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REVIEW

Search for bioactive constituents from several medicinal foods: hepatoprotective, antidiabetic, and antiallergic activities

Toshio Morikawa

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Abstract In the course of our characterization studies on bioactive constituents from medicinal herbs, several medicinal foods and their constituents were found to show the following biological activities: (1) hepatoprotective sesquiterpenes from Zedoariae Rhizoma (2) α-glucosidase and aldose reductase inhibitors form *Salacia* spices and the flowers of *Chrysanthemum indicum*, and (3) anti-allergic and antiinflammatory diaryloheptanoids from the barks of *Myrica rubra* and *Acer nikoense*.

Keywords Medicinal food · Hepatprotective activity · Aldose reductase inhibitor · Degranulation inhibitor · Nitric oxide production inhibitor · Structural requirement

Introduction

Various food materials are used as herbal medicines in traditional, alternative, and/or complementary medicines all over the world. These medicinal foods are known to have not only nutritive and taste values but also medicinal effects and they are prescribed in various traditional preparations. This is refered to as ''natural medicines and foods from the same source (薬食同源)'' in ancient Chinese philosophy [1]. However, the bioactive principles and pharmaceutical properties of these medicinal foods remain uncharacterized except for a few examples. To address this, our research work is focused on the search for bioactive constituents from medicinal foods. In this review, we described the following topics: (1) hepatoprotective sesquiterpenes from Zedoariae Rhizoma [2–8]. The sesquiterpene constituents from this medicinal food were found to show protective effects for D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-activated acute liver injury in mice. To clarify the mechanism of this action, the effects of the active sesquiterpenes on D-GalN-induced cytotoxicity in primary cultured rat hepatocytes (in vitro), nitric oxide (NO) production in LPS-activated mouse peritoneal macrophages (in vitro), and D-GalN/tumor necrosis factor-α (TNF-α)-activated liver injury in mice (in vivo) were examined. The sesquiterpene constituents from this medicinal food also exhibited vasorelaxtant activity [3, 6]. (2) α-Glucosidase and aldose reductase inhibitors form *Salacia* spices [9–13] and the flowers of *Chrysanthemum indicum* [14–16]. In particular, flavonoid constituents were found to show aldose reductase inhibitory activities and several structural requirements of flavonoids were suggested [13, 17]. Furthermore, polyacetylene and flavonoid constituents from *C. indicum* were found to show LPS-activated NO production inhibitory activity [15]. (3) Diaryloheptanoid constituents from the bark of *Myrica rubra* [18, 19] and *Acer nikoense* [20, 21] were found to show inhibitory effects on the release of β-hexosaminidase in RBL-2H3 cells and LPS-activated NO production in mouse peritoneal macrophages.

T. Morikawa (✉)
Pharmaceutical Research and Technology Institute,
Kinki University, 3-4-1 Kowakae, Higashi-osaka,
Osaka 577-8502, Japan
e-mail: morikawa@kindai.ac.jp

Zedoariae Rhizoma [2–8]

Chemical constituents

The Zingiberaceae plant *Curcuma zedoaria* Roscoe (common name: zedoary) has been widely cultivated as a vegetable or spice in South and Southeast Asian countries. The rhizomes of this plant (Zedoariae Rhizoma), which is listed in the Japanese pharmacoepia, are used as a stimulant, stomachic, carminative, diuretic, anti-diarrheal, anti-emetic, anti-pyretic, depurator, and also to clean and cure ulcers, wounds, and other kinds of skin disorders in India and Southeast Asian countries. In Japanese and Chinese traditional medicine, Zedoariae Rhizoma has been prescribed as a stomachic, emmenagogue, and for the treatment of ''Oketsu'' syndrome caused by blood stagnation in various traditional preparations. During the course of our characterization studies on bioactive constituents of natural medicines and medicinal foodstuffs, we have found that 43 sesquiterpenes (**1**–**43**) and two diarylheptanoid (**44**, **45**) constituents were isolated from the 80% aqueous acetone extract of Zedoariae Rhizoma cultivated in Szechwan province, China. Among the constituents, six carabrane-type sesquiterpenes, curcumenolactones A (**1**), B (**2**), and C (**3**), 4*S*-dihydrocurcumenone (**4**), and curcarabranols A (**5**) and B (**6**), six guaiane- and *seco*-guaiane-type sesquiterpenes, 4-epicurcumenol (**19**), neocurcumenol (**20**), gajutsulactones A (**21**) and B (**22**), and zedoarolides A (**23**) and B (**24**), and a eudesmane-type sesquiterpene, zedoarofuran (**38**), were isolated as new compounds.

The stereostructures of these compounds (**1**–**6**, **19**–**24**, **38**) were determined by UV, CD, IR, ^{1}H and ^{13}C NMR, which were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), homo- and heterocorrelation spectroscopy (^{1}H–^{1}H and ^{13}C–^{1}H COSY), HMBC, and NOESY experiments, and MS spectroscopic analyses [2–7]. To clarify the absolute stereostructures of the 8-positions of **1**–**3**, **23**, and **24** were determined by the CD spectrum of the α,β-unsaturated-γ-lactone moiety [22, 23]. The absolute stereostructures of **4**–**6** and **20** were clarified on the basis of chemical relations to known compounds (**7**, **25**) and the application of the modified Mosher's method [24] as shown in Fig. 1.

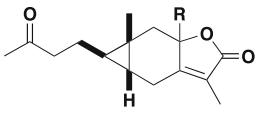
Hepatoprotective activity

The 80% aqueous acetone extract of this medicinal food was found to show a protective effect on D-GalN/LPS-induced acute liver injury in mice (Fig. 2) [2, 8]. As shown in Fig. 3, the aqueous acetone extract showed an inhibitory effect on the increase in serum GPT (s-GPT) and serum GOT (s-GOT) induced by D-GalN/LPS in mice at doses of 12.5–50 mg/kg p.o., whose protective activity was stronger than that of natural hepatoprotective agents, curcumin (**44**) and silymarin in this model.

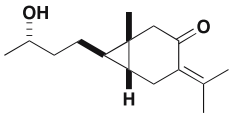
To clarify the active constituents from this medicinal food, the principal 11 sesquiterpene constituents were examined. As the result, curcumenone [**7**, inhibition of s-GPT: 90.1%; s-GOT: 88.8% at a dose of 50.0 mg/kg, p.o., respectively], furanodiene (**9**, 72.9%, 74.8%), germacrone (**12**, 82.9%, 78.6%), 13-hydroxygermacrone (**13**, 61.7%, 54.9%), curdione (**16**, 76.6%, 75.1%), neocurdione (**17**, 59.6%, 59.0%), dehydrocurdione (**18**, 49.7%, 46.5%), curcumenol (**25**, 72.4%, 75.0%), isocurcumenol (**26**, 77.3%, 80.6%), aerugidiol (**27**, 88.0%, 89.8%), and zedoarondiol (**32**, 60.8%, 54.9%), were found to inhibit the increase in s-GPT and s-GOT levels. In particular, **16**, **17**, **25**, and **26** potently inhibited the increase in s-GPT and s-GOT levels at a dose of 12.5 mg/kg, p.o. [2, 4, 8] (Fig. 4).

