

Recent Progress in Study on the Biologically-Active Natural Products

Degranulation Inhibitors from Medicinal Plants in Antigen-Stimulated Rat Basophilic Leukemia (RBL-2H3) Cells

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Mast cells and basophils play important roles in both immediate- and late-phase reactions of type 1 allergy. Histamine, which is released from mast cells and basophils stimulated by an antigen or degranulation inducers, is usually determined as a degranulation marker in experiments on immediate allergic reactions *in vitro*. β -Hexosaminidase is also stored in secretory granules of the cells and is released concomitantly with histamine when the cells are immunologically activated, and recently this enzyme activity in the medium has been used as a marker of the degranulation. In this paper, we review our studies on the search for degranulation inhibitors, such as flavonoids, stilbenes, and curcuminoids, from medicinal plants using rat basophilic leukemia (RBL-2H3) cells.

Key words degranulation; inhibitor; RBL-2H3 cell; flavonoid; stilbene; curcuminoid

1. Introduction

Recently, an increase in the number of patients with allergies such as pollinosis and asthma in Japan has necessitated the search for more effective, safe antiallergic agents, although excellent antiallergic agents have been developed. Mast cells and basophils play important roles in both immediate- and late-phase reactions of type 1 allergy. The aggregation of high-affinity Fc ϵ receptor I (Fc ϵ RI) by antigens results in tyrosine phosphorylations, Ca²⁺ release from intracellular Ca²⁺ stores and influx *via* Ca²⁺ release-activated Ca²⁺ channels. The elevation of intracellular free Ca²⁺ ([Ca²⁺]_i) level plays an essential role in the degranulation process.^{1–4)}

Histamine, which is released from mast cells and basophils stimulated by an immunoglobulin E (IgE)/antigen or a degranulation inducer, is usually determined as a degranulation marker of immediate allergic reactions in *in vitro* experiments. β -Hexosaminidase is also stored in secretory granules of the cells, and is released concomitantly with histamine when the cells are immunologically activated. Therefore, this enzyme activity is used as a marker of degranulation of cells, and this assay has been used for the evaluation of antiallergic compounds as an alternative to passive cutaneous anaphylaxis (PCA) reactions in laboratory animals.⁵⁾ In the course of our search for bioactive constituents from medicinal plants, we have isolated various degranulation inhibitors from medicinal plants using rat basophilic leukemia (RBL-2H3) cells. For example, flavonoids from various medicinal plants including the aerial parts of *Cissus sicyoides*^{6–8)}, stilbenes, stilbene dimers, and phenanthrenes from the rhizomes of *Rheum undulatum*, the tuber of *Gymnadenia conopsea*, and the whole plant of *Cyperus longus*^{9–11)}, diarylheptanoids including curcuminoids from the barks of *Myrica rubra* and *Acer nikoense* and the rhizomes of *Curcuma zedoaria* and *C. comosa*^{12–15)}, 3-phenyl-isocoumarins from the processed leaves of *Hydrangea macrophylla* var. *thunbergii*^{16,17)}, phenylpropanoids from the rhizomes of *Alpinia galanga*¹⁸⁾, β -carboline-type alkaloids

from the roots of *Stellaria dichotoma*¹⁹⁾, sesquiterpenes from the rhizomes of *Hedychium coronarium* and the fruits of *Alpinia oxyphylla*^{20,21)}, meroterpenes from the seeds of *Psoralea corylifolia*²²⁾, and acylated oleanane-type triterpene saponin from *Camellia sinensis* and *C. assamica*^{23–25)} were reported.

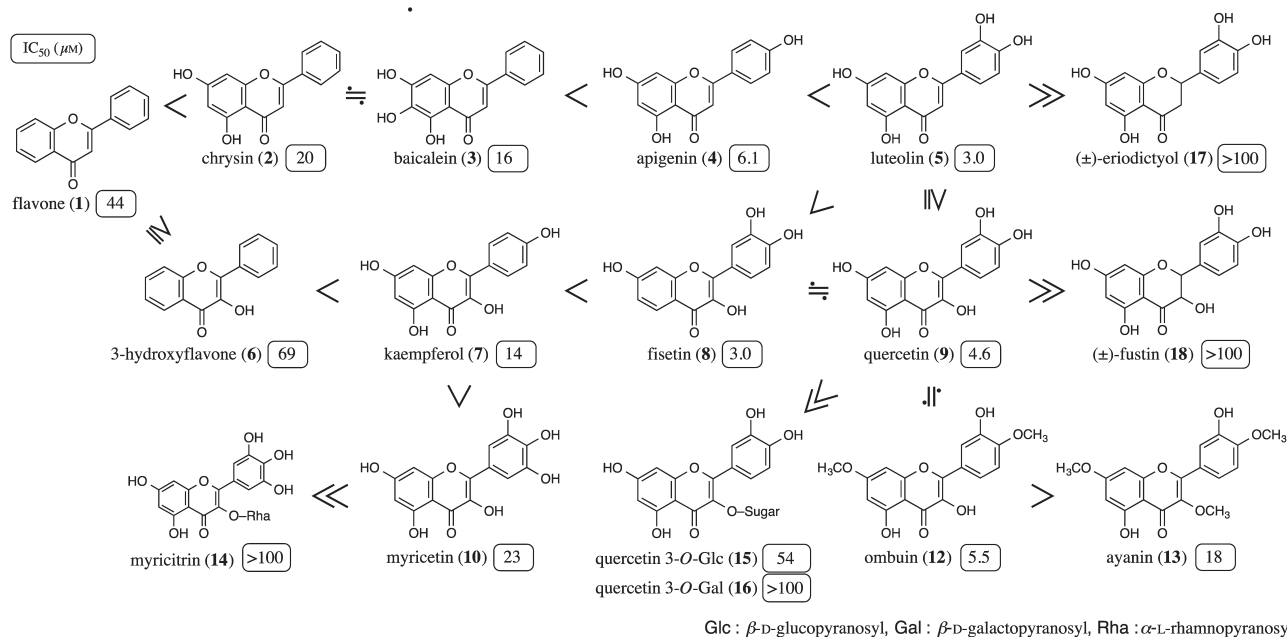
In this review, we focus on flavonoids, stilbenes, and curcuminoids from several medicinal plants with antiallergic activity in *in vitro* experiments, their structural requirements for antiallergic activity, and some mechanisms of action.

