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Inhibition of Adenosine 3′,5′-Cyclic Monophosphate Phosphodiesterase by Components of *Sophora flavescens* AITON[1)]

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Adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase inhibitors contained in the roots of *Sophora flavescens* AITON were identified as flavonoids, kushenol A (**9**), kurarinone (**5**), and kuraridin (**22**). The structure–inhibitory activity relationships were studied in 30 flavonoids. *O*-Methylation of a hydroxyl group on an aromatic ring decreased the activity. The prenyl group is important for high inhibitory activity. A kinetic study revealed that nor-kurarinone, kurarinol and kuraridin non-competitively inhibit cAMP phosphodiesterase.

Keywords——cAMP phosphodiesterase; inhibitor; *Sophora flavescens*; flavonoid; kushenol A; kurarinone; nor-kurarinone; kurarinol; kuraridin

Introduction

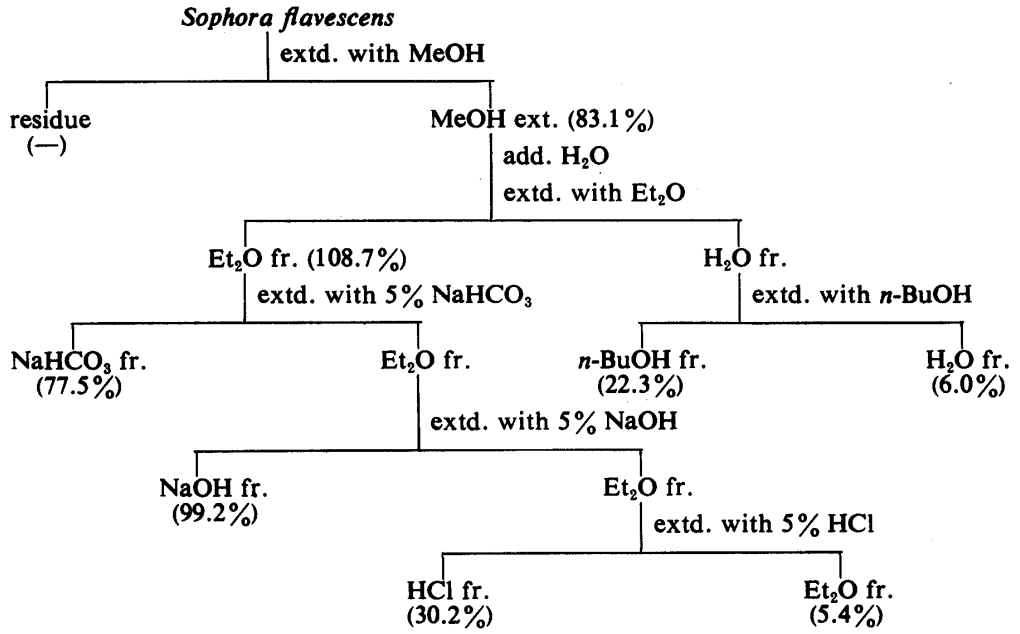
Adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase is a useful tool for screening biologically active compounds contained in medicinal plants. In previous papers, we have reported on cAMP phosphodiesterase inhibitors contained in various medicinal plants, *i.e.*, *Anemarrhena asphodeloides* 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,[8)] *Panax japonicus* C. A. MEYER,[8)] *Cassia obtusifolia* L.,[9)] *Cassia tora* L.,[9)] *Morus alba* L.[10)] and *Olea* and *Fraxinus* barks.[1)]

This paper deals with the identification of cAMP phosphodiesterase inhibitors present in the root of *Sophora flavescens* AITON, which has been used as a bitter stomachic, diuretic, antiphlogistic, antidiarrheal and anthelmintic in Chinese traditional medicine. The structure–activity relationships in 17 compounds identified as components of the root of *Sophora flavescens* AITON and 13 analogous compounds were investigated, and the results are discussed.