D-GalN/LPS-induced liver injury is recognized as developing from immunological responses [25]. This type of liver injury occurs in two ways, as shown in Fig. 2: (1) depletion of uridine triphosphate and increased sensitivity of hepatocytes to TNF-α are induced by D-GalN; (2) release of proinflammatory mediators, such as TNF-α, from LPS-activated macrophages (Kupffer's cell) vide infra. Apoptosis of hepatocytes induced by TNF-α is reported to be important in D-GalN/LPS-induced liver injury [26]. In order to elucidate the mechanisms of action of the constituents of Zedoariae Rhizoma, we examined the protective effect against D-GalN-induced cytotoxicity in primary cultured rat hepatocytes [2, 4] and NO production, which is a marker of activation of mouse peritoneal macrophages stimulated by LPS [2, 6, 7]. As a result, germacrone (**12**, inhibition: 59.8 ± 6.3% at 100 μM), curdione (**16**, 77.1 ± 5.8%), neocurdione (**17**, 44.6 ± 5.3%), and curcumenol (**25**, 25.1 ± 5.3%) inhibited D-GalN-induced cytotoxicity, while the other compounds did not [curcumenone (**7**, –12.2 ± 2.6%), furanodiene (**9**, –0.5 ± 0.2%), 13-hydroxygermacrone (**13**, –21.7 ± 3.0%), dehydrocurdione (**18**, –6.3 ± 0.3%), isocurcumenol (**26**, 14.2 ± 5.9%), aerugidiol (**31**, –41.5 ± 8.0%), zedoarondiol (**32**, –35.6 ± 7.9%), and curcumin (**44**, –44.3 ± 0.3%)]. In addition, curcumenone (**7**, IC_{50} = 82 μM), furanodiene (**9**, 75 μM), neocurdione (**17**, 98 μM), 13-hydroxycurdione (**13**, 98 μM), curcumenol (**25**, 55 μM), and isocurcumenol (**26**, 57 μM) were found to inhibit NO production. Curcumin (**44**) also inhibited NO production (13 μM), although cytotoxicity was also observed (viability 4%

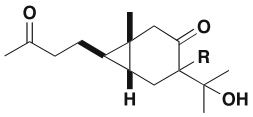
Carabrane-type sespuiterpenes



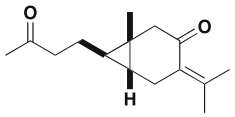
curcumenolactone A (**1**) : R=β-H
curcumenolactone B (**2**) : R=α-H
curcumenolactone C (**3**) : R=α-OH



4*S*-dihydrocurcumenone (**4**)

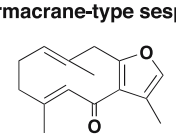


curcarabranol A (**5**) : R=β-H
curcarabranol B (**6**) : R=α-H

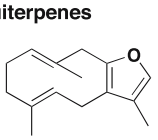


curcumenone (**7**)

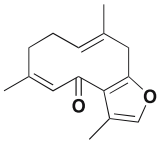
Germacrane-type sespuiterpenes



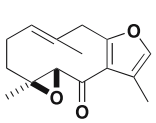
furanodienone (**8**)



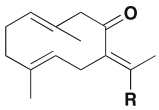
furanodiene (**9**)



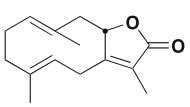
isofuranodienone (**10**)



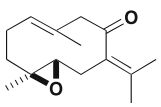
zederone (**11**)



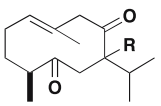
germacrone (**12**) : R=CH_3
13-hydroxygermacrone (**13**) : R=CH_2OH



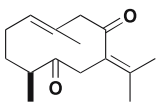
glechomanolide (**14**)



(+)-germacrone 4,5-epoxide (**15**)

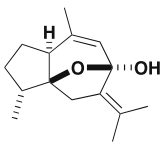


curdione (**16**) : R=β-H
neocurdione (**17**) : R=α-H

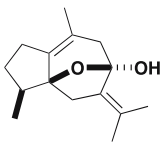


dehydrocurdione (**18**)

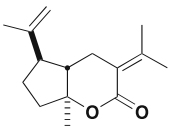
Guaiane-type sespuiterpenes



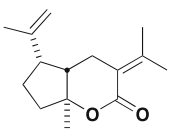
4-epicurcumenol (**19**)



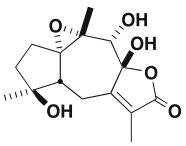
neocurcumenol (**20**)



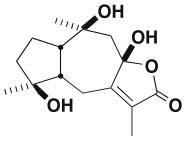
gajutsulactone A (**21**)



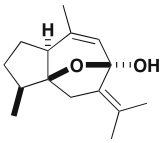
gajutsulactone B (**22**)



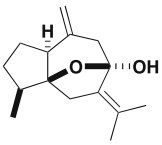
zedoarolide A (**23**)



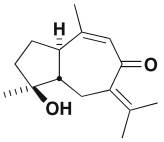
zedoarolide B (**24**)



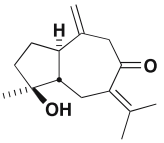
curcumenol (**25**)



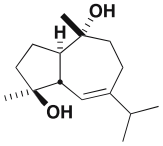
isocurcumenol (**26**)



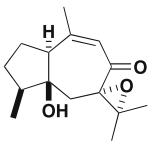
procurcumenol (**27**)



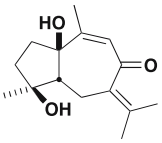
isoprocurcumenol (**28**)



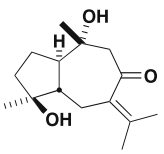
alismoxide (**29**)



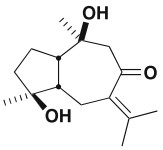
7α,11α-epoxy-5β-hydroxy-9-guaiaen-8-one (**30**)



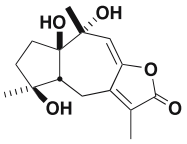
aerugidiol (**31**)



zedoarondiol (**32**)

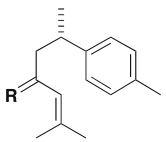


isozedoarondiol (**33**)



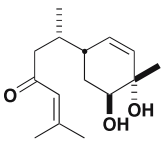
zedoalactone B (**34**)

Bisaborane-type sespuiterpenes

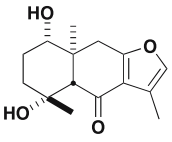


(+)-*ar*-turmerone (**35**) : R= O
bisacumol (**36**) : R=α-OH, β-H

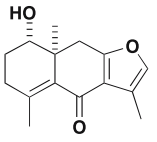
Eudesmane-type sespuiterpenes



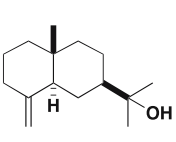
bisacurone (**37**)



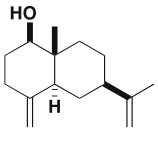
zedoarofuran (**38**)



curcolone (**39**)

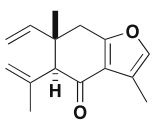


β-eudesmol (**40**)



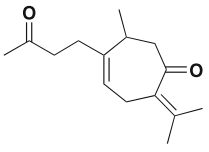
β-dictyopterol (**41**)

Elemane-type sespuiterpenes



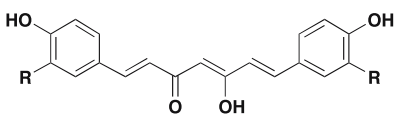
curzerenone (**42**)

Xanthane-type sespuiterpenes



curcumadione (**43**)

