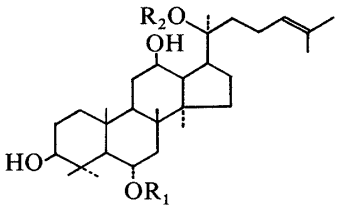
Saponin	R_1	R_2	IC 50 ($\times 10^{-5}$ M)
[20(*S*)-Protopanaxadiol]	H	H	9.2
Ginsenoside-Ra_1	-G-^{2}-G	-G-^{6}-A(p)-^{4}-X(p)	7.6
Ginsenoside-Ra_2	-G-^{2}-G	-G-^{6}-A(f)-^{2}-X(p)	35.5
Ginsenoside-Rb_1	-G-^{2}-G	-G-^{6}-G	13.7
Ginsenoside-Rb_2	-G-^{2}-G	-G-^{6}-A(p)	19.9
Ginsenoside-Rb_3	-G-^{2}-G	-G-^{6}-X	8.3
Ginsenoside-Rc	-G-^{2}-G	-G-^{6}-A(f)	26.4
Ginsenoside-Rd	-G-^{2}-G	-G	8.4
20(*S*)-Ginsenoside-Rg_3	-G-^{2}-G	H	2.4
Notoginsenoside-R_3	-G	-G-^{6}-G	20.6
Notoginsenoside-Fa	-G-^{2}-G-^{2}-X(p)	-G-^{6}-G	21.0
Notoginsenoside-Fc	-G-^{2}-G-^{2}-G	-G-^{6}-X(p)	7.2
Notoginsenoside-Fe	-G	-G-^{6}-X(f)	8.1
Gypenoside-IX	-G	-G-^{6}-A(f)	0.5
Chikusetsusaponin Ia	-G-^{6}-X	H	32.5
Chikusetsusaponin III	-G-^{6}-X (2-G)	H	11.5

G, glucose; A, arabinose; X, xylose; (p), pyranosyl type; (f), furanosyl type.

triterpenes).

The inhibitory activity of saponins and prosapogenins containing 20(*S*)-protopanaxadiol was high, whereas saponins containing 20(*S*)-protopanaxatriol as the sapogenin showed

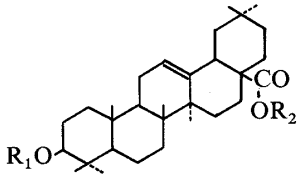
TABLE V. Inhibitory Effect of Saponins [20(*S*)-Protopanaxatriol] on Cyclic AMP Phosphodiesterase



Saponin	R_1	R_2	IC 50 ($\times 10^{-5}$ M)
[20(*S*)-Protopanaxatriol]	H	H	16.8
Ginsenoside-Re	–G–(2)–R	–G	>100
Ginsenoside-Rf	–G–(2)–G	H	30.0
Ginsenoside-Rg_1	–G	–G	43.1
Ginsenoside-Rg_2	–G–(2)–R	H	>100
Notoginsenoside-R_1	–G–(2)–X	–G	>100

G, glucose; R, rhamnose; X, xylose.

TABLE VI. Inhibitory Effect of Saponins [Oleanolic Acid] on Cyclic AMP Phosphodiesterase



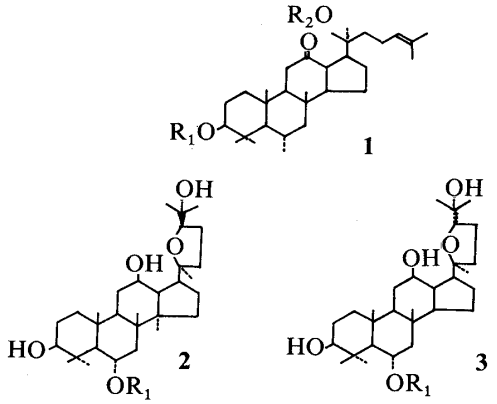
Saponin	R_1	R_2	IC 50 ($\times 10^{-5}$ M)
[Oleanolic acid]	H	H	27.8
Chikusetsusaponin IV	–GA–(4)–A(f)	–G	1.2
Chikusetsusaponin IVa	–GA	–G	9.7
Chikusetsusaponin V	–GA–(2)–G	–G	5.8
Tarasaponin VI (=Prochikusetsusaponin IV)	–GA–(4)–A(f)	H	1.7
Tarasaponin VII	–A–(3)–G, 2–X	H	1.8
Tarasaponin V	–A–(3)–G, 2–X	–G	1.1

GA, glucuronic acid; G, glucose, A, arabinose; X, xylose; (f), furanosyl type.

lower activity. In addition, saponins containing oleanolic acid showed high inhibitory activity. Han *et al.* examined the metabolism of Ginseng saponins in animals[8] and showed that the glycosidic bond at C-20 is hydrolyzed with dehydration by the gastric juice *in vivo* to yield a mixture of 20(*S*)- and 20(*R*)-ginsenoside-Rg_3, which showed very high inhibitory activity against cyclic AMP phosphodiesterase.

