[Chem. Pharm. Bull. 32(8)3075—3078(1984)]

Inhibitors of Adenosine 3′,5′-Cyclic Monophosphate Phosphodiesterase in Cassia Seed[1)]

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(Received December 20, 1983)

Aurantio-obtusin and obtusin were identified as inhibitors of adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase in seeds of *Cassia obtusifolia* L. and *Cassia tora* L. The structure–activity relationships of 22 anthraquinones and 17 analogous compounds were studied. Anthraquinones of the emodin and obtusin types were generally more inhibitory towards cAMP phosphodiesterase than other types of anthraquinones, anthrones and lactones.

Keywords——*Cassia obtusifolia*; *Cassia tora*; aurantio-obtusin; obtusin; phosphodiesterase inhibitor

Adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase is a useful tool for screening biologically active compounds contained in medicinal plants. The biological actions of traditional medicinal drugs are sometimes very difficult to detect by *in vivo* tests such as blind screening methods due to their mildness.

We have already reported on cAMP phosphodiesterase inhibitors contained in various medicinal plants, *Anemarrhena asphodeloides* BUNGE,[2)] *Forsythia suspensa* VAHL.,[3)] *Polygala tenuifolia* WILLD.,[4)] *Citrus reticulata* BLANCO.,[5)] *Iris florentina* L.,[5)] *Picrasma quassioides* BENNET.,[6)] *Phyllostachys nigra* MUNRO var. *henonis* STAPF.,[7)] *Phragmites communis* TRINN.,[7)] *Panax ginseng* C. A. MEYER[1)] and *Panax japonicus* C. A. MEYER.[1)] The present paper deals with the identification of cAMP phosphodiesterase inhibitors contained in the seeds of *Cassia obtusifolia* L. and *Cassia tora* L., which have been used as aperient, antiasthenic and diuretic agents and also to improve visual acuity in Chinese medicine. Some constituents, hydroxyanthraquinones[8)] and naphthopyrones[9)] have been isolated.

Results and Discussion

Hot aqueous extracts of commercial Cassia seeds showing inhibitory activity against beef heart cAMP phosphodiesterase, obtained previously,[2)] were extracted with chloroform. The cAMP phosphodiesterase inhibitors in the Cassia seeds were completely extracted with chloroform from the aqueous solution, and therefore the compounds contained in the chloroform-soluble fraction were examined. This fraction was refractionated by silica gel thin-layer chromatography (TLC) and the resulting fractions were assayed for inhibitory activity against cAMP phosphodiesterase. The most active fraction consisted mainly of two compounds with *Rf* values of 0.40 and 0.50 on TLC.

In order to characterize the active compounds, Cassia seeds were extracted with chloroform on a large scale. The extract was then fractionated by Takido's method[8)] to yield two active compounds. The active compounds showing *Rf* 0.40 and 0.50 were identified as

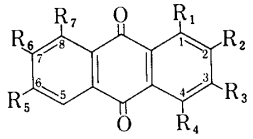
aurantio-obtusin (**19**) and obtusin (**21**) by comparison of their TLC behavior and infrared (IR) spectra with those of authentic samples. Therefore, anthraquinones previously isolated from *Cassia obtusifolia* L. and *Cassia tora* L. were also tested for inhibitory effect against cAMP phosphodiesterase. In addition to compounds **19** and **21**, compounds **11**, **15**, **18** and **22**

TABLE I. Inhibitory Activity of Cassia Seed Anthraquinones on cAMP Phosphodiesterase

Sample	IC_{50} ($\times 10^{-5}$ M)	Source
Chrysophanol (**7**)	21.3	C.o. & C.t.
Obtusifolin (**9**)	>100	C.o.
Emodin (**11**)	7.0	C.o.
Physcion (=Parietin, **14**)	>100	C.o. & C.t.
Questin (**15**)	6.1	C.o. & C.t.
1-Desmethylaurantio-obtusin (**18**)	13.1	C.o.
Aurantio-obtusin (**19**)	11.2	C.o. & C.t.
1-Desmethylchryso-obtusin (**20**)	35.2	C.o.
Obtusin (**21**)	7.6	C.o. & C.t.
Chryso-obtusin (**22**)	10.9	C.o. & C.t.

C.o.: *Cassia obtusifolia* L.
C.t.: *Cassia tora* L.

TABLE II. Inhibitory Activity of Anthraquinones on cAMP Phosphodiesterase



Compd. No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	IC_{50} ($\times 10^{-5}$ M)	Reference
1	OH	H	H	H	H	H	H	>100	10
2	OH	H	H	H	H	H	OH	>100	11
3	OH	OAc	H	H	H	H	H	47.9	12
4	OH	H	OAc	H	H	H	H	6.0	13
5	OH	H	H	OMe	H	H	H	>100	14
6	H	H	OBz	Br	H	H	H	68.5	15
7[a]	OH	H	Me	H	H	H	OH	21.3	8*a*
8	OH	H	COOH	H	H	H	OH	12.1	16
9[a]	OMe	OH	Me	H	H	H	OH	>100	8*a*
10	OH	H	Me	OH	H	H	OH	>100	17
11[a]	OH	H	Me	H	OH	H	OH	7.0	18
12	OH	H	CH_2OH	H	OH	H	OH	9.3	19
13	OH	H	COOH	H	OH	H	OH	12.0	19
14[a]	OH	H	Me	H	OMe	H	OH	>100	8*a*
15[a]	OH	H	Me	H	OH	H	OMe	6.1	20
16	OH	H	Me	OH	OH	H	OH	>100	21
17	OH	H	Me	H	OH	Cl	OH	3.4	22
18[a]	OH	OH	Me	H	OH	OMe	OH	13.1	23
19[a]	OMe	OH	Me	H	OH	OMe	OH	11.2	8*b*
20[a]	OH	OH	Me	H	OMe	OMe	OMe	35.2	23
21[a]	OMe	OH	Me	H	OMe	OMe	OH	7.6	8*b*
22[a]	OMe	OH	Me	H	OMe	OMe	OMe	10.9	8*b*

a) Cassia seed anthraquinone.