Diarylheptanoids



curcumin (**44**) : R=OMe
bis (4-hydroxycinnamoyl)methane (**45**) : R=H

Scheme 1 Chemical constituents from Zedoariae Rhizoma

at 100 μM). In addition, curcumin (**44**) was reported to inhibit TNF-α production in the LPS-stimulated human monocytic macrophage cell line, 7 Mono Mac 6 [27]. These in vitro findings led us to suggest the following possible mechanisms of action for the hepatoprotective effect, although the bioavailabilities and metabolites of the compounds have not clarified: (1) inhibition of the activation of macrophages by LPS [curcumenone (**7**), furanodiene (**9**), 13-hydroxygermacrone (**13**), isocurcumenol (**26**), and curcumin (**44**)]; (2) protection of hepatocytes against the toxicity of D-GalN [germacrone (**12**) and curdione (**16**)]; and (3) both effects (1) and (2) [neocurdione (**17**) and curcumenol (**25**)]. Since overproduction of NO is known to be a cause of inflammation, immunological responses, and endotoxin-induced shock [28], these sesquiterpenes (**7**, **9**, **13**, **26**) and **44** may be effective in the treatment of inflammation and endotoxic shock. On the other hand, dehydrocurdione (**18**), aerugidiol (**31**), and zedoarondiol (**32**) lacked inhibitory activity against NO production and protection against the cytotoxicity of D-GalN. Therefore, the metabolites of **18**, **31**, and **32** might be effective in the treatment of D-GalN/LPS-induced liver injury, although other mechanisms exist. When we examined the D-GalN/TNF-α-induced liver injury model, eight sesquitepenes (**7**, **9**, **12**, **13**, **16**–**18**, **25**) also inhibited the increase in s-GPT and s-GOT levels (inhibition: 67.5–76.4% at a dose of 50.0 mg/kg, p.o.). These in vivo results suggest that those sesquiterpenes protect the liver against the toxicity of D-GalN or reduce the effects of TNF-α. However, the detailed mechanisms of action of the active constituents should be studied further.

Vasorelaxtant activity

The relaxant effects of the 80% aqueous acetone extract of Zedoariae Rhizoma on high concentration of potassium cation (K^+) and DL-noradrenaline (NA)-induced contractions in rat thoracic aorta were examined. Cumulative application of the 80% aqueous acetone extract (6.25–50 μg/ml) inhibited the sustained contractions induced by high K^+ [IC_{50} = 18 μg/ml, (nifedipine, 6.4 nM)]. However, this extract did not inhibit the contractions induced by NA in isolated rat aortic strips. Among the isolated constituents from this medicinal food, 16 sesquiterpenes [curcumenolactone B (**2**, IC_{50} = 100 μM), furanodiene (**9**, 67 μM), zederone (**11**, 46 μM), germacrone (**12**, 19 μM), 13-hydroxygermacrone (**13**, 52 μM), glechomanolide (**14**, 35 μM), neocurdione (**17**, 54 μM), 4-epicurcumenol (**19**, 90 μM), neocurcumenol (**20**, 85 μM), curcumenol

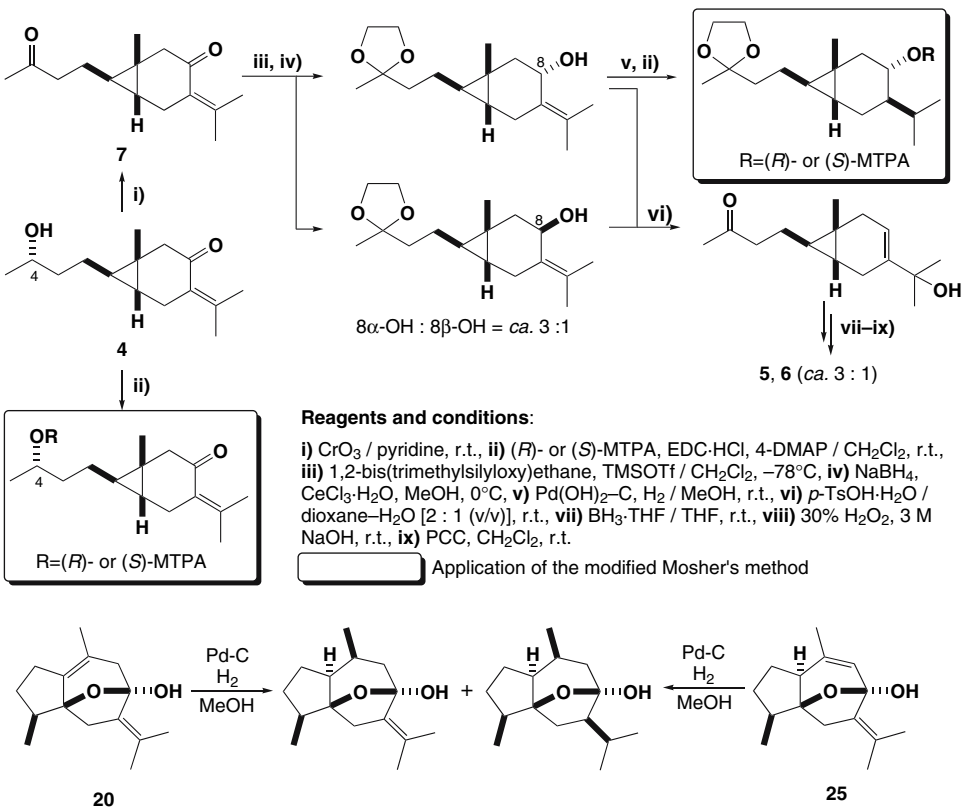


Fig. 1 Absolute stereostructures of **4**–**7** and **20**

(**25**, 92 μM), isocurcumenol (**26**, 26 μM), (+)-*ar*-turumerone (**35**, 38 μM), bisacumol (**36**, 37 μM), *β*-eudesmol (**40**, 16 μM, *β*-dictyopterol (**41**, 9 μM), and curzerenone (**42**, 38 μM)] and two diarylheptanoids [curcumin (**44**, 32 μM) and *bis*(4-hydroxycinnamoyl)methane (**45**, 22 μM)] relaxed the sustained contractions induced by high K^+. Especially, five sesquiterpenes (**12**, **14**, **26**, **40**, **41**) showed potent relaxation, and these results suggested that the activities of germacrane- and eudesmane-type sesquiterpenes were stronger than those of the other type of sesquiterpenes. Furthermore, compounds having *exo*-methylene moiety, such as **26** and **40**–**42**, showed potent activity. On the other hand, polyoxygenated sesquiterpenes such as