On the other hand, Brekhman *et al.*[9] and Saito *et al.*[10] reported that diol congeneric saponins showed sedative action and triol ones stimulated the central nervous system. Hiai *et al.*[11] reported differences in the effects on plasma corticosterone. It appears that the effects of Ginseng saponins on corticosterone secretion and on cyclic AMP phosphodiesterase activity

TABLE VII. Inhibitory Effect of Saponins [Various Types of Triterpene] on Cyclic AMP Phosphodiesterase



Saponin	Compd. No.	R_1	R_2	IC 50 ($\times 10^{-5}$ M)
Chikusetsusaponin LT_5	1	–G	–G$\overset{6}{-}$G	3.0
Chikusetsusaponin LN_4	1	–G$\overset{6}{-}$X	–G$\overset{6}{-}$A(p)	1.6
Pseudoginsenoside-F_{11}	2	–G$\overset{2}{-}$R		>100
Majonoside-R_1	3	–G$\overset{2}{-}$G		>100

G, glucose; X, xylose; A, arabinose; R, rhamnose; (p), pyranosyl type.

TABLE VIII. Effect of Ginseng Saponins on Plasma Corticosterone and Cyclic AMP Phosphodiesterase

Ginsenoside	ED 50 (μmol/kg) for corticosterone secretion-inducing activity	IC 50 ($\times 10^{-5}$ M) for cAMP phosphodiesterase
Rd	7.1	8.4
Rb_2	20	19.9
Rc	44	26.4
Rb_1	112	13.7
Re	107	>100
Rg_1	>160	43.1
Rg_3[20(*S*)][a]	<28	2.4
Rg_3[20(*R*)][b]	<26	0.5

Cf. a) Ref. 28. *b*) Ref. 29.

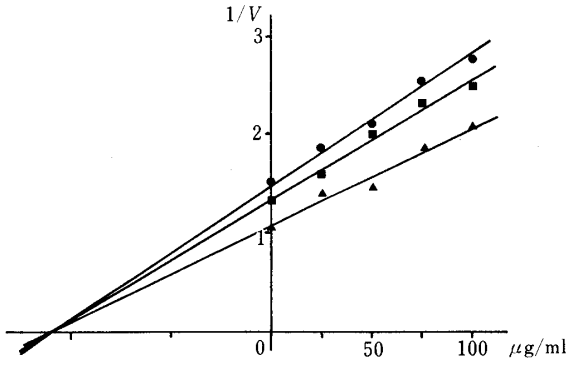


Fig. 1. Inhibition of Cyclic AMP Phosphodiesterase by Ginsenoside-Rd (Dixon Plots)

The assay was carried out by the method described in a previous paper.[2]
Substrate concentration (^{3}H-cAMP): 50 μM (●), 75 μM (■) and 100 μM (▲).
Enzyme concentration: 4.5 mU (Boehringer).

are roughly in parallel (Table VIII).

Ginsenoside-Rd (diol congener), ginsenoside-Rg_1 (triol congener) and chikusetsusaponin V (oleanolic acid congener) showed non-competitive ($K_i = 11.8 \times 10^{-5}$ M), mixed ($K_i =$

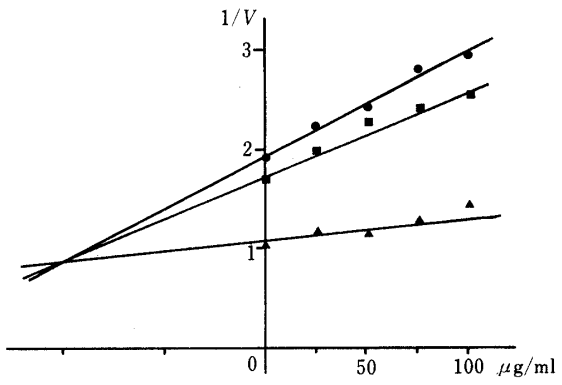


Fig. 2. Inhibition of Cyclic AMP Phosphodiesterase by Ginsenoside-Rg_1 (Dixon Plots)

The assay was carried out by the method described in a previous paper.[2]
Substrate concentration (^{3}H-cAMP): 50 μM (●), 75 μM (■) and 100 μM (▲).
Enzyme concentration: 4.5 mU (Boehringer).

