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Inhibition of Adenosine 3′,5′-Cyclic Monophosphate Phosphodiesterase by Phenolic Constituents of Mulberry Tree[1)]

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Adenosine 3′,5′-cyclic monophosphate (cyclic AMP) phosphodiesterase inhibition by phenolic constituents of mulberry tree was studied. The structure–inhibitory activity relationships were studied in 56 derivatives of prenylflavonoids, prenylchalcones and Diels–Alder type adducts. Diels–Alder type adducts of chalcones and dehydroprenylflavonoids or dehydroprenyl-2-arylbenzofurans differed from prenylflavonoids and prenylchalcones in the inhibitory activity and inhibitory type (determined by means of Dixon plots).

Keywords——cyclic AMP phosphodiesterase; inhibitor; *Morus alba*; prenylflavonoid; Diels–Alder type adduct

We have already reported many adenosine 3′,5′-cyclic monophosphate (cyclic AMP) phosphodiesterase inhibitors contained in medicinal plants; several phenol compounds were identified from *Citrus reticulata*,[2)] *Iris florentina*,[2)] *Phyllostachys nigra* var. *henonis*[3)] and *Phragmites communis*.[3)]

On the other hand, it has been suggested[4)] that hypotensive constituents of the root bark of the mulberry tree (*Morus alba* L. and other plants of the genus Morus) are phenolic compounds. Nomura *et al.*[6-8,10,11b-23,25-31)] have isolated many phenolic constituents from this plant, so these phenolic constituents were examined in order to elucidate the structure–activity relationships, and the results are discussed.

Results and Discussion

Many phenolic constituents of the root bark of the cultivated mulberry tree, the Chinese crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi"), and *Morus alba* callus tissues have been isolated by Nomura *et al.*[6-8,10,11b-23,25-31)] These constituents and their derivatives were tested for inhibitory activity against beef heart cyclic AMP phosphodiesterase using the method reported in the previous paper.[5)] The assay consists of a two-step isotopic procedure. Tritium-labeled cyclic AMP is hydrolyzed to 5′-AMP by phosphodiesterase and the 5′-AMP is then further hydrolyzed to adenosine by snake venom nucleotidase. The anion-exchange resin binds all charged nucleotides and leaves [^{3}H]adenosine as the only labeled compound to be counted. The results are summarized in Table I.

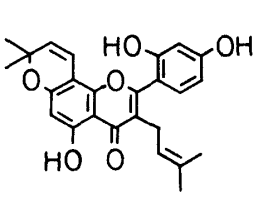
These samples may be grouped into six categories, (1) prenylflavonoids and prenylchalcones, (2) Diels–Alder type adducts of chalcones and dehydroprenylflavonoids, (3) Diels–Alder type adducts of chalcones and dehydroprenyl-2-arylbenzofurans, (4) Diels–Alder type adducts of chalcones and dehydroprenylchalcones, (5) Diels–Alder type adducts of prenylphenols and dehydroprenylphenols and, (6) the others. The congeners whose phenolic hydroxyl groups are not substituted, compounds **1**—**12**, belonging to group (1), showed

Table I. Inhibitory Activity of Phenolic Compounds on Cyclic AMP Phosphodiesterase