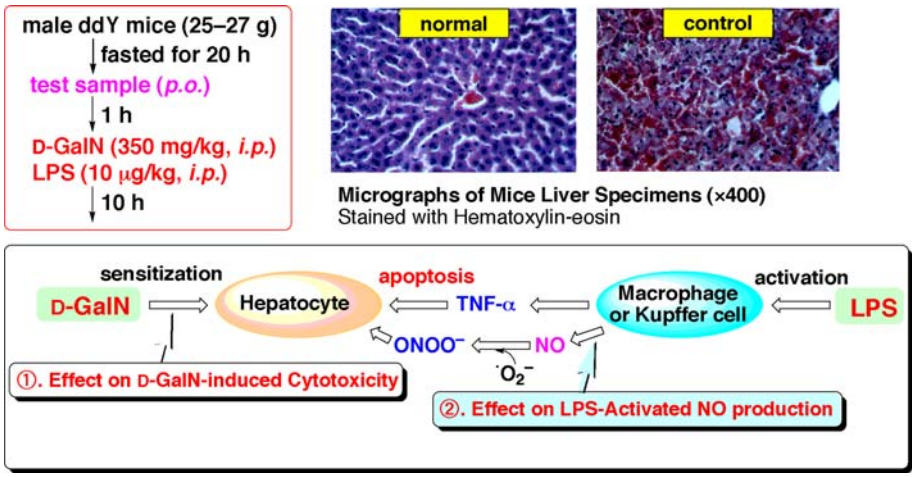


Fig. 2 Mechanisms of D-Galn/LPS-induced liver injury

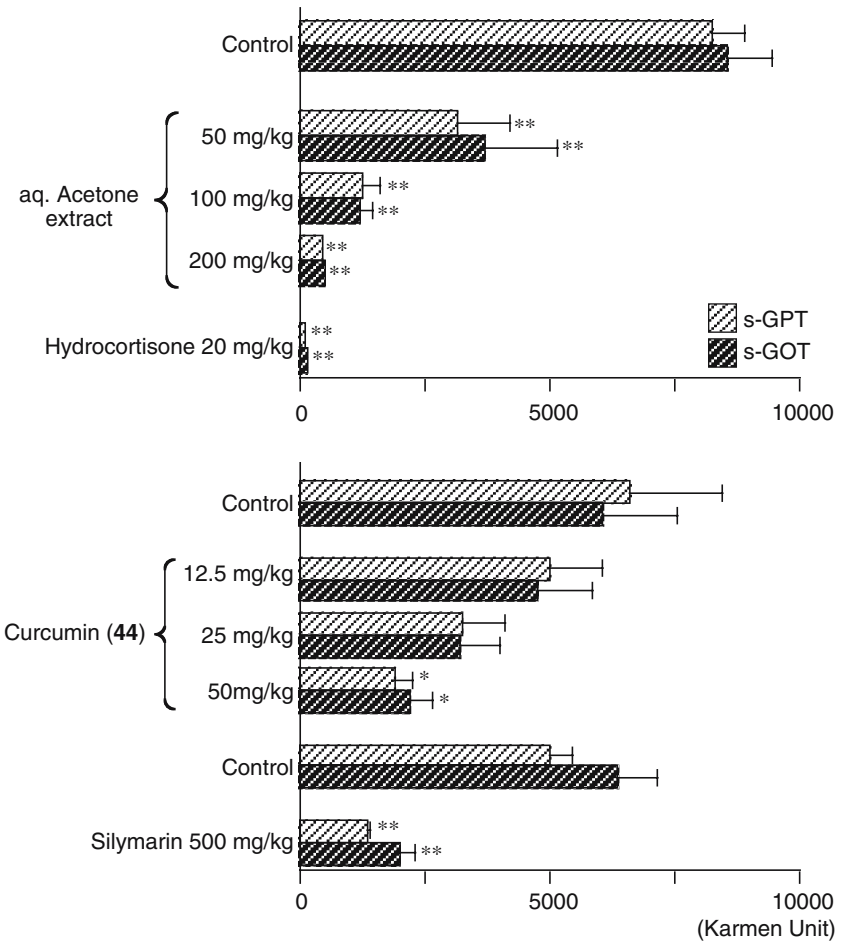


Fig. 3 Inhibitory effects of aqueous acetone extract from Zedoariae Rhizoma on D-GalN/LPS-induced liver injury in mice. Male ddY mice weighing about 25–27 g (6 weeks old) were fasted for 20 h before the experiment. D-GalN (350 mg/kg) and LPS (10 μg/kg) dissolved in saline were injected intraperitoneally to produce liver injury. The test sample was given orally 1 h before D-GalN/LPS injection. Blood samples were collected 10 h after D-GalN/LPS injection. *Each column* represents the mean with standard error, SE (n = 4–12, $^*P < 0.05$, $^{**}P < 0.01$)

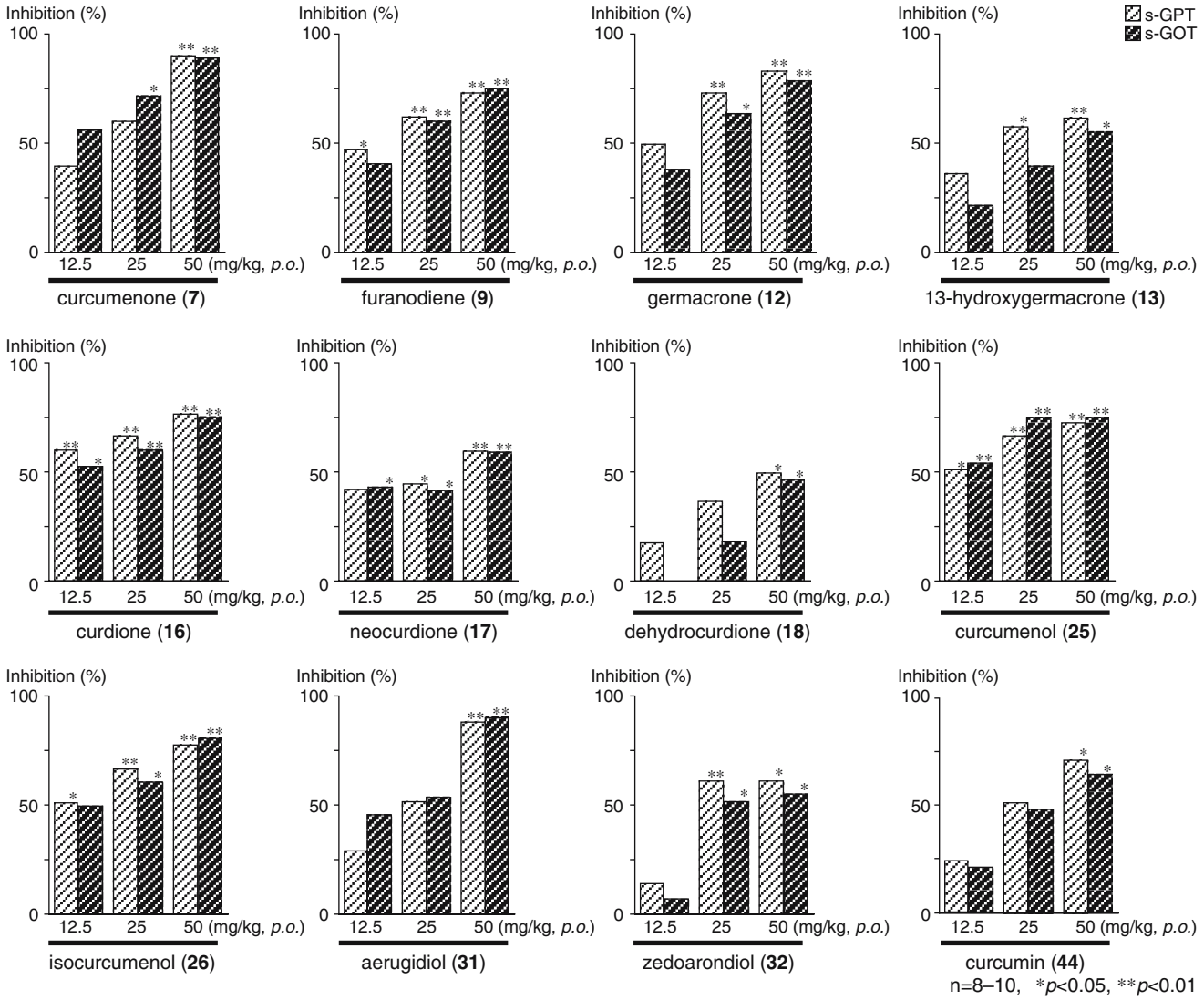


Fig. 4 Inhibitory effects of the constituents of Zedoariae Rhizoma on D-GalN/LPS-induced liver injury in mice

(**24**, **31**–**34**, **37**) showed weak activity. These results led us to deduce the following structural requirements of the isolated sesquiterpenes for the activity: (1) germacrane- and eudesmane-type sesquiterpenes were active structures; (2) *exo*-methylene moiety enhanced the activity; and (3) polyoxygenated sesquiterpenes exhibited weak activity. In addition, inhibitory effects of the isolated constituents on NA-induced contractions were examined in isolated thoracic aorta of rat. As the result, two diarylheptanoids (**44**, **45**) slightly inhibited the contractions. However, other sesquiterpene constituents did not show the significant inhibition. It is well known that high K^+-induced contractions in smooth muscles are the result of an increase in intracellular Ca^{2+}, and calcium antagonists such as nifedipine inhibit the voltage-dependent calcium channel, thereby inhibiting the contractions in the depolarized aortic strips, although they show weak inhibitory effects on the NA-induced contractions [29]. These active compounds (**2**, **9**, **11**–**14**, **17**, **19**, **20**, **25**, **26**, **35**, **36**, **40**–**42**, **44**, **45**) relaxed high K^+-induced contractions by their calcium channel blocking activities like nifedipine. The vasorelaxant effect of these active sesquiterpenes may be related to the traditional medicinal value of Zedoariae Rhizoma as the treatment effect of ''Oketsu'' syndrome caused by blood stagnation.