2. Flavonoids

Flavonoids, which are widely distributed in the plant kingdom, are recognized to have various biological activities including antiallergic actions, and various *in vitro* and *in vivo* studies of the antiallergic effects of flavonoids were reported. However, their structure–activity relationships were not discussed satisfactorily because of insufficient numbers of examples *in vitro* and/or technical limitations *in vivo*.

2.1. Structural Requirements of Flavonoids for the Antigen-Induced Release of β -Hexosaminidase from RBL-2H3 Cells To clarify the structural requirements of flavonoids for antiallergic activity, we reported the inhibitory effects of more than 50 flavonoids on the release of β -hexosaminidase induced by dinitrophenylated bovine serum albumin (DNP-BSA) as an antigen from RBL-2H3 cells sensitized with anti-DNP IgE, which is a convenient screening test for the immediate phase of type I antiallergic reactions.⁶⁾ Figure 1 shows representative flavones and flavonols and their IC₅₀ values. Among them, luteolin (**5**, IC₅₀=3.0 μ M), fisetin (**8**, 3.0 μ M), and diosmetin (2.1 μ M) were found to show potent inhibitory activity without direct inhibition of the enzyme activity of β -hexosaminidase, and the results suggested the following structural requirements of flavonoids: 1) The C-2/C-3 double bond of flavones and flavonols is essential for the activity, 2) the glycoside moiety markedly reduced the activity, 3) as the hydroxy groups at the C-5, C-6, C-7, C-3', and C-4' positions increased in

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Fig. 1. Effects of Flavones and Flavonols on Release of β-Hexosaminidase Induced by Antigen in RBL-2H3 Cells^{a)}

a) Data taken from ref. 6.

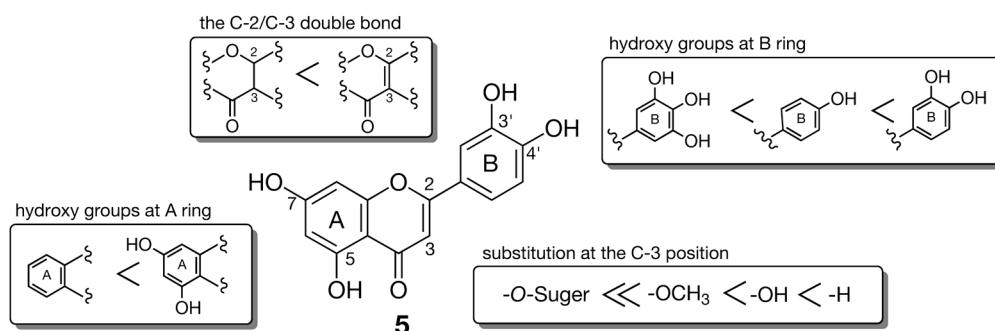


Fig. 2. Structural Requirements of Flavones and Flavonols for the Activity

number, the inhibitory activities became more potent, 4) the flavonols with a pyrogallol-type moiety (the 3',4',5'-trihydroxy group) at the B ring showed less activity than those with a *p*-phenol-type moiety (the 4'-hydroxy group) or catechol-type moiety (the 3',4'-dihydroxy group) at the B ring, 5) Flavones tended to show stronger activity than flavonols, 6) Methylation of flavonols at the C-3 position reduced the activity, although several exceptions were observed⁶⁾ (Fig. 2). However, isoflavones and flavan-3-ols showed less activity (>100 μM).

