Communications to the Editor

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EFFECTS OF SOME PHENOLICS ON THE PROSTAGLANDIN SYNTHESIZING ENZYME SYSTEM

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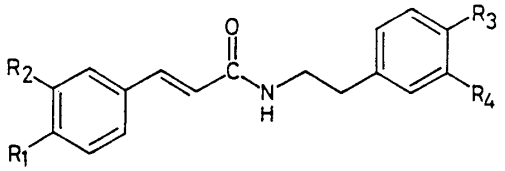
N-*cis*- and N-*trans*-feruloyltyramines were isolated as the inhibitors of prostaglandin (PG) biosynthesis from *Ipomoea aquatica*. Aromatic amides consisting of naturally occurring cinnamic acids and β-phenethylamines were synthesized and tested for their effects on PG biosynthesis. Of those caffeoyl-β-phenethylamine(CaP) stimulated PG biosynthesis at a lower concentration and inhibited at a higher concentration. It has been shown that CaP inhibits cyclooxygenase and stimulates peroxidase in PG biosynthesis. *o*- and *m*-Hydroxycinnamic acids were found to act as "tryptophan-like cofactors" even at a very high concentration where *p*-coumaric acid significantly inhibit PG biosynthesis.

KEYWORDS——prostaglandin synthetase inhibitor; aromatic amide; caffeoyl-β-phenethylamine; cyclooxygenase; peroxidase; PGE_2 synthetase; feruloyltyramine

Some plant constituents inhibit *in vitro* prostaglandin(PG) biosynthesis.[1] In previous papers, we reported the isolation of inhibitors of PG biosynthesis from several medicinal plants used in Oriental medicine.[2-5] *Ipomoea aquatica* Forsk (Convolvulaceae), a folk medicine used in southeast Asia,[6] was found to contain substances that inhibit *in vitro* PG biosynthesis. Chromatographic separation of an acetone extract of the stems of this plant yielded N-*trans*- and N-*cis*-feruloyltyramines(FeT, **1** and **2**) along with scopoletin and umbelliferone as constituents that inhibit PG synthetase. N-Feruloyltyramines (FeT, **1** and **2**) have been isolated from *Solanum melongena*,[7] *Capsicum annum*[8] and *Tinospora tuberculata*.[9] Following this finding, we synthesized N-cinnamoyl-β-phenethylamine derivatives[10] which consist of different combinations of naturally occurring cinnamic acids and β-phenethylamines to clarify the structural requirement for the inhibition of PG biosynthesis. Their inhibition of PG biosynthesis was determined under a reported bioassay condition[1a,11] and the results are summarized in Table I. Of the



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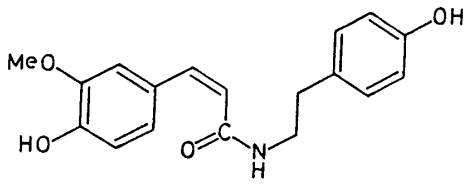

2

Table I. 50% Inhibition Concentration of Cinnamoyl-β-phenethylamine

R_1	R_2	R_3	R_4	Compounds		IC_{50}(μM)
OH	OMe	OH	H	Feruloyltyramine	(**1**;FeT)	210
H	H	H	H	Cinnamoyl-β-phenethylamine	(**3**;CiP)	180
H	H	OH	H	Cinnamoyltyramine	(**4**;CiT)	120
H	H	OH	OH	Cinnamoyldopamine	(**5**;CiD)	90
OH	H	H	H	p-Coumaroyl-β-phenethylamine	(**6**;CoP)	100
OH	H	OH	H	p-Coumaroyltyramine	(**7**;CoT)	280
OH	H	OH	OH	p-Coumaroyldopamine	(**8**;CoD)	230
OH	OH	H	H	Caffeoyl-β-phenethylamine	(**9**;CaP)	80
OH	OH	OH	H	Caffeoyltyramine	(**10**;CaT)	210
OH	OH	OH	OH	Caffeoyldopamine	(**11**;Cad)	270

compounds tested N-cinnamoyldopamine (CiD, **5**) and N-caffeoyl-β-phenethylamine (CaP, **9**) were the strongest inhibitors. Observed structure-activity relationships are well in accord with our previous observation that relatively strong inhibitors possess free phenolic and lipophylic groups. A significant inhibitory effect of CiP (**3**) without the phenolic group may be caused by strong binding of its aromatic rings to the enzyme as was observed in di- and tri-phenylacrylonitriles, which have no phenolic group.[12] In order to determine the concentration-dependent effects of FeT (**1**) and CaP (**9**) on PG biosynthesis, the initial velocity of oxygen up-take was measured with an oxygen electrode at different concentrations.[1a,11] FeT (**1**) was significantly inhibitory at a lower concentration range (5-200 μM), whereas CaP (**9**) increased the reaction rate by 5-27% in the same range. CaP (**9**) inhibited the reaction at a concentration higher than 200 μM (Table II). The stimulatory effect of CaP (**9**) on PG biosynthesis seemed to be caused by the stimulation of hydroperoxidase, since it is augumented by the presence of cofactors such as tryptophan, and phenol, hydroquinone, epinephrine and uric acid which are called "tryptophan-like cofactors".[13]