Results and Discussion

In order to identify the cAMP phosphodiesterase inhibitors, roots of *Sophora flavescens* AITON were extracted with hot methanol and the extracts were fractionated as shown in Chart 1. The sodium hydroxide- and sodium bicarbonate-soluble fractions showed considerable inhibition of cAMP phosphodiesterase.

The sodium hydroxide-soluble fraction, which showed relatively high inhibitory activity, was further fractionated by silica gel column chromatography, centrifugally accelerated



[inhibition %: 100 μg/ml]

Chart 1

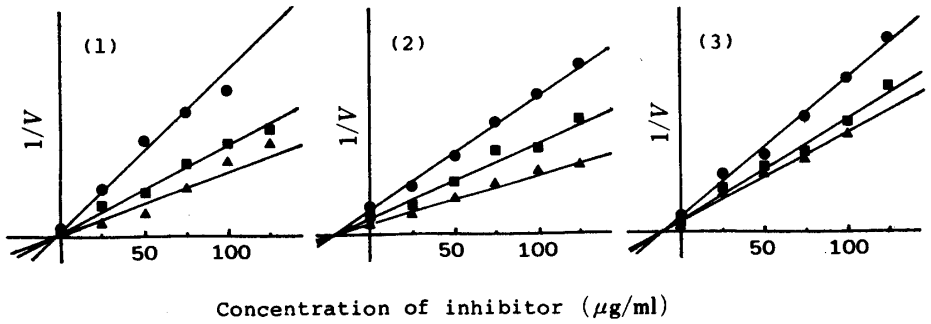


Fig. 1. Dixon Plots of the Inhibition of cAMP Phosphodiesterase by Nor-kurarinone (1), Kurarinol (2) and Kuraridin (3)

The assay was carried out by the method described in a previous paper.[5]
Substrate concentration (^{3}H-cAMP): 50 μM (●), 75 μM (■), 100 μM (▲). Enzyme amount: 4.5 mU (Boehringer).

chromatography (Chromatotron) and preparative thin-layer chromatography (TLC), and each fraction was tested for cAMP phosphodiesterase inhibition. Nor-kurarinone (**1**), iso-kurarinone (**4**), kushenol A (**9**) and kuraridin (**22**) were identified as active components.

For studies on structure–activity relationships, flavonoids which had been isolated from *Sophora flavescens* in structural studies[11-17] were tested for inhibition of cAMP phosphodiesterase. We have already reported that a polymethoxy flavone, nobiletin,[5] was more active than the corresponding flavone with free hydroxyl groups, desmethoxynobiletin,[5] but on the other hand, morusin (**12**), with free hydroxyl groups, was more active than morusin monomethylether (**13**) or trimethylether (**14**).[10]

The activities of trimethyl nor-kurarinone (**7**), tetramethyl nor-kurarinone (**8**) and trimethyl kuraridin (**24**), were lower than those of the corresponding flavonoids, **1** and kuraridin (**22**), with free hydroxyl groups. This is similar to the case of **12** and morachalcone A (**25**). Isokurarinone (**4**) and **13**, which have a methoxyl group at the C-2′ position in place of a hydroxyl group in **1** and **12**, respectively, showed lower decreases in inhibitory effect on

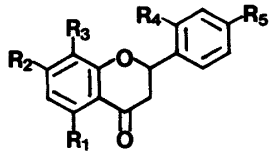
TABLE I. Inhibitory Activity of Flavonoids on cAMP Phosphodiesterase