In type 1 allergy, IgE antibodies bind to FcεRI on the surface of mast cells and basophils. After sensitization with IgE antibodies, exposure to the same allergen cross-links the bound IgE on sensitized cells, resulting in degranulation and the secretion of physiologically active mediators such as histamine, leukotrienes (LTs), and prostaglandins (PGs) that cause the inflammation of the surrounding tissues. Type 1 allergy is further classified into immediate- and late-phase reactions. The immediate-phase reaction occurs within minutes after the exposure to an allergen and is caused by the release of vasoactive amines and lipid mediators. The late-phase reaction occurs 2–4 h after the exposure and is mediated by the release of cytokines such as interleukins (ILs)-3, 4, 5, and 6; tumor necrosis factor α (TNF-α); and granulocyte-macrophage

Table 1. Effects of Several Flavonoids on Release of TNF-α and IL-4 in RBL-2H3 Cells^{a)}

	IC ₅₀ (μM)	
	TNF-α	IL-4
Apigenin (4)	5.3	3.6
Luteolin (5)	5.8	3.7
Diosmetin	5.3	3.0
Fisetin (8)	4.2	3.8
Quercetin (9)	5.6	7.5

TNF-α and IL-4 levels in the medium were determined 4 h after the stimulation. a) Data taken from ref. 6.

colony-stimulating factor (GM-CSF). The late-phase reaction is caused by release of mediators such as major basic protein (MBP), eosinophil cationic protein (ECP), and LTC₄ from eosinophils and are dependent on the activity of Th₂ cells.^{1–4)}

Several flavones [apigenin (4), luteolin (5)], flavonols [kaempferol (7), quercetin (9)] inhibit the antigen-induced release of TNF-α and IL-4 in sensitized RBL-2H3 cells⁶⁾ (Table 1). In addition, a chalcone (isoliquiritigenin) also inhibits degranulation and the release of TNF-α and IL-4.^{7,8)} These

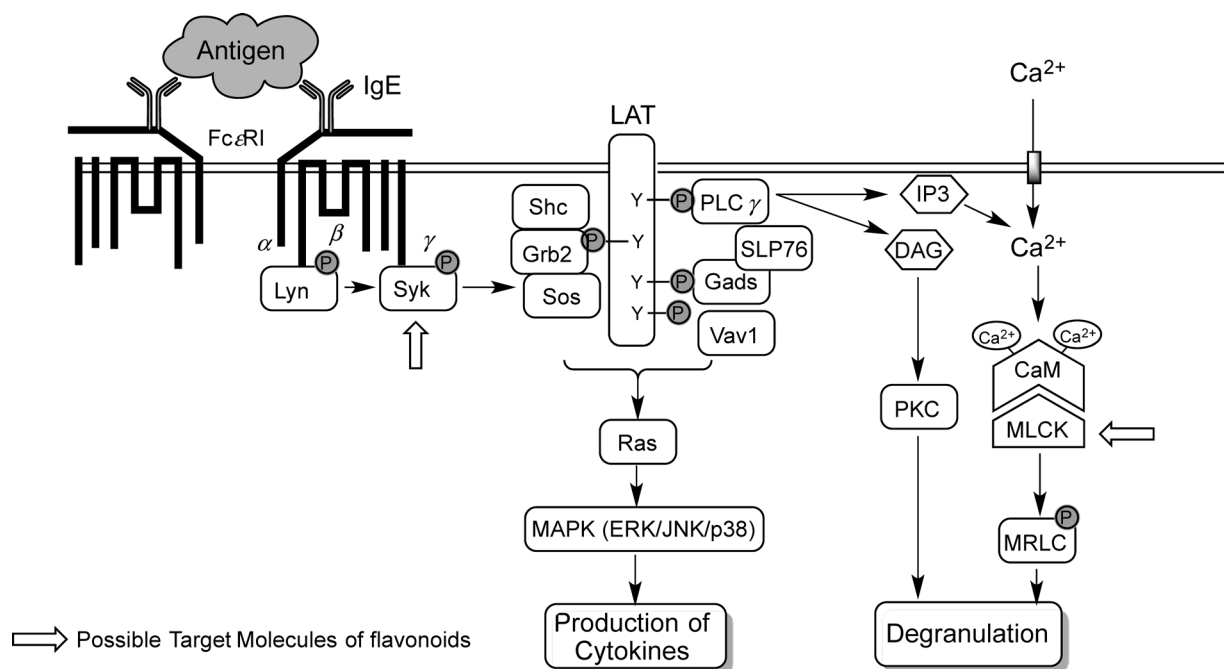


Fig. 3. FcεRI Signaling Pathway and Possible Target Molecules of Flavonoids

CaM: calmodulin, DAG: diacylglycerol, ERK: extracellular signal-regulated kinase, Gads: grb2-related adaptor downstream of src, Grb2: growth factor receptor binding protein 2, IP3: inositol-1,4,5-trisphosphate, JNK: Jun amino-terminal kinase, LAT: linker for activation of T cells, Lyn: a Src family tyrosine-protein kinase, MLCK: myosin-light chain kinase, MRLC: myosin regulatory light chain, p38: p38 mitogen-activated protein kinase, PKC: protein kinase C, PLCγ: phospholipase Cγ, Ras: a small G-protein, SLP76: SH2-containing leukocyte-specific protein of 76 kDa, Shc: (Src homology 2 domain containing) transforming protein, Sos: son of sevenless, Syk: spleen tyrosine kinase, Vav1: vav 1 guanine nucleotide exchange factor.

findings suggest that these active flavonoids could be effective for the treatment of late-phase as well as immediate-phase reactions.