The stem and roots of *Salacia* species [9–13]

Recently we reported the antidiabetogenic constituents from *Salacia* species in a review including our research, including α-glucosidase and aldose reductase inhibitors

from the roots and stems of *Salacia reticulata*, *S. oblonga*, and *S. chinensis* [30]. The details have been described in Ref. [30].

The flowers of *C. indicum* [14–16]

Chemical constituents

The flowers of *C. indicum* L. (Compositae) [Yagikka (野菊花) in Japanese] is prescribed for anti-inflammatory, analgesic, and antipyretic purposes and the treatment of eye disease in Chinese traditional preparations. The flowers of *C. indicum* and *C. morifolium* [Kangiku (甘菊) in Japanese] are listed in Japanese pharmacoepia as treatments of cephalalgia, vertigo, and eye inflammation. As chemical constituents of this plant, several bisaborane- and guaiane-type sesquiterpenes and flavonoids have been isolated from the flowers of *C. indicum*. However, the pharmacological activity and bioactive constituents of this medicinal food remain uncharacterized. In the course of our studies on bioactive principles of medicinal foods, we found that the methanolic extract from the flowers of *C. indicum* exhibited potent inhibitory activities on rat lens aldose reductase [14, 16] and LPS-activated NO production in mouse peritoneal macrophages [15]. By bioassay-guided separation, 11 sesquitepenens (**46**–**56**), two polyacetylenes (**57**, **58**), 12 aromatic compounds (**59**–**70**) including 10 flavonoids were isolated from the flowers of Chinese *C. indicum*. Among the isolated constituents, seven sesquiterpenes, kikkanols A (**46**), B (**47**), C (**48**), D (**49**), D monoacetate (**50**), E (**51**), F (**52**), and F monoacetate (**53**), two flavanone glycosides, (2*S*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**59**) and (2*R*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**60**), and a phenylbutanoid glycoside, (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-*β*-D-glucopyranoside (**69**), were isolated as new compounds. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence, which included application of the modified Mosher's method [14–16].

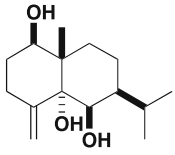
Inhibitory effects of the constituents from *C. indicum* on rat lens aldose reductase

Aldose reductase as a key enzyme in the polyol pathway is reported to catalyze the reduction of glucose to sorbitol. In normal tissue, aldose reductase has low substrate affinity to glucose, so that the conversion of glucose to sorbitol is only slightly catalyzed. However, in diabetes mellitus, the increased availability of glucose in insulin-insensitive tissues such as lens, nerve, and retina leads to the increased formation of sorbitol through the polyol pathway. Sorbitol does not readily diffuse across cell membranes and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataracts, neuropathy, and retinopathy. These findings suggest that aldose reductase inhibitor prevents the conversion of glucose to sorbitol and may have the capacity of preventing and/or treating several diabetic complications [31]. Since the flowers of *C. indicum* have proved useful for the treatment of eye disease in Chinese traditional medicine, we examined the inhibitory activity of the methanolic extract from the flowers of *C. indicum* (IC_{50} = 3.5 μg/ml) and the components isolated from the active fractions [the ethyl acetate (EtOAc)-soluble fraction: IC_{50} = 1.3 μg/ml and the *n*-butanol (BuOH)-soluble fraction: IC_{50} = 3.5 μg/ml] on rat lens aldose reductase. As the result, several flavonoids, (2*S*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**59**, IC_{50} = 2.1 μM), (2*R*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**60**, 1.5 μM), luteolin (**62**, 0.45 μM), luteolin 7-*O*-*β*-D-glucopyranoside (**63**, 0.99 μM), luteolin 7-*O*-*β*-D-glucopyranosiduronic acid (**64**, 3.1 μM), and acacetin 7-*O*-(6″-α-L-rhamnopyranosyl)-*O*-*β*-D-glucopyranoside (**66**, 4.7 μM), and chlorogenic acid (**70**, 1.8 μM) showed potent inhibitory activity. Another flavonoid constituents, eupatilin (**61**, 25 μM), apigenin 7-*O*-*β*-D-glucopyranoside (**65**, 23 μM), diosmetin 7-*O*-*β*-D-glucopyranoside (**66**, 23 μM), and quercetin 3,7-di-*O*-*β*-D-glucopyranoside (**68**, 84 μM), and two sesquiterpenes, clovanediol (**54**, 96 μM) and caryolane 1,9*β*-diol (**55**, 45 μM), exhibited weak inhibitory activity. Taking this data into account, these results indicated that several flavonoids (**59**, **60**, **62**–**64**, **66**) and chlorogenic acid (**70**) are active principles of this medicinal food [14, 16].

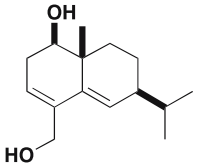
Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity

During the course of our investigation of aldose reductase inhibitors from medicinal foods, various flavonoid constituents were commonly isolated as active components (vide ante). To clarify the structural requirements of flavonoids for rat lens aldose reductase inhibitory activity, 94 compounds including 19 flavones, 37 flavonols, eight flavanones, a dihydroflavonol, 12 isoflavones, three flavan-3-ols, and 14 stilbenes, were examined [13, 17]. Among them, quercitrin (**71**, IC_{50} = 0.15 μM) [18, 19], guaijaverin (**72**, 0.18 μM) [32, 33], and desmanthin-1 (**73**, 0.082 μM) [32, 33] were found to show potent inhibitory activity. The inhibitory

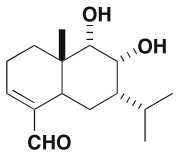
Sesquiterpenes



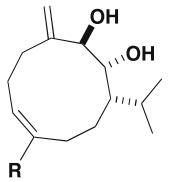
kikkanol A (**46**)



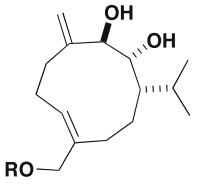
kikkanol B (**47**)



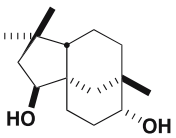
kikkanol C (**48**)



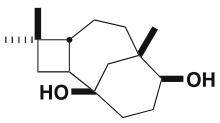
kikkanol D (**49**): R=CH_2OH
kikkanol D monoacetate (**50**): R=CH_2OAc
kikkanol E (**51**): R=CHO



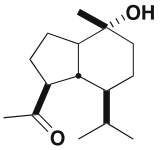
kikkanol F (**52**): R=H
kikkanol F monoacetate (**53**): R=Ac



clovanediol (**54**)