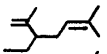
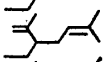
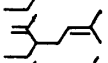
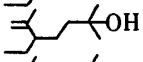
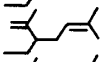
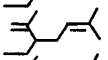
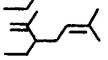
Sample	IC_{50} ($\times 10^{-5}$ M)	Reference
Flavanone		
Nor-kurarinone (**1**)[a]	3.9	14
Kushenol E (**2**)[a]	4.0	17
Kushenol B (**3**)[a]	3.1	16
Isokurarinone (**4**)[a]	5.7	15
Kurarinone (**5**)[a]	2.5	14
Kurarinol (**6**)[a]	6.3	15
7,2′,4′-TriMe nor-kurarinone (**7**)	>500	14
5,7,2′,4′-TetraMe nor-kurarinone (**8**)	>500	14
Kushenol A (**9**)[a]	1.4	16
Isoxanthohumol (**10**)[a]	>500	13
Kuwanone E (**11**)	1.7	10
Flavone		
Morusin (**12**)	1.1	10
2′-OMe morusin (**13**)	23.7	10
Morusin triMe (**14**)	>500	10
Kuwanone C (**15**)	3.8	10
Flavanonol		
Kushenol H (**16**)[a]	17.2	17
Flavonol		
Kushenol C (**17**)[a]	6.3	16
Kushenol G (**18**)[a]	7.0	17
Kaempferol (**19**)	20.6	5
Morin (**20**)	21.9	Commercial[b]
Quercetin (**21**)	31.7	5
Chalcone		
Kuraridin (**22**)[a]	3.1	14
Kuraridinol (**23**)[a]	8.7	15
2,4,4′-TriMe kuraridin (**24**)	>500	14
Morachalcone A (**25**)	11.5	10
2,4,4′-TriMe morachalcone A (**26**)	>500	10
Isoflavone		
Formononetin (**27**)[a]	20.9	5
Maackiain (**28**)[a]	>500	11
Pterocarpin (**29**)[a]	>500	11
Trifolirhizin (**30**)[a]	>500	12
Cf. rapaverine	3.0	Commercial[c]

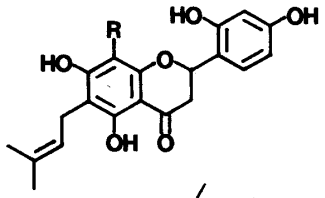
a) These compounds were identified from *Sophora flavescens*. *b*) Sigma Chemical Company. *c*) Tokyo Kasei Kogyo Co., Ltd.

cAMP phosphodiesterase. Trimethyl kuraridin (**24**) and trimethyl morachalcone A (**26**) which are methylated derivatives of **22** and morachalcone A (**25**), respectively, also lost inhibitory activity. Namely, *o*-methylation of a hydroxyl group on an aromatic ring decreased the activity.

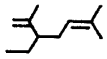
Kushenol E (**2**), which has two prenyl groups at the C-6 and C-8 positions of the simple flavanone, has strong inhibitory activity on cAMP phosphodiesterase with nearly the same IC_{50} as kuwanone C (**15**), which has two prenyl groups at the C-3 and C-8 positions of the simple flavone. Kushenol B (**3**) which has an additional prenyl group at the C-6 position as compared with **1**, having a prenyl group at C-8, also showed strong inhibitory activity. Moreover, these flavonoids have the same hydroxyl group substitution. Thus, the prenyl

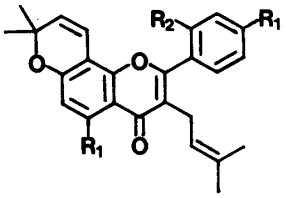


	R_1	R_2	R_3	R_4	R_5
1	OH	OH		OH	OH
4	OH	OH		OMe	OH
5	OMe	OH		OH	OH
6	OMe	OH		OH	OH
7	OH	OMe		OMe	OMe
8	OMe	OMe		OMe	OMe
9	OH	OH		OH	—
10	OMe	OH		—	OH

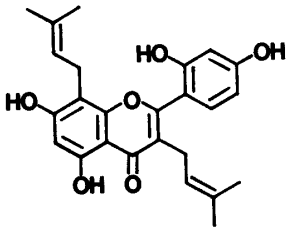


2 : R= 

3 : R= 



	R_1	R_2
12	OH	OH
13	OH	OMe
14	OMe	OMe



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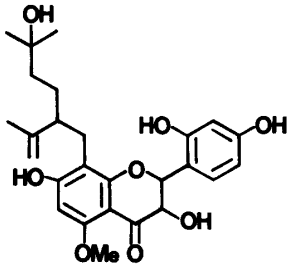