Sample	IC_{50} ($\times 10^{-5}$ M)	Reference
(1) Prenylflavonoids and prenylchalcones		
Morusin (**1**)	1.1	6
Morusin hydroperoxide (**2**)	4.3	7
Cyclomorusin (**3**)	6.5	6
Oxydihydromorusin (=morusinol, **4**)	5.0	8*a*, 32
Kuwanon C (**5**)	3.8	8*a*
Tetrahydrokuwanon C (**6**)	0.4	9
Kuwanon D (**7**)	0.4	10
Kuwanon E (**8**)	1.7	10
Compound A (**9**)	>500	6
Morachalcone A (**10**)	11.5	11*a*, 12
Sanggenon A (**11**)	17.2	13
Sanggenon B (**12**)	4.8	14
2′-OMe morusin (**13**)	23.7	7
Morusin diAc (**14**)	194	6
Morusin triMe (**15**)	>500	6
Tetrahydrokuwanon C tetraMe (**16**)	>500	9
Kuwanon E triAc (**17**)	>500	10
Morachalcone A triMe (**18**)	>500	15, 16
Sanggenon A triMe (**19**)	23.4	17
Sanggenon B pentaMe (**20**)	17.2	14
(2) Adducts of chalcones and dehydroprenylflavonoids		
Kuwanon G (**21**)	2.2	18
Kuwanon H (**22**)	1.3	15
Sanggenon C (**23**)	0.1	19
Sanggenon D (**24**)	2.6	20
Kuwanon G hexaMe (**25**)	>500	18*a*
Kuwanon G heptaMe (**26**)	>500	18*a*
Kuwanon G heptaMe (**27**)	114	18*a*
Kuwanon G D-octaMe (**28**)	76.1	18*a*
Sanggenon C octaMe (**29**)	16.5	19
Sanggenon C octaMe (**30**)	73.2	21
(3) Adducts of chalcones and dehydroprenyl-2-arylbenzofurans		
Chalcomoracin (**31**)	1.1	11
Kuwanon P (**32**)	1.8	22
Albanol B (**33**)	0.9	23, 24
Mulberrofuran A (**34**)	2.6	25
Mulberrofuran C (**35**)	1.1	26
Mulberrofuran F (**36**)	6.4	23
Mulberrofuran G (**37**)	1.0	23
Mulberrofuran H (**38**)	5.8	27
Mulberrofuran I (**39**)	2.5	28
Mulberrofuran J (**40**)	0.2	29
Kuwanon P octaMe (**41**)	10.7	22
Mulberrofuran C heptaMe (**42**)	7.5	26
Mulberrofuran F pentaMe (**43**)	30.0	23
Mulberrofuran G pentaMe (**44**)	0.9	23
Mulberrofuran I pentaMe (**45**)	27.5	28
Mulberrofuran J heptaMe (**46**)	27.3	29
(4) Adducts of chalcones and dehydroprenylchalcones		
Kuwanon I (**47**)	0.6	16
Kuwanon J (**48**)	2.4	11*b*

TABLE I. (continued)

Sample	IC_{50} ($\times 10^{-5}$ M)	Reference
Kuwanon Q (**49**)	0.3	30
Kuwanon R (**50**)	2.2	30
Kuwanon I hexaMe (**51**)	7.0	16
Kuwanon R heptaMe (**52**)	19.5	30
(5) Adducts of prenylphenols and dehydroprenylphenols		
Kuwanon M (**53**)	4.8	31
(6) The others		
2′-Hydroxy-2,4,4′-triMe chalcone (**54**)	>500	16, 18*a*
Compound 10 (**55**)	340	18*b*
Compound 11 (**56**)	>500	18*b*



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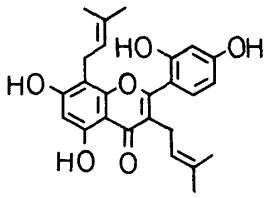

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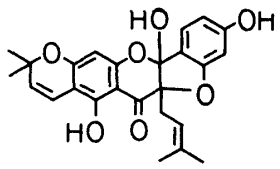

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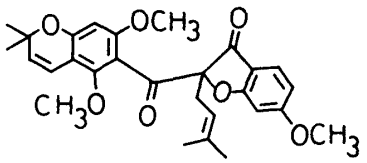

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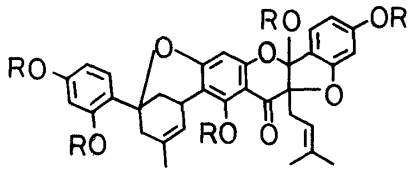


12 : R=H

20 : R=CH_3

strong inhibitory activity except for compound **9**. Substitution of phenolic hydroxyl groups of these compounds clearly decreased the inhibitory effect. However, in the case of sanggenones A (**11**) and B (**12**) which are prenylflavonoids having a hemiketal moiety in the structure, substitution had no effect.

The other groups (2)—(5) showed strong inhibitory effects, especially in the case of phenolic compounds whose hydroxyl groups are not substituted. The permethylate derivatives of the inhibitors belonging to groups (3) and (4) did not show greatly decreased inhibitory activities. The Diels–Alder type adducts of chalcones and dehydroprenyl 2-arylbenzofurans (3) or dehydroprenylchalcones (4) showed lower decreases in inhibitory effect upon substitution of the hydroxyl groups than the adducts of chalcones and dehydroprenylflavonoids (2).