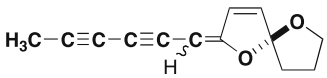


caryolane 1,9β-diol (**55**)



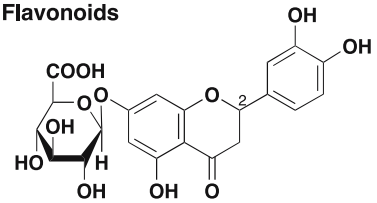
oplopanone (**56**)

Polyacetylenes

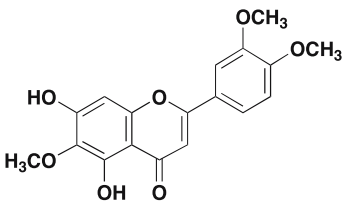


cis-spiroketalenolether polyyne (**57**)
trans-spiroketalenolether polyyne (**58**)

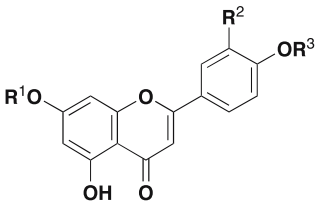
Flavonoids



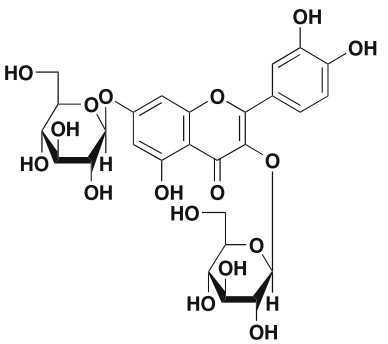
(2*S*)-eriodictyol 7-*O*-β-D-glucopyranosiduronic acid (**59**): 2*S*
(2*R*)-eriodictyol 7-*O*-β-D-glucopyranosiduronic acid (**60**): 2*R*



eupatilin (**61**)

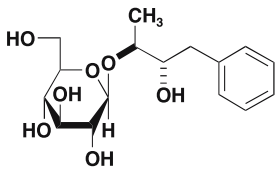


	R^1	R^2	R^3
luteolin (**62**):	H	OH	H
luteolin 7-*O*-β-D-glucopyranoside (**63**):	Glc	OH	H
luteolin 7-*O*-β-D-glucopyranosiduronic acid (**64**):	GlcA	OH	H
apigenin 7-*O*-β-D-glucopyranoside (**65**):	Glc	H	H
diosmetin 7-*O*-β-D-glucopyranoside (**66**):	Glc	OH	CH_3
acacetin 7-*O*-(6''-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**67**):	Rha^{6}—Glc	H	CH_3

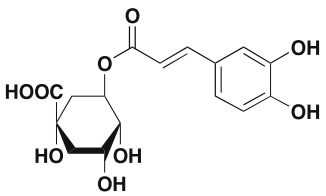


quercetin 3,7-di-*O*-β-D-glucopyranoside (**68**)

Other aromatics



(2*S*, 3*S*)-1-phenyl-2,3-butanediol 3-*O*-β-D-glucopyranoside (**69**)



chlorogenic acid (**70**)

Glc : β-D-glucopyranosyl
GlcA: β-D-glucopyranosiduronic acid
Rha : α-L-rhamnopyranosyl

Scheme 2 Chemical constituents from the flowers of *C. indicum*

activities of these compounds were equivalent to that of a commercial synthetic aldose reductase inhibitor, epalrestat (0.072 μM) as shown in Fig. 5.

In addition, the following structural requirements of flavonoids for aldose reductase inhibitory activity were suggested (a part of the result shown in Fig. 6): (1) the