The enzyme system of PG biosynthesis used in our studies involves three different reaction steps, cyclooxygenase, hydroperoxidase and PGE_2 synthetase. In order to clarify in which step CaP (**9**) acts as an inhibitor or a promotor, PG

Table II. Concentration-Dependent Effects of CaP on Oxygen Uptake in PG Biosynthesis

Sample \ mM	0.02	0.08	0.2	0.8	2.0
CaP (**9**)	105	121	127	75	23
Aspirin	90	82	75	62	48

Figures indicate reaction % when control is taken as 100%.

endoperoxide synthetase was purified from sheep seminal vesicles according to the method described by Yamamoto et al. for the purification of bovine seminal vesicle enzyme.[14] Radioactive endoperoxides, [^{14}C]-PGG_2 and [^{14}C]-PGH_2 were prepared from [^{14}C]-AA with purified enzyme by reported methods with some modifications.[13a,15] The effects of CaP (**9**) were investigated in each reaction step by using a microsomal preparation and PG endoperoxide synthetase. The conversion of AA into PGG_2 (cyclooxygenase) and PGG_2 into PGH_2 (hydroperoxidase) were measured with purified PG endoperoxide synthetase which possesses cyclooxygenase and hydroperoxidase activities. The conversion of PGH_2 into PGE_2 (PGE_2 synthetase) and AA into PGE_2 (PG synthesis) was determined with a microsomal preparation of sheep seminal vesicles. The results are summarized in Table III. The PG synthesis and cyclooxygenase reactions were strongly inhibited by CaP (**9**) at a concentration of 200 μM. In the hydroperoxidase reaction Cap (**9**) was significantly stimulative. On the other hand, it did not show any significant effect on PGE_2 synthetase. The results indicate that the apparent effect of CaP (**9**) in PG biosynthesis is the sum of the inhibitory effect in the cyclooxygenase reaction and the stimulative effect in the hydroperoxidase reaction.

Table III. Inhibition of Cyclooxygenase, Hydroperoxidase and PG Synthesis by CaP

Preparation	Endoperoxide synthetase				Microsomes			
Reaction	Cyclooxygenase		Hydroperoxidase		PGE_2 synthetase		PG synthesis	
Substrate and product	AA	PGG_2	PGG_2	PGH_2	PGH_2	PGE_2	AA	PGE_2
Cap(**9**) 0μM	14.5	85.5	47.1	52.9	4.5	95.5	12.9	87.1
Cap(**9**) 200μM	85.4	14.6	28.2	71.8	5.7	94.3	75.6	24.4

Figures indicate the ratios of substrates and products when their sums of those are taken 100.

The tryptophan-like cofactor activity of phenolic compounds is of interest from the point of the regulation of PG biosynthesis. Since PGs have a remarkably wide variety of physiological actions, compounds that alter the biosynthesis and metabolism of PGs are expected to have various physiological effects. Next we tested the cofactor activity of cinnamic acid derivatives and related phenolic compounds in PG biosynthesis, because caffeic acid was reported to stimulate PG biosynthesis.[1d] The results are summarized in Table IV. Except for those lacking the phenolic group, all the compounds tested were more or less stimulative. Coniferyl alcohol, which lacks the carboxyl group, strongly inhibited PG biosynthesis even at a low concentration. It is rather surprising that a slight change of structure, especially in the isomers of coumaric acids, caused a remarkable difference in the effects on PG biosynthesis. Most of the derivatives of cinnamic acids were stimulative at lower concentrations, but were inhibitory at higher concentrations, as was observed in CaP (**9**). On the contrary, meta-and ortho-coumaric acids were not significantly inhibitory even at a higher concentration. Since the details of the mechanism of the hydroperoxidase reaction have not been clarified,[16] the different behavior of hydroxycinnamic acids may give some

clue to clarify the effect of tryptophan-like cofactors on PG endoperoxide synthetase.

Table IV. Effects of Hydroxycinnamic Acids and Related Compounds

mM / Sample	0.37	1.11	3.33	10.0
Caffeic acid	210.5	201.8	145.9	49.2
Ferulic acid	191.4	191.1	157.1	98.1
Isoferulic acid	172.4	185.9	170.4	109.6
p-Coumaric acid	171.0	178.4	173.3	50.8
o-Coumaric acid	171.0	173.9	172.5	153.8
m-Coumaric acid	174.2	178.4	214.4	206.2
Protocatechuic acid	174.2	168.4	183.3	154.5
Homovanillic acid	146.3	174.3	177.1	101.4
Cinnamic acid	102.3	99.3	91.2	101.4
p-Methoxycinnamic acid	93.7	88.7	85.4	88.5
Coniferyl alcohol	35.7	25.1	18.3	19.1

Figures indicate reaction % when control is taken as 100%.

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