Nikaido *et al.* reported[2] that in the case of flavonoids with five or six substituents, polymethoxy derivatives (*e.g.* nobiletin and irigenin, contained in *Citrus reticulata* and *Iris florentina*, respectively) were more effective inhibitors of cyclic AMP phosphodiesterase than the corresponding flavonoids with free hydroxyl groups. It is interesting that the inhibition by simple flavonoids differs from that by complex phenolic compounds.

Three inhibitors of cyclic AMP phosphodiesterase were investigated by the method described in the previous paper.[2] These inhibitors were selected from group (1) to group (3)

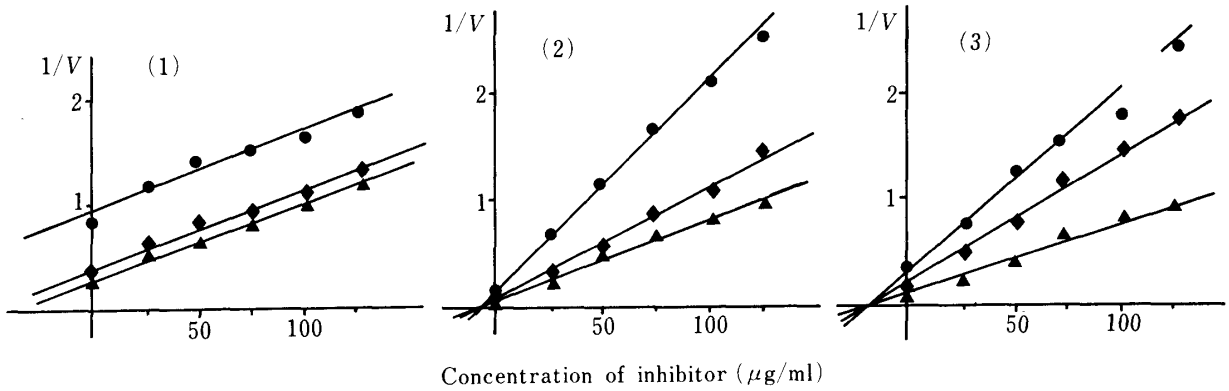
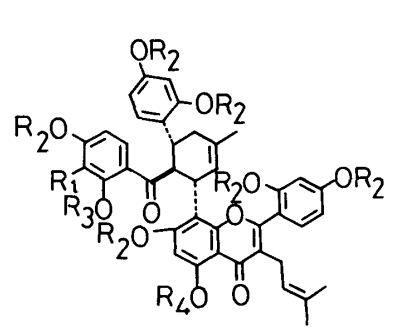


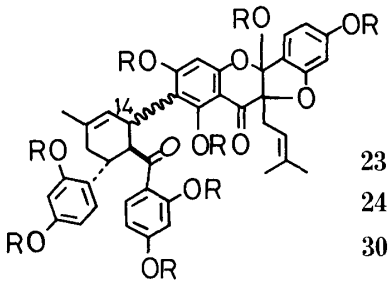
Fig. 1. Inhibition of Cyclic AMP Phosphodiesterase by Morusin (**1**), Kuwanon G (**2**) and Mulberrofuran G (**3**) (Dixon Plots)

The assay was carried out by the method described in a previous paper.[2]
Substrate concentration (^{3}H-cyclic AMP): 50 μM (●), 75 μM (◆), 100 μM (▲).
Enzyme amount: 4.5 mU (Boehringer).
V: nmol of hydrolyzed cyclic AMP/mg prot. min.

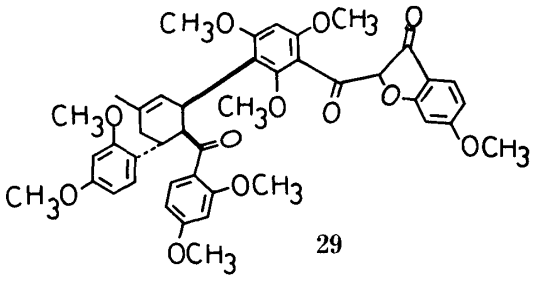


21 : $R_1=R_2=R_3=R_4=H$
22 : $R_1=$ —⟨ , $R_2=R_3=R_4=H$

25 : $R_1=R_3=R_4=H$, $R_2=CH_3$
26 : $R_1=R_4=H$, $R_2=R_3=CH_3$
27 : $R_1=R_3=H$, $R_2=R_4=CH_3$
28 : $R_1=H$, $R_2=R_3=R_4=CD_3$