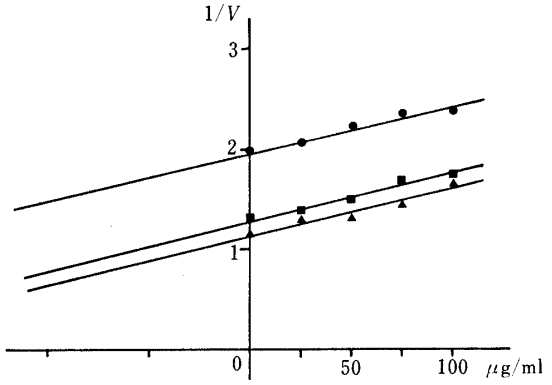


Fig. 3. Inhibition of Cyclic AMP Phosphodiesterase by Chikusetsusaponin V (Dixon Plots)

The assay was carried out by the method described in a previous paper.[2]
Substrate concentration (^{3}H-cAMP): 50 μM (●), 75 μM (■) and 100 μM (▲).
Enzyme concentration: 4.5 mU (Boehringer).

12.7×10^{-5} M) and uncompetitive inhibition patterns in Dixon plots, respectively (Figs. 1, 2 and 3). Thus, the mode of inhibition of cyclic AMP phosphodiesterase activity by these saponins may be related to their biological activities.

Experimental

Liquid scintillation counting was done with an Aloka LSC-903 instrument. Silica gel 60 Merck, precoated plates, 0.25 mm) was used for TLC and detection was achieved by illumination with an ultraviolet (UV) lamp or by spraying 10% H_2SO_4 followed by heating. For column chromatography, Silica gel C-200 (Wako) was used.

Assay Method for Cyclic AMP Phosphodiesterase——Samples were tested for cyclic AMP phosphodiesterase activity in duplicate by the method described in a previous paper.[2] All inhibitors were added as solutions in DMSO. The presence of DMSO in the assay medium at up to 2% concentration is known to have no effect on the enzyme activity. The IC 50 value is the concentration of a compound required to give 50% inhibition of cyclic AMP phosphodiesterase activity.

Extraction and Separation——The dried roots of *Panax ginseng* C. A. MEYER and rhizomes of *Panax japonicus* C. A. MEYER were purchased from Uchida Pharmacy for Oriental Medicine (Tokyo). A 20 g sample of each material was extracted with MeOH (200 ml × 3) under reflux for 3 h. The MeOH extract (5.35 and 6.50 g) was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer gave a solid residue ($CHCl_3$-soluble fraction; 0.39 and 0.13 g) on removal of the solvent by evaporation, and the $CHCl_3$-insoluble fraction was extracted three times with *n*-BuOH (*ca.* 10 volumes). On removal of the solvent under reduced pressure, the *n*-BuOH extract gave a residue (*n*-BuOH fraction; 1.5 and 4.11 g), and the aqueous layer was frozen and dried to give a powder-like product (*n*-BuOH-insoluble fraction; 2.98 and 1.79 g). These extracts were tested for inhibitory activity against cyclic AMP phosphodiesterase at a concentration of 100 μg/ml.

Purified Saponins——Purified ginsenoside-Ro, -Ra_1, -Ra_2, -Rb_1, -Rb_2, -Rb_3, -Rc, -Rd, -Re, -Rf, -Rg_1 and -Rg_2 were obtained from *Panax ginseng* root in the course of structural studies.[12-16] Purified chikusetsusaponin I, Ia, III, IV, IVa and V were obtained from *Panax japonicus* rhizome and leaves in the course of structural studies.[17-21] Pseudoginsenoside-F_{11} (from leaves of *P. pseudoginseng* subsp. *himalacius*),[22] notoginsenoside-R_1, -R_3, -Fa, -Fc, -Fe and gypenoside (from roots or leaves of *P. notoginseng*),[23-25] majonoside-R_1 (from rhizomes of *P. japonicus* var. *major*)[26] and tarasaponins V, VI and VII (from roots of *Aralia elata*),[27] prosapogenins (20(*S*)-ginsenoside-Rg_3 and 20(*R*)-ginsenoside-Rg_3)[8] and sapogenins (20(*S*)-protopanaxadiol, 20(*S*)-protopanaxatriol and oleanolic acid) were prepared in our laboratories (Tanaka and Shoji).

Dixon Plots——The assay was carried out by the method described in the previous paper.[4] Substrate concentration was 50, 75 and 100 μM. Concentration of cyclic AMP phosphodiesterase (Boeringer) was 4.5 mU.

References and Notes

1) A part of this work was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka.

April 1982 and the 29th Annual Meeting of the Japanese Society of Pharmacognosy, Sapporo, September 1982. This paper forms Part VII of "Inhibitors of Cyclic AMP Phosphodiesterase in Medicinal Plants." Part VI: T. Nikaido, Y. I. Sung, T. Ohmoto and U. Sankawa, *Chem. Pharm. Bull.*, **32**, 578 (1984).
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