2.2. Possible Target Molecules of Flavonoids In FcεRI signal transduction, the nuclear factor of activated T cells (NFAT) and activator protein 1 (AP-1), which is composed of c-fos and c-jun, induce the transcription of target genes of cytokines by binding at gene promoters and enhancers.^{1-4,26} Several flavonoids such as luteolin (5) and quercetin (9) were reported to inhibit IgE-mediated Ca²⁺ influx and the activation of protein kinase C, extracellular signal-regulated kinases (ERKs) and c-jun NH₂-terminal kinase (JNK), but not the activation of p38 mitogen-activated protein kinase (p38 MAPK), and then inhibit the release of LTs and PGD₂ and expression of GM-CSF mRNA in cultured human mast cells.²⁷ Hirano *et al.* also reported that luteolin (5) inhibited phosphorylation of c-jun and DNA-binding activity of AP-1 in a human basophilic cell line (KU812) induced by A23187 and 12-myristate 13-acetate (PMA).²⁸

In our study, kaempferol (7) and quercetin (9), which showed significant inhibition of the release of TNF-α and IL-4 in RBL-2H3 cells, markedly inhibited phosphorylation of ERK1/2 and JNK/SAPK, but obvious inhibition of the phosphorylation of p38 MAPK was not observed, consistent with a previous report.⁸

Shichijo *et al.* reported that flavonoids inhibited the activation of spleen tyrosine kinase (Syk) which plays a pivotal role in FcεRI-mediated degranulation of mast cells.²⁹ In addition, luteolin (5) and quercetin (9) were reported to inhibit calcium ionophore A2318-induced histamine release in human cultured mast cells,²⁷ and the calmodulin pathway, at least myosin-light chain kinase (MLCK), calmodulin-dependent protein kinases (CaMKs), and calcineurin, and positively regulate

Ca²⁺-induced degranulation in RBL-2H3 cells.³⁰ Ludowyke *et al.* reported that Ca²⁺-induced degranulation correlated with the phosphorylation of myosin light chain by protein kinase C,³¹ whereas kaempferol (7) was reported to inhibit MLCK of bovine aorta.³² The FcεRI signaling pathway is partly shown in Fig. 3.^{1-4,26,30,31,33}

In our study using commercially available kinases (Z'-LYTE assay kit, Invitrogen, Carlsbad, CA, U.S.A.), 7 and 9 inhibited the enzyme activity of Syk and MLCK2 partly correlated with their effects on the degranulation of RBL-2H3 cells. Furthermore, 7 and 9 inhibited the phosphorylation of the linker for activation of T cells (LAT) which is a target protein of Syk, and myosin regulatory light chain (MRLC, myosin light chain 2) which is a target protein of MLCK.³⁰ These findings suggest that the possible target molecules of antiallergic activity in RBL-2H3 are Syk and MLCK, although further studies are needed to clarify the mechanism of action (Fig. 3).

3. Stilbenes

The rhizomes of *R. undulatum* (Polygonaceae) have been used as a remedy for blood stagnation syndrome as well as a purgative agent in Japanese, Korean, and Chinese traditional medicines. This rhubarb has been considered to have a less purgative effect but to be more effective in treating blood stagnation syndrome than other rhubarbs³⁴ and contains stilbenes such as rhaponticin, piceatannol 3'-O-β-D-glucopyranoside, rhapontigenin, piceatannol as the principal constituents.^{35,36} Stilbenes have been reported to have antibacterial, anti-inflammatory, anti-tumor, antimalarial, and anti-aging effects.³⁷⁻⁴¹ We previously reported the antioxidant activity of stilbenes isolated from this herb and their inhibitory effects on nitric oxide production from macrophages stimulated by a lipopolysaccharide (LPS).^{35,36} In addition, the effects of the