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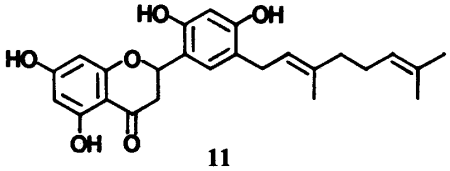

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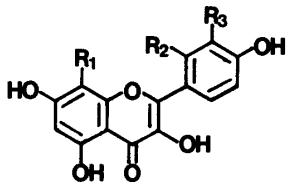
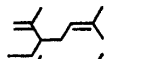
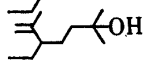
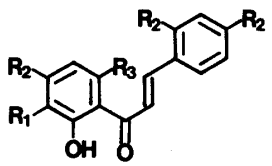
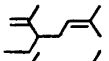
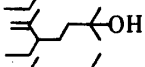
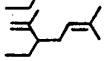
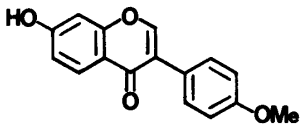


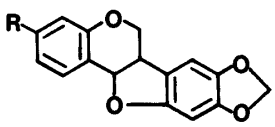
	R_1	R_2	R_3
17		OH	—
18		OH	—
19	—	—	—
20	—	OH	—
21	—	—	OH



	R_1	R_2	R_3
22		OH	OMe
23		OH	OMe
24		OMe	OMe
25		OH	—
26		OMe	—



27



28 : R=OH

29 : R=OMe

30 : R=O-glu

Chart 2

group is important for high inhibitory activity toward cAMP phosphodiesterase.

In addition, **1**, **3**, **4**, kurarinone (**5**), **9**, kushenol C (**17**) and **22**, which have a lavandulyl(5-methyl-2-isopropenyl-hex-4-enyl) or a geranyl side chain attached to the aromatic ring and have not more than one methoxyl group, showed strong inhibitory activity.

Kurarinol (**6**), in which H_2O is added to the lavandulyl side chain of **5**, showed slightly lower inhibitory activity than **5**, and kushenol H (**16**), which has a hydroxyl group at the C-3 position of **6**, showed much lower inhibitory activity than **6**. On the other hand, a flavonol, kushenol G (**18**), in which H_2O is added to the lavandulyl side chain in **17**, did not show lower inhibitory activity. Morin (**20**) has the same hydroxyl group substitution as **18**. Since **20** shows inhibitory activity as low as that of kaempferol (**19**), it appears that the lavandulyl side chain is related to the inhibitory activity in flavonols. A chalcone, kuraridinol (**23**), in which H_2O is added to the lavandulyl side chain of **22**, showed lower inhibitory activity, as in the case of **5**

and **6**.

A weak inhibitor, formononetin (**27**), was also isolated from the sodium hydroxide-soluble fraction. Maackiain (**28**) and trifolirhizin (**30**), which had been isolated from *Sophora flavescens* before,[12] did not show inhibitory activity.

Three inhibitors of cAMP phosphodiesterase were further investigated by preparing Dixon plots by the method described in the previous paper.[5] Compounds **1** and **6** were selected from among flavanone congeners with a lavandulyl or 5-hydroxy-2-isopropenyl-5-methylhexyl side chain, and **22** was selected from among chalcone congeners. Compounds **1**, **6** and **22** showed non-competitive kinetic patterns and the K_i values determined from the Dixon plots were 7.1×10^{-6}, 4.3×10^{-5} and 2.5×10^{-5} M respectively (Fig. 1). The substitution patterns of these compounds resemble that of morusin, but these compounds differed kinetically from **12**, which showed an uncompetitive pattern.[10]