23 : R = H, 14α H
24 : R = H, 14β H
30 : R = CH_3, 14α H



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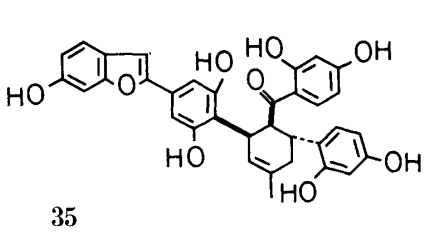

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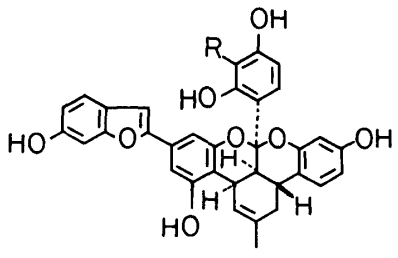

36 : R = —⟨
37 : R = H

because they showed strong inhibitory effects and were available in sufficient amounts for the assay. It was found that morusin (**1**, group 1), kuwanon G (**21**, group 2) and mulberrofuran G (**37**, group 3) showed uncompetitive, non-competitive ($K_i = 1.2 \times 10^{-5}$ M) and non-competitive

($K_i = 3.7 \times 10^{-5}$ M) inhibition patterns, respectively, in Dixon plots (Fig. 1).

Nomura *et al.* tested the hypotensive constituents of mulberry tree and found that kuwanons G (**21**)[18] and H (**22**),[15] and sanggenons C (**23**)[19] and D (**24**)[20] of group 2, and mulberrofurans C (**35**),[26] F (**36**)[23] and G (**37**)[23] of group 3 showed clear hypotensive effects, but kuwanon C (**5**),[8b] oxydihydromorusin (=morusinol, **4**)[32b] and sanggenon B (**12**) of group 1 did not; further, sanggenon B showed a hypertensive effect.[33]

Thus, the mode of inhibition of cyclic AMP phosphodiesterase activity by these compounds may be related to their hypotensive effects. Further work on the hypotensive effects of other constituents of mulberry tree is desirable.

Experimental

Assay Method for Cyclic AMP Phosphodiesterase——Samples were tested for cyclic AMP phosphodiesterase activity in duplicate by the method described in a previous paper.[5] 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 cyclic AMP phosphodiesterase activity. Liquid scintillation counting was done with an Aloka LSC-903 instrument.

Authentic Phenol Compounds——The authentic samples which were used for tests of inhibitory action on cyclic AMP phosphodiesterase were isolated or prepared during structural studies (Table I).

Dixon Plots——The assay was carried out by the method described in the previous paper.[2] Substrate concentration was 50, 75 or 100 μM. The amount of cyclic AMP phosphodiesterase (Boehringer) used was 4.5 mU.