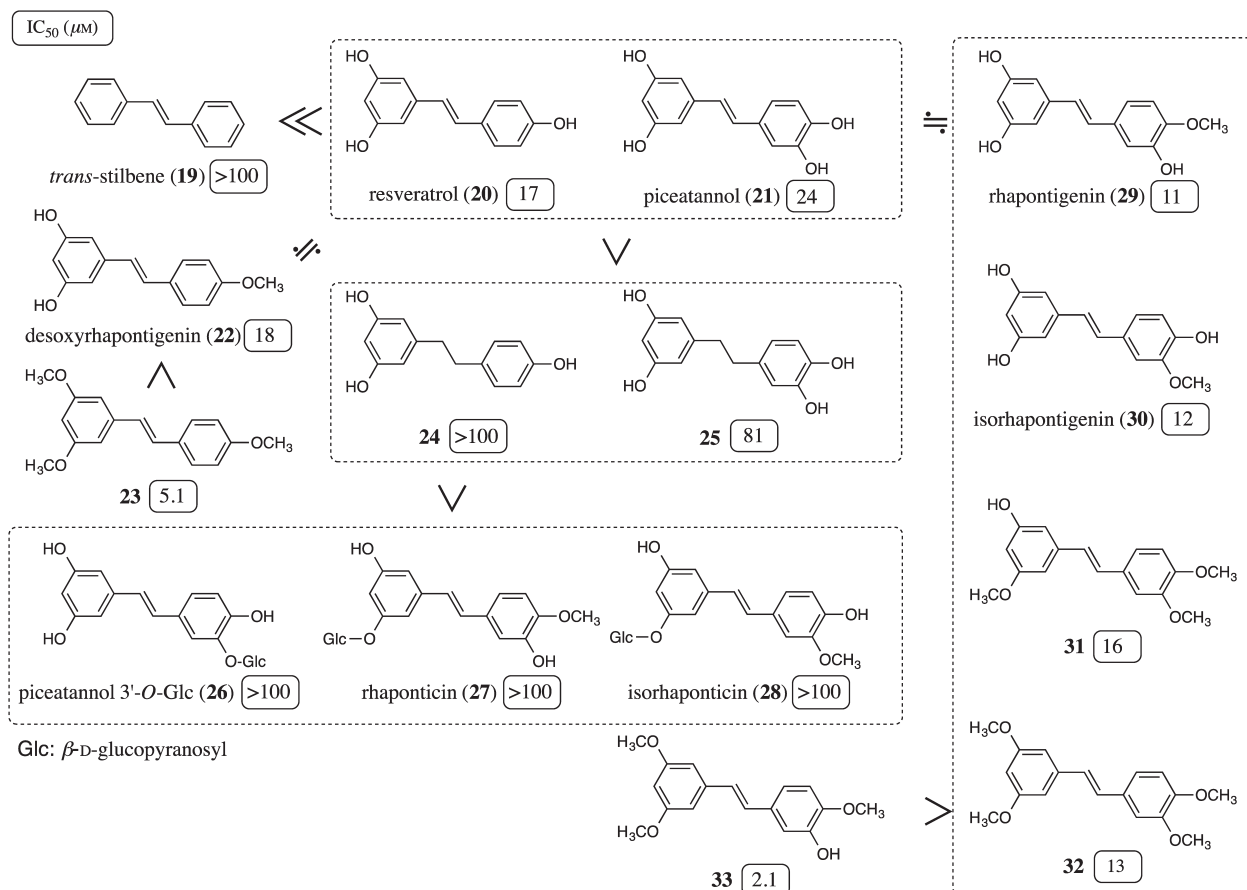


Fig. 4. Effects of Stilbenes and Dihydrostilbenes on Release of β-Hexosaminidase Induced by Antigen in RBL-2H3 Cells^{a)}

^{a)} Data taken from ref. 9.

principal stilbene constituents on antigen-induced histamine release from rat peritoneal mast cells, 48-h homologous PCA in rats (type I allergic model), and sheep blood cell-induced delayed-type hypersensitivity in mice (type IV allergic model) were also reported.⁴²⁾ However, the structure–activity relationships of stilbenes for antiallergic activity were not studied in sufficient detail.

In our study, the methanolic extract also inhibited the release of β-hexosaminidase induced by the antigen in the sensitized RBL-2H3 cells. Therefore, the effects of principal stilbenes isolated from this herb and their related compounds on the antigen-induced degranulation in the RBL-2H3 cells were examined.⁹⁾

3.1. Structural Requirements of Stilbenes for the Antigen-Induced Release of β-Hexosaminidase from RBL-2H3 Cells Among the compounds tested, resveratrol (20, IC₅₀=17 μM), piceatannol (21, 24 μM), desoxyrhaphontigenin (22, 18 μM), trimethylresveratrol (23, 5.1 μM), rhaphontigenin (29, 11 μM), isorhaphontigenin (30, 12 μM), 3,3',4'-trimethylpiceatannol (31, 16 μM), tetramethylpiceatannol (32, 13 μM), and 3,5,4'-trimethylpiceatannol (33, 2.1 μM) exhibited potent activity, and their activities were stronger than those of two antiallergic agents, tranilast and ketotifen fumarate. *trans*-Stilbene (19, >100 μM), which lacks oxygen functions on the aromatic rings, was inactive. Our results suggested that stilbenes substituted with methoxy groups at the C-3, C-5, and C-4' positions exhibited higher activity than those with hydroxy groups. The substitution of glycoside moiety on the ring markedly de-

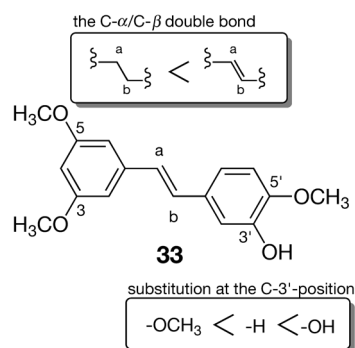


Fig. 5. Structural Requirements of Methoxystilbenes for the Activity

creased the activity (Fig. 4).

Our results suggested that the positions of the methoxy groups of stilbenes were important for their antiallergic activity. Briefly, 33 (2.1 μM) with methoxy groups at the C-3, C-5, and C-4' positions exhibited activity *ca.* 8-fold higher than that of 31 (16 μM) with methoxy groups at the C-3, C-3', and C-4' positions. In addition, the three compounds 23, 32, and 33, which have three methoxy groups at the C-3, C-5, and C-4' positions but have a different group substituted at the C-3' position, also showed different IC₅₀ values (Fig. 5).