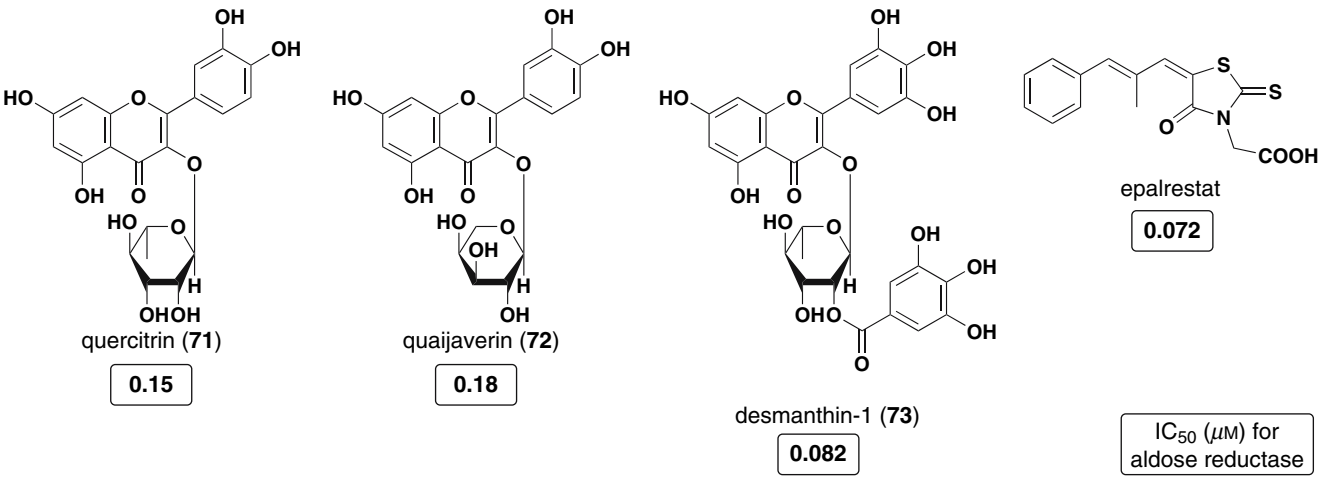


Fig. 5 Structures of aldose reductase inhibitors

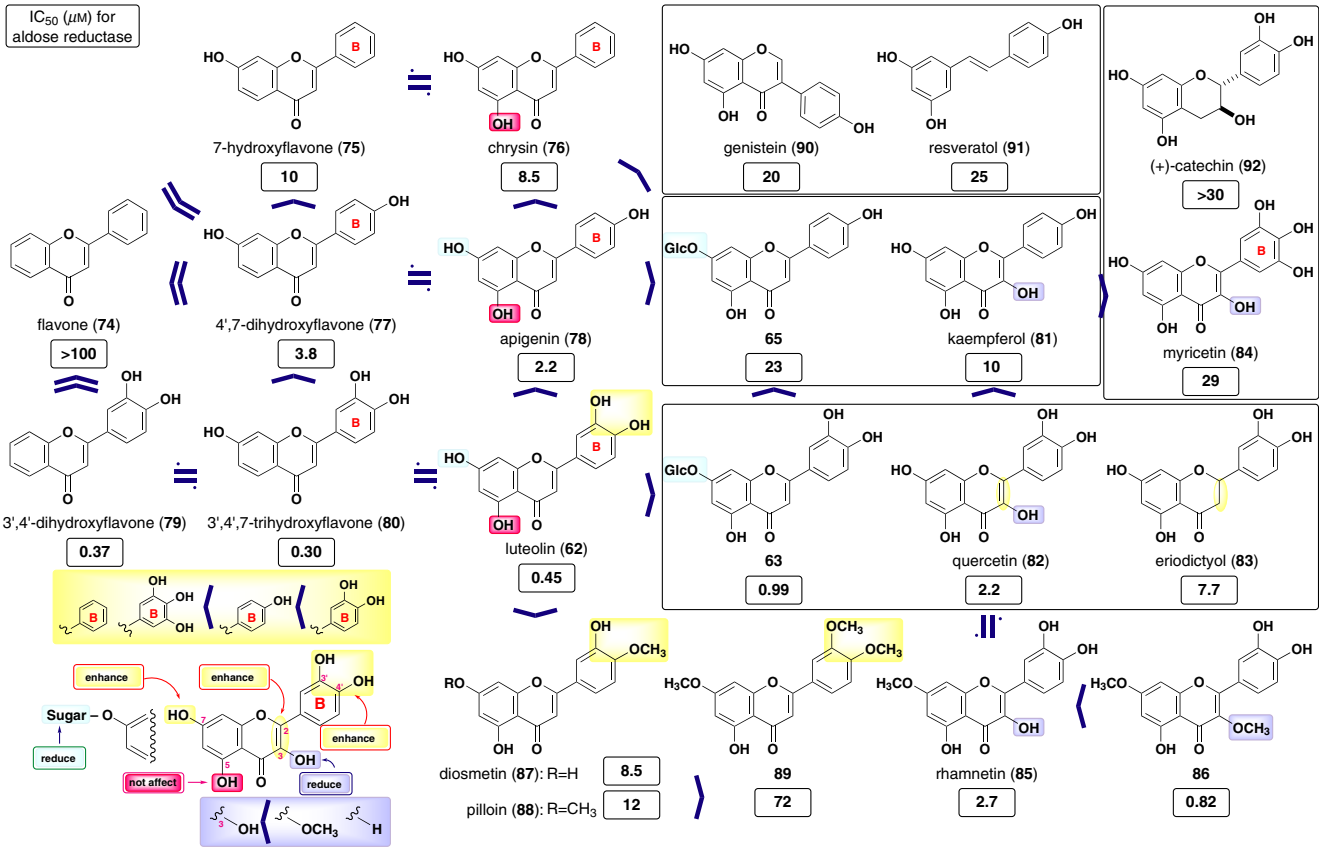


Fig. 6 Structural requirements of flavonoids for aldose reductase inhibitory activity

flavones and flavonols having the 7-hydroxyl and/or catechol moiety at the B ring (the 3′,4′-dihydroxyl moiety) exhibit strong activity [flavone (**74**, >100 μM) < 7-hydroxyflavone (**75**, 10 μM) < 3′,4′-dihydroxyflavone (**79**, 0.37 μM) ≒ 3′,4′,7-trihydroxyflavone (**80**, 0.30 μM)]; (2) the 5-hydroxyl moiety does not affect activity [75 ≒ chrysin (**76**, 8.5 μM), 4′,7-dihydroxyflavone (**77**, 3.8 μM) ≒ apigenin (**78**, 2.2 μM), **80** ≒ luteolin (**62**, 0.45 μM)]; (3) the 7-*O*-glucosyl moiety reduce the activity [**78** > apigenin 7-*O*-β-D-glucopyranoside (**65**, 23 μM), **62** > luteolin 7-*O*-β-D-glucopyranoside (**63**, 0.99 μM)]; (4) the 3-hydroxyl

moiety reduce the activity (flavonols exhibited weaker activity than those of the corresponding flanones) [**78** > kaempferol (**81**, 10 μM), **62** > quercetin (**82**, 2.2 μM)]; (5) the 2–3 double bond enhances activity [**82** > eriodictyol (**83**, 7.7 μM)]; (6) the flavones and flavonols having the catechol moiety at the B ring exhibit stronger activity than those having the pyrogallol moiety (the 3′,4′,5′-trihydroxyl moiety) [**81** > myricetin (**84**, 29 μM)]; (7) isoflavones, stilbenes, and flavan-3-ols exhibited weaker activity than those of the corresponding flavones [**78** > genistein (**90**, 20 μM) and resveratrol (**91**, 25 μM), **62** > (+)-catechin (**92**, >30 μM)].

Inhibitory effects of the constituents from *C. indicum* on LPS-activated NO production in mouse peritoneal macrophages

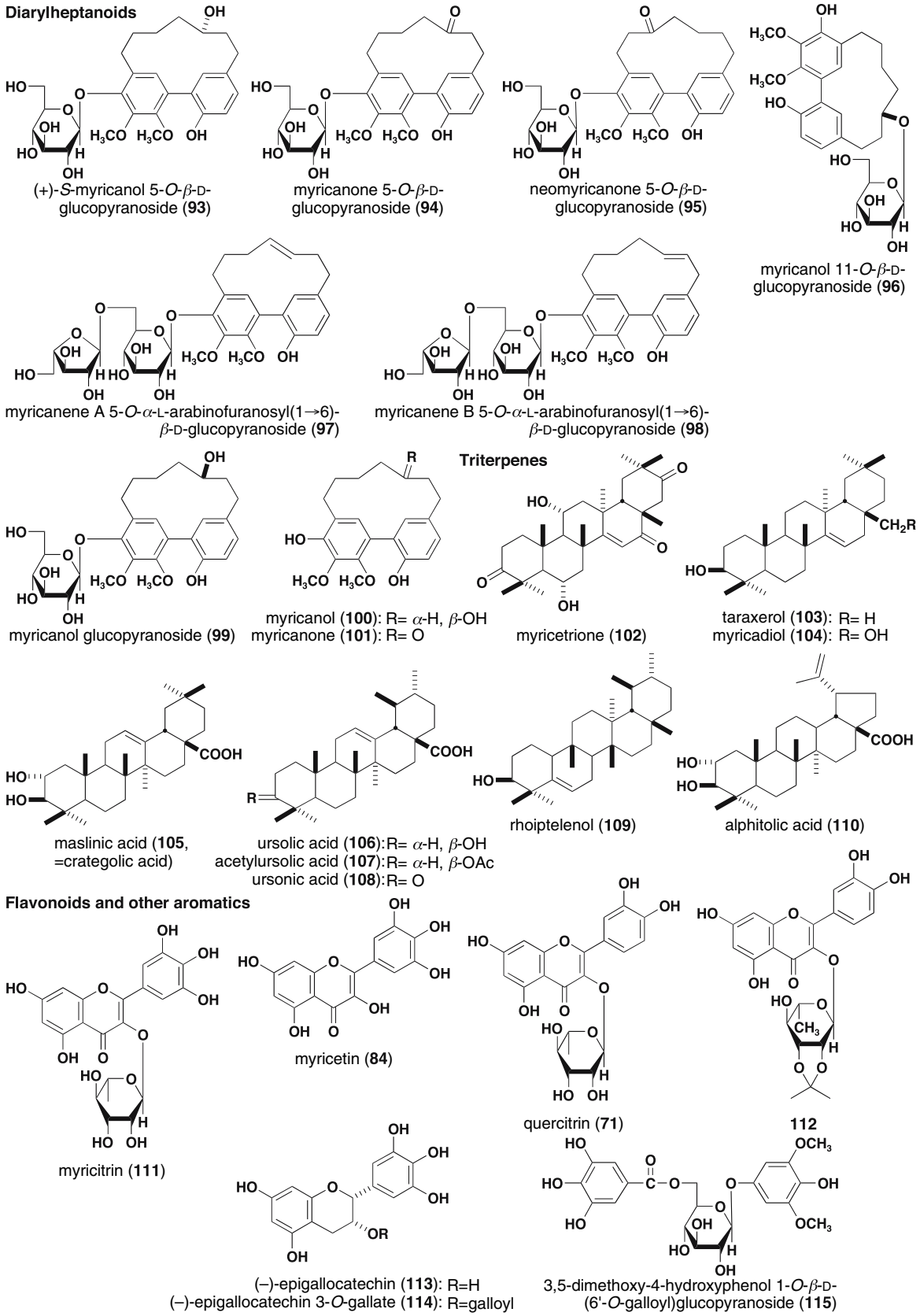
The inorganic free radical NO has been implicated in physiological and pathological processes such as vasodilation, nonspecific host defense, ischemia reperfusion injury, and chronic or acute inflammation [34]. NO is produced by the oxidation of L-arginine catalyzed by NO synthase (NOS). In the NOS family, inducible NOS in particular is involved in pathological overproduction of NO, and can be expressed in response to pro-inflammatory agents such as interleukin (IL)-1β, TNF-α, and LPS in various cell types including macrophages, endothelial cells, and smooth muscle cells. Nuclear factor (NF)-κB is a major transcription factor involved in iNOS, TNF-α, IL-1β, and IL-8 gene expression. NF-κB activation involves dissociation of an inhibitory subunit, IκB, which keeps NF-κB in the cytoplasm, thereby preventing activation of the target gene in the nucleus. Cellular signals lead to phosphorylation of IκB following elimination of IκB from NF-κB by proteolytic degradation. Then, the activated-NF-κB is released and translocated into the nucleus to activate transcription of its target genes [35]. Therefore, inhibition of iNOS enzyme activity or iNOS induction and inhibition of NF-κB activation may be of therapeutic benefit in various types of inflammation [35–37]. Since the flowers of *C. indicum* have been prescribed for anti-inflammatory purpose, we examined the inhibitory effect of the methanolic extract from on LPS-activated NO production in mouse peritoneal macrophages [15]. As the result, the methanolic extract of this medicinal food showed inhibitory activity (IC_{50} = 89 μg/ml). Next, we examined the inhibitory effects of the constituents (**46–58**, **61**, **62**) from the active fraction [the EtOAc-soluble fraction (IC_{50} = 17 μg/ml)] on LPS-activated NO production. Among them, two polyacetylene compounds, *cis*-spiroketalenoether polyyne (**57**, IC_{50} = 38 μM) and *trans*-spiroketalenoether polyyne (**58**, 60 μM) and two flavones, eupatilin (**61**, 42 μM) and luteolin (**62**, 20 μM), were found to inhibit NO production. The inhibitory activity of these compounds and the extract from the flowers of *C. indicum* on NO production may be important evidence substantiating the traditional effects of this medicinal food.