TABLE III. Inhibitory Activity of Anthrones, Lactones, Bianthraquinones and Other Related Compounds on cAMP Phosphodiesterase

Compound No.	Compound name	IC_{50} ($\times 10^{-5}$ M)
23	Chrysophanol-9-anthrone	47.9
24	Emodin-9-anthrone	>100
25	Physcion-9-anthrone	>100
26	Toralactone	13.2
27	Isotoralactone	37.1
28	Cassialactone	>100
29	Rubrofusarin	14.7
30	Iridoskyrin	>100
31	Skyrin	>100
32	Luteoskyrin	17.5
33	Penicilliopsin	>100
34	Averufin	3.9
35	Germichrysone	30.4
36	Torosachrysone	26.0
37	Scytalone	>100
38	Flaviolin	22.1
39	Torachrysone	18.5

showed higher inhibitory effects than other anthraquinones (Table I).

Various other free anthraquinones contained in these plants and related compounds were also assayed to investgate the structure–activity relationship (Tables II and III).

The results showed that the presence of a substituent at the 3-position is required for inhibitory activity. In the congeners **1**—**8** which have one to three substituents attached to the anthraquinone skeleton, the presence of an oxygen atom at the 6-position was not always required, but in th congeners **9**—**22** which have four to six substituents, it was required for inhibitory activity. The congeners **16**—**22** which have five and six substituents showed a fair inhibitory activity except for compounds **16** and **20**.

The anthrones, lactones and other anthraquinones **23**—**39** were generally not markedly inhibitory, except for **34**. It seems that the presence of hydroxyl groups at the β-position the anthraquinone skeleton is necessary for inhibitory activity towards cAMP phosphodiesterase. Interestingly, most of the strong inhibitors among the above compounds are components of Cassia seed.

Experimental

The following instruments were used to obtain the physical data. The liquid scintillation counter used was an Aloka LSC-903. Silica gel 60 (Merck, precoated plate, 0.25 mm) was used for TLC with 0.5 N oxalic acid as the solvent, and detection was done 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 Pure Chemicals Co., Ltd.) was used. Infrared (IR) spectra were recorded on a Hitachi EPI-G3 spectrometer. All melting points were determined with a micromelting point apparatus, and are uncorrected.

Medicinal Plant——Cassiae Semen (Japanese name "Ketsumeishi") was purchased from Uchida Pharmacy for Oriental Medicine (Tokyo).

Assay Method for cAMP Phosphodiesterase——Samples were tested for cAMP phosphodiesterase activity in duplicate by the method described in the previous paper.[2] All the inhibitors were added as solutions in dimethylsulfoxide (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 compound required for 50% inhibition of cAMP phosphodiesterase activity.

Enzymes and Chemicals——Beef heart phosphodiesterase and cAMP were obtained from Sigma, and [^{3}H]-

cAMP from the Radiochemical Centre. Papaverine, a reference inhibitor, was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo).

Extraction and Separation——Commercial Cassia seeds (10 g) were extracted three times with hot water (150 ml each) for 6 h and the combined extract was concentrated, frozen and dried to give a powder (1.34 g), which was dissolved in water and fractionated with $CHCl_3$ (50 ml × 3). Evaporation of the solvent under reduced pressure gave a solid residue ($CHCl_3$ fraction; 0.14 g). The aqueous layer was frozen and dried to give a powder ($CHCl_3$-insoluble fraction; 1.14 g). These extracts were tested for inhibitory effect on cAMP phosphodiesterase (88.1% and not observed, respectively). Aurantio-obtusin (**19**) and obtusin (**21**), which showed spots of *Rf* 0.40 and 0.50 [solvent; C_6H_6–AcOEt (4 : 1)], in the $CHCl_3$-soluble fraction were the main active constituents.

In order to identify biologically active components, commercial Cassia seeds (100 g) were extracted with $CHCl_3$ (500 ml × 3) under reflux for 4 h. Each fraction was tested for inhibitory effect against cAMP phosphodiesterase.

Aurantio-obtusin (19)——The $CHCl_3$ extract (4.2 g) was chromatographed on a silica gel column with benzene and $CHCl_3$. The active fraction eluted with benzene–$CHCl_3$ (9 : 1) was refractionated by preparative TLC [solvent; benzene–AcOEt (4 : 1)]. The main active compound in this fraction showed *Rf* 0.40 on TLC and was identified as aurantio-obtusin (**19**) by mixed mp determination [mp 263—265 °C, lit.,[8b] mp 265—266 °C)], and comparison of the TLC behavior and IR spectrum with those of an authentic sample.

Obtusin (21)——The fraction eluted with benzene on silica gel column chromatography was refractionated by preparative TLC. The active compound showed *Rf* 0.50 on TLC and was identified as obtusin (**21**) by mixed mp determination [mp 241—243 °C, lit.,[8b] mp 242—243 °C)] and comparison of the TLC behavior and IR spectrum with those of an authentic sample.

Authentic Anthraquinones, Anthrones and Lactones——The authentic samples which were used for tests of inhibitory action on cAMP phosphodiesterase were isolated or prepared during structural studies (Tables II and III).

References and Notes

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