Inamori *et al.* reported that the inhibitory effect of piceatannol (21) was greater than that of the α,β-dihydro derivative (25) on antigen-induced histamine release in rat peritoneal mast cells.⁴³⁾ Consistent with that report, our results showed

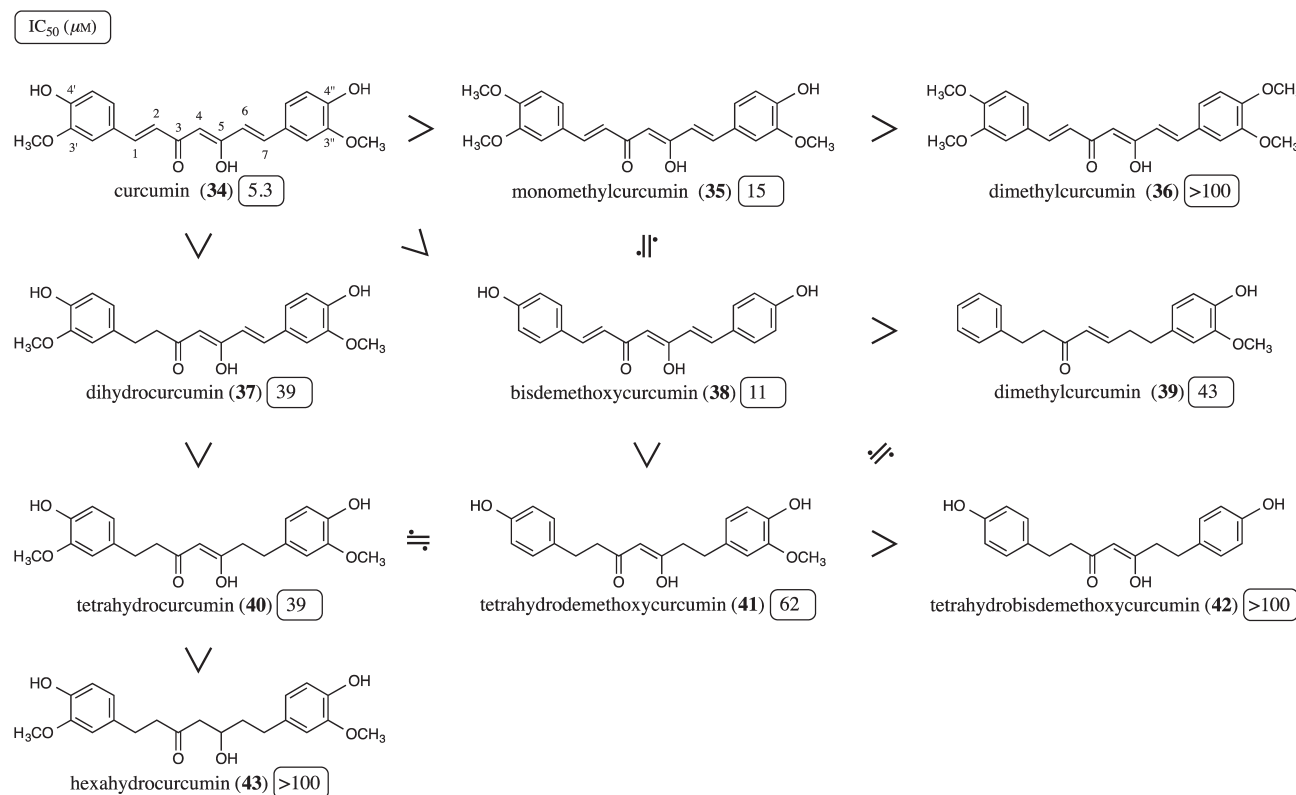


Fig. 6. Effects of Curcuminoids on Release of β -Hexosaminidase Induced by Antigen in RBL-2H3 Cells^{a)}

a) Data taken from ref. 14.

that the activities of resveratrol (**20**, $17\mu M$) and **21** ($24\mu M$) were greater than those of dihydroresveratrol (**24**, $>100\mu M$) and **25** ($81\mu M$), respectively. Thus, the C- α /C- β double bond is essential for higher activity.

Cheong *et al.* also reported the inhibitory effects of hydroxystilbenes on the release of β -hexosaminidase,⁴⁴⁾ and they came to two main conclusions; 1) The antiallergic effect decreased in proportion to the number of methoxy groups, and hydroxy substituents on the benzene rings might be important for the activity, 2) glycoside substitution of stilbenes decreased the activity. Our results supported the previous report and suggested some additional or revised structural requirements: 1) The oxygen functions ($-OCH_3$, $-OH$) are essential and their positions on aromatic rings were important for the activity, especially that of methoxy groups, which differed from the previous report,⁴⁴⁾ 2) the glycoside moiety markedly decreased the activity, 3) The C- α /C- β double bond increased the activity, 4) the substitution group at the C-3' position in trimethylresveratrol was preferably $OH > H > OCH_3$ for the activity.

Furthermore, **20**, **21**, **23**, and **33** inhibited the release of β -hexosaminidase by ionomycin, and their IC_{50} values (IC_{50} =23, 31, 8.5, $2.9\mu M$, respectively) were similar to those by the antigen (17, 24, 5.1, $2.1\mu M$, respectively) in RBL-2H3 cells.