The bark of *M. rubra* and *A. nikoense* [18–21]

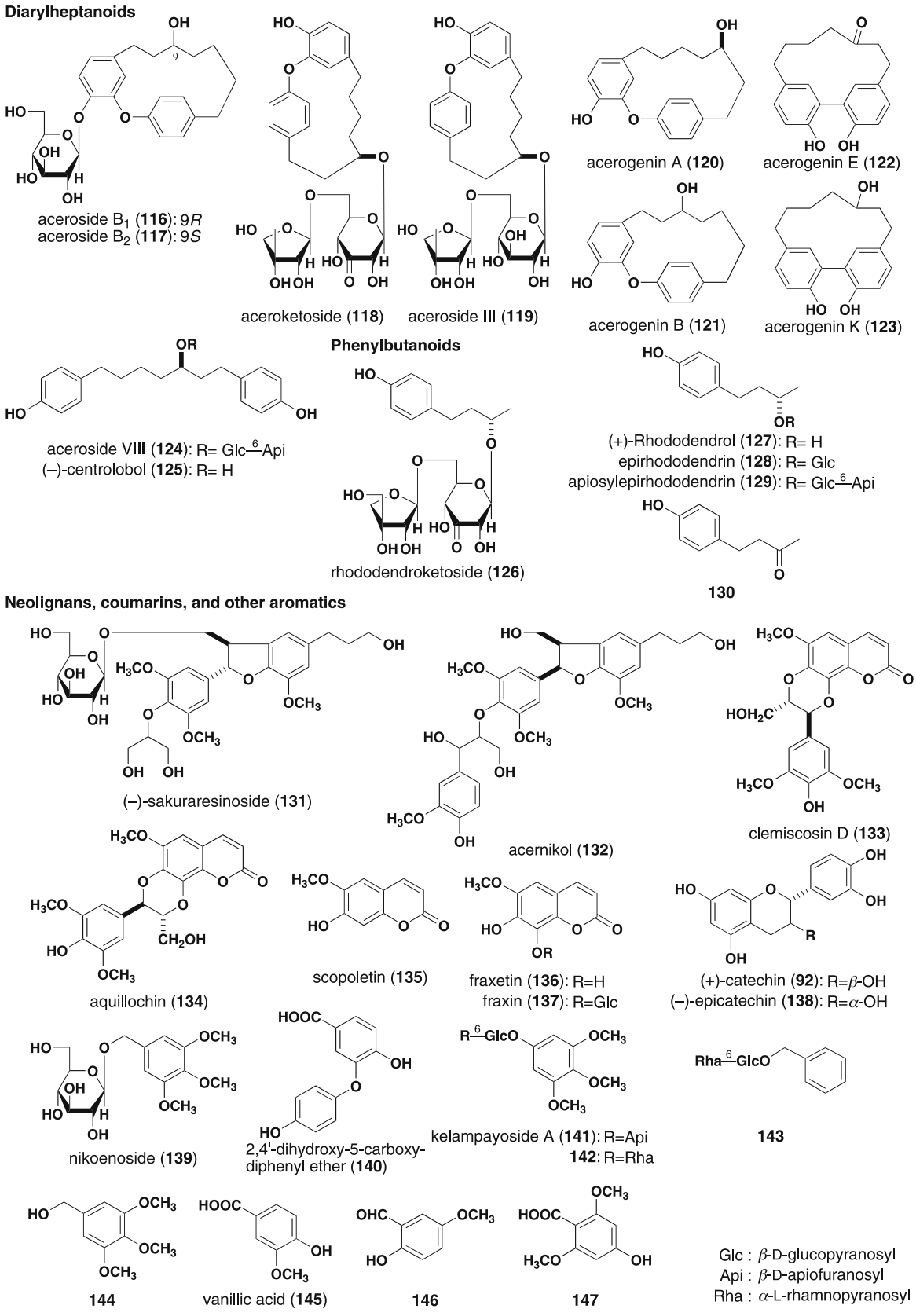
Chemical constituents

The Myricaceae plant *M. rubra* Seib. et Zucc. is widely distributed in China, Taiwan, Japan, and Korea. The bark of *M. rubra* has been used locally as an astringent, antidote, and antidiarrheic in Japanese folk medicine, and has also been used externally for burns and skin diseases in Chinese traditional medicine. On the other hand, the bark of *A. nikoense* Maxim. (Aceraceae), which is indigenous to Japan, has been used as a folk medicine for hepatic disorders and eye diseases. As a part of our characterization studies on bioactive constituents from natural medicines, we found that the methanolic extracts of the bark of *M. rubra* [18, 19] and *A. nikoense* [20, 21] exhibited inhibitory activities on LPS-activated NO production in mouse peritoneal macrophages and the release of β-hexosaminidase from RBL-2H3 cells. From the bark of *M. rubra*, nine diarylheptanoids (**93–101**), nine triterpenes (**102–110**), and seven aromatic compounds (**71**, **84**, **111–115**) were isolated [18, 19]. Among the isolated constituents, six diarylheptanoids, (+)-*S*-myricanol 5-*O*-β-D-glucopyranoside (**93**), myricanone 5-*O*-β-D-glucopyranoside (**94**), neomyricanone 5-*O*-β-D-glucopyranoside (**95**), myricanol 11-*O*-β-D-glucopyranoside (**96