References and Notes

1) This paper forms Part IX of "Inhibitors of Cyclic AMP Phosphodiesterase in Medicinal Plants." Part VIII: T. Nikaido, T. Ohmoto, U. Sankawa, S. Kitanaka, and M. Takido, *Chem. Pharm. Bull.*, **34**, 3075 (1984).
2) T. Nikaido, T. Ohmoto, U. Sankawa, T. Hamanaka, and K. Totsuka, *Planta Med.*, **46**, 162 (1982).
3) T. Nikaido, Y-I. Sung, T. Ohmoto, and U. Sankawa, *Chem. Pharm. Bull.*, **32**, 578 (1984).
4) *a*) T. Ohishi, *Sanshi Sikenjo Iho*, **59**, 1 (1941); *b*) B. Suzuki and T. Sakuma, *ibid.*, **59**, 9 (1941).
5) T. Nikaido, T. Ohmoto, H. Noguchi, T. Kinoshita, H. Saitoh, and U. Sankawa, *Planta Med.*, **43**, 18 (1981).
6) T. Nomura, T. Fukai, S. Yamada, and M. Katayanagi, *Chem. Pharm. Bull.*, **26**, 1394 (1978).
7) T. Nomura, T. Fukai, S. Yamada, and M. Katayanagi, *Chem. Pharm. Bull.*, **26**, 1431 (1978).
8) *a*) T. Nomura, T. Fukai, and M. Katayanagi, *Chem. Pharm. Bull.*, **26**, 1453 (1978); *b*) unpublished data.
9) T. Nomura, Y. Sawaura, T. Fukai, S. Yamada, and S. Tamura, *Heterocycles*, **9**, 1355 (1978).
10) T. Nomura and T. Fukai, *Planta Med.*, **42**, 79 (1981).
11) *a*) M. Takasugi, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, *Chem. Lett.*, **1980**, 1573; *b*) S. Ueda, T. Nomura, T. Fukai, and J. Matsumoto, *Chem. Pharm. Bull.*, **30**, 3042 (1982).
12) The compound was also obtained from the root bark of *Morus alba* L. and *M. lhou* (*Ser.*) Koidz. (T. N., unpublished data).
13) T. Nomura, T. Fukai, and Y. Hano, *Planta Med.*, **47**, 30 (1983).
14) T. Nomura, T. Fukai, Y. Hano, and S. Urano, *Planta Med.*, **47**, 95 (1983).
15) T. Nomura, T. Fukai, and T. Narita, *Heterocycles*, **14**, 1943 (1980).
16) T. Nomura, T. Fukai, J. Matsumoto, A. Imashimizu, S. Terada, and M. Hama, *Planta Med.*, **46**, 167 (1982).
17) Y. Hano, M. Itoh, N. Koyama, and T. Nomura, *Heterocycles*, **22**, 1791 (1984).
18) *a*) T. Nomura and T. Fukai, *Chem. Pharm. Bull.*, **28**, 2548 (1980); *b*) T. Nomura, T. Fukai, T. Narita, S. Terada, J. Uzawa, Y. Iitaka, M. Takasugi, S. Ishikawa, S. Nagao, and T. Masamune, *Tetrahedron Lett.*, **22**, 2195 (1981).
19) T. Nomura, T. Fukai, Y. Hano, and J. Uzawa, *Heterocycles*, **16**, 2141 (1981).
20) T. Nomura, T. Fukai, Y. Hano, and J. Uzawa, *Heterocycles*, **17**, 381 (1982).
21) Y. Hano, M. S. Thesis, Toho University 1982.
22) Y. Hano, S. Takizawa, E. Mizuno, and T. Nomura, *Chem. Pharm. Bull.*, **31**, 2936 (1983).
23) T. Fukai, Y. Hano, K. Hirakura, T. Nomura, J. Uzawa, and K. Fukushima, *Heterocycles*, **22**, 473 (1984).
24) A. V. Rama Rao, V. H. Deshpande, R. K. Shastri, S. S. Tavale, and N. N. Dhaneshwar, *Tetrahedron Lett.*, **24**, 3013 (1983).
25) T. Nomura, T. Fukai, J. Uno, and T. Arai, *Heterocycles*, **9**, 1593 (1978).
26) T. Nomura, T. Fukai, J. Matsumoto, and T. Ohmori, *Planta Med.*, **46**, 28 (1982).
27) T. Fukai, Y. Hano, K. Hirakura, T. Nomura, and J. Uzawa, *Chem. Pharm. Bull.*, **32**, 808 (1984).
28) Y. Hano, T. Fukai, T. Nomura, J. Uzawa, and K. Fukushima, *Chem. Pharm. Bull.*, **32**, 1260 (1984).
29) T. Fukai, Y. Hano, K. Hirakura, T. Nomura, J. Uzawa, and K. Fukushima, *Heterocycles*, **22**, 1007 (1984).

30) S. Ueda, J. Matsumoto, and T. Nomura, *Chem. Pharm. Bull.*, **32**, 350 (1984).
31) T. Nomura, T. Fukai, Y. Hano, and H. Ikuta, *Heterocycles*, **20**, 585 (1983).
32) *a*) C. Konno, Y. Oshima, and H. Hikino, *Planta Med.*, **32**, 118 (1977); *b*) Y. Oshima, Ph. D. Thesis, Tohoku University, 1981.
33) The compound (**12**) showed no hypotensive effect in Wistar rats.