Our previous study on the effects of stilbenes against nitric oxide production in LPS-activated macrophages indicated structural requirements similar to those for the antiallergic activity, except that the C- α /C- β double bond of stilbenes did not affect nitric oxide production,³⁶⁾ whereas the C- α /C- β double bond is essential for the antiallergic activity.

Active stilbenes (**20**, **21**, **23**, **33**) with degranulation inhibi-

tory activity on the release of TNF- α and IL-4 in RBL-2H3 cells were also investigated, and **33** exhibited potent activity against TNF- α and IL-4 release (IC_{50} =10, $39\mu M$, respectively), whereas **20** exhibited the most potent activity against IL-4 with an IC_{50} value of $32\mu M$. Trimethylresveratrol (**23**), which is active against the release of β -hexosaminidase (IC_{50} = $5.1\mu M$), exhibited only moderate or less activity against the release of TNF- α and IL-4 (64 , $>100\mu M$, respectively). These results indicate that the mechanism of action of **23** on degranulation is somewhat different from that on the release of TNF- α and IL-4. Piceatannol (**21**) exhibited moderate activity against both TNF- α and IL-4, with IC_{50} values of 30 and $72\mu M$, respectively.⁹⁾

Regarding the stilbene-related compounds, 3-phenyl-isocoumarins [e.g. thunberginol B (5,6,3',4'-tetrahydroxy 3-phenyl-isocoumarin)] from the process leaves of *Hydrangea macrophylla* var. *thunbergii* also showed the inhibitory activity with an IC_{50} value of $5.7\mu M$, and the 3,4-dihydro derivatives also showed weaker activity. In addition, the gene expression of several cytokines and c-fos in RBL-2H3 cells treated with thunberginol B was similar to that of a flavone, luteolin (**5**).^{16,17)} Stilbene derivatives and phenanthrenes from the tubers of *G. conopsea* also showed moderate activity.¹⁰⁾

3.2. Possible Target Molecules of Piceatannol (21) Piceatannol (**21**) was reported to be a selective inhibitor of Syk in RBL-2H3, and the inhibition of Syk has been considered to be an inhibitory mechanism of degranulation by **21**.^{45,46)} However, our results, in which **21** inhibited a calcium ionophore ionomycin-stimulated degranulations, suggested that other mechanisms such as the inhibition of Ca^{2+} influx or degranulation mechanisms after Ca^{2+} influx as well as the in-

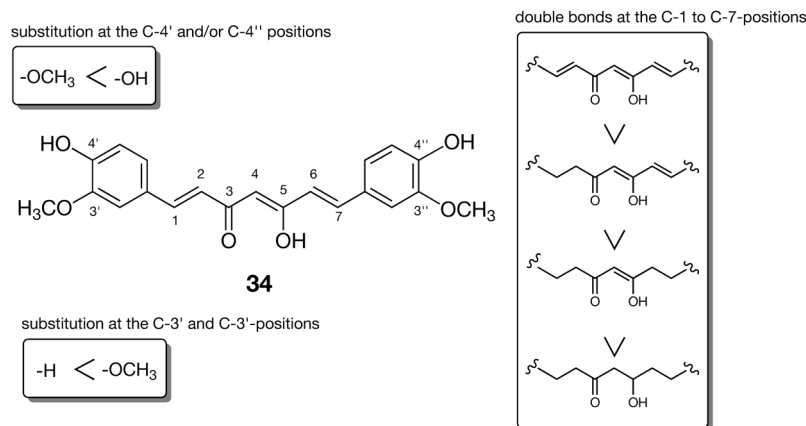


Fig. 7. Structural Requirements of Curcuminoids for the Activity

hibition of Syk were involved in the inhibitory activity of **21**.⁹⁾

4. Curcuminoids

The rhizomes of *C. zedoaria* have been used as anti-inflammatory and antifatulence agents as well as a spice and fragrance in Asia. Pharmacological studies of this herb resulted in reports on the antiinflammatory effects of the extract and sesquiterpenes were reported.^{47–50)} In our previous studies, we reported the hepatoprotective, vasodilative, and nitric oxide production-inhibitory activities of various sesquiterpenes and curcuminoids isolated from the rhizomes of *C. zedoaria* cultivated in China.^{51–56)}

In our study, the 80% aqueous acetone extract of the dried rhizomes of *C. zedoaria* cultivated in Thailand (Thai zedoary)⁵⁷⁾ was found to inhibit the release of β -hexosaminidase induced by the antigen in sensitized RBL-2H3 cells and ear PCA reactions in mice.¹⁴⁾ Four curcuminoids and two bisabolane-type sesquiterpenes were isolated from the active fraction. Therefore, the effects of curcuminoids and their derivatives on the release of β -hexosaminidase induced by the antigen in sensitized RBL-2H3 cells were examined.