Experimental

The following instruments were used to obtain the physical data. The liquid scintillation counter used was an Aloka LSC-903. Silica gel 60 (Kieselgel 60 F_{254}, Merck) was used for TLC, and detection was achieved by illumination with an ultraviolet (UV) lamp, by spraying 1 N $FeCl_3$ or by spraying 10% H_2SO_4 followed by heating. On preparative TLC (Kieselgel 60 GF_{254}, Merck, 0.5 mm), detection was also achieved by UV illumination. For column chromatography, Silica gel C-200 (Wako) was used. For centrifugally accelerated chromatography (Chromatotron), Silica gel 60 (Kieselgel 60 GF_{254}, Merck, 1, 2 and 4 mm) was used. Infrared (IR)spectra were recorded on a Hitachi 260-30 infrared spectrophotometer. UV spectra were recorded on a Hitachi 340 recording spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded with Hitachi R-900 and JEOL GX-400 spectrometers. The mass (MS) spectra were measured with a JEOL JMS-01SG-2 mass spectrometer.

Assay Method for cAMP Phosphodiesterase——Samples were tested for cAMP phosphodiesterase activity in duplicate by the method described in a previous paper.[2] All 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 a compound required to give 50% inhibition of cAMP phosphodiesterase activity.

Dixon Plots——The assay was carried out by the method described in the previous paper.[5] Substrate concentration was 50, 75 or 100 μM. The amount of cAMP phosphodiesterase (Boehringer) used was 4.5 mU.

Extraction and Separation——The dried roots of *Sophora flavescens* (3 kg, purchased from Uchida Pharmacy for Oriental Medicine, Tokyo) were extracted twice with hot MeOH (6000 ml each) for 3 h and the precipitate was removed by decantation. The extract was concentrated, and the residue was dissolved in water and extracted 3 times with Et_2O. The Et_2O solution was shaken with 5% $NaHCO_3$, 5% NaOH, and 5% HCl. Each fraction was tested for inhibitory effect on cAMP phosphodiesterase. The NaOH fraction was found to be the most active, so it was separated by column chromatography, centrifugally accelerated chromatography, and preparative TLC.

Authentic Flavonoids——The authentic samples which were used for tests of inhibitory action on cAMP phosphodiesterase had been isolated or prepared during structural studies.

Nor-kurarinone (1)——This was identified by comparison (mmp, TLC, and UV, IR, NMR and MS spectra) with an authentic sample.

Methylation of 1 (Formation of 7, 8 and 24)——Methylation of **1** was carried out by the reported method.[14] The products were identified from the TLC behavior, and UV, IR, NMR and MS spectral data.

Isokurarinone (4)——This was identified by comparison (mmp, TLC, and UV, IR, NMR and MS spectra) with an authentic sample.

Kushenol A (9)——This was identified from the UV, IR, NMR and MS spectral data.[16]

Isoxanthohumol (10)——This was identified from the UV, IR, NMR and MS spectra data.[13]

Kuraridin (22)——This was identified by comparison (TLC, and UV, IR, NMR and MS spectra) with an authentic sample.

Methylation of 22 (Formation of 24)——Methylation of **22** was carried out by the reported method.[14] **24** was identified from the TLC behavior, and UV, IR, NMR and MS spectral data.

Formononetin (27)——This was identified by comparison (mmp, TLC, and UV and IR spectra) with an authentic sample.

References and Notes

1) A part of this study was presented at the 105th Annual Meeting of the Phamaceutical Society of Japan,

Kanazawa, April 1985. This paper forms Part XI of "Inhibitors of Cyclic AMP Phosphodiesterase in Medicinal Plants." Part X: S. Nishibe, H. Tsukamoto, S. Hisada, T. Nikaido, T. Ohmoto and U. Sankawa, *Shoyakugaku Zasshi*, **40**, 89 (1986).
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