4.1. Structural Requirements of Curcuminoids for the Antigen-Induced Release of β -Hexosaminidase from RBL-2H3 Cells Curcumin (**34**), monomethylcurcumin (**35**), and bisdemethoxycurcumin (**38**) exhibited potent inhibitory activities with IC_{50} values of 5.3, 15, and $11\ \mu\text{M}$, respectively. Other compounds showed moderate or weak activity¹⁴⁾ (Fig. 6). Yano *et al.* reported that the extract of the rhizomes of *Curcuma longa* and several curcuminoids isolated from the active fraction inhibited histamine release induced by compound 48/80, concanavalin A, and/or calcium ionophore A23187 in rat peritoneal mast cells.⁵⁸⁾ However, the structure-activity relationships of antiallergic activity of curcuminoids have not been sufficiently studied.

Regarding the structural requirements of curcuminoids for antiallergic activity, when the conjugated olefins at the C-1 to C-7 positions and the C-3' and C-3'' methoxy groups decreased in number, the activities decreased. This indicates the importance of the conjugated olefins and methoxy groups of curcuminoids. However, the conjugated olefins seem to be more important than the methoxy groups for the activity. Compound **38** was reported to show stronger activity than **34** against compound 48/80-induced histamine release in rat peritoneal mast cells.⁵⁸⁾ However, in our study, the activity of

34 was greater than that of **38** against antigen-IgE mediated degranulation in RBL-2H3 cells.

Compound **34** with the C-4' hydroxy group exhibited activity *ca.* 3-fold more potent than **35** with the C-4' methoxy group. Moreover, **36** with both the C-4' and C-4'' methoxy groups exhibited less activity. These findings suggested that at least one *p*-hydroxy group of the aromatic rings of **34** was essential to exhibit the activity, and we concluded that the conjugated olefins at the C-1 to C-7 positions and the C-4' and C-4'' hydroxy groups of curcuminoids are essential for the activity, whereas the C-3' and C-3'' methoxy groups only enhanced the activity¹⁴⁾ (Fig. 7).

Calcium ionophores are compounds that enhance Ca^{2+} influx into the cell by increasing the membrane permeability of Ca^{2+} . The increase in $[\text{Ca}^{2+}]_i$ is followed by the degranulation of mast cells or basophils and activates the formation of inflammatory mediators such as PGs and LTs.^{1–3)} Yano *et al.* reported that curcumin (**34**) inhibited histamine release induced by concanavalin A, compound 48/80, and A23187 in rat peritoneal mast cells, and they concluded that **34** potentially suppressed the histamine release, probably through the inhibition of the degranulation process following a rise in $[\text{Ca}^{2+}]_i$ levels.⁵⁸⁾ Consistent with that report, **34** and **38** inhibited A23187 and ionomycin-induced degranulation of RBL-2H3 cells, and their IC_{50} values (7.2 and $13\ \mu\text{M}$ for A23187, 6.0 and $15\ \mu\text{M}$ for ionomycin) were nearly the same as those of the antigen (5.3 and $11\ \mu\text{M}$).¹⁴⁾

Curcumin (**34**) was previously reported to inhibit the production of $\text{TNF-}\alpha$ and IL-1 in a human macrophage cell line induced by LPS and to inhibit IL-12 production in mouse splenic macrophages induced by LPS,^{59–61)} but the effects of **34** on the antigen-induced production of cytokines in basophils was not investigated. In our study, **34** inhibited the release of $\text{TNF-}\alpha$ and IL-4 with IC_{50} values of 20 and $18\ \mu\text{M}$ respectively, while the values of **38** were 38 and $34\ \mu\text{M}$.¹⁴⁾

4.2. Possible Molecular Targets of Curcumin (34) Regarding the mechanism of action of curcuminoids, Lee *et al.* reported that **34** inhibited the activity of Syk which plays a pivotal role in Fc ϵ RI-mediated degranulation of mast cells.⁶²⁾ However, a previous study⁵⁸⁾ and our results, showing that **34** inhibited calcium ionophore A23187-stimulated degranulation, suggested that other mechanisms such as degranulation mechanisms after Ca^{2+} influx as well as the inhibition of Syk were involved, as for flavonoids and stilbenes.

Curcumin (**34**) has poor bioavailability and is metabolized to less active reduction metabolites such as **40** and **43** and their glucuronides.⁶³ However, Li *et al.* reported that the oral administration of **34** significantly attenuated IgE/antigen-induced passive systemic anaphylaxis in mice, as determined by serum LTC₄, PGD₂, and histamine levels.⁶⁴

5. Conclusion

Several antiallergic agents clinically used, such as tranilast and ketotifen fumarate, show weak inhibitions of the degranulation in rat peritoneal mast cells and RBL-2H3 cells *in vitro* (IC₅₀>100 μM), contrary to our expectations. We found many degranulation inhibitors with a variety of chemical structures from medicinal plants using RBL-2H3 cells. Recently, many researchers have also reported various active constituents using similar methods, and these active compounds may be promising resources for developing new antiallergic agents. However, most active constituents found in *in vitro* examinations have not yet been examined in animal experiments (*in vivo*), except for several flavonoids, stilbenes, and curcuminoids. Their effectiveness should be confirmed in *in vivo* experiments. In addition, since the antiallergic constituents such as flavonoids are contained in foods as well as medicinal plants, the relation between eating habits and allergic conditions of patients should be investigated.

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Conflict of Interest The authors declare no conflict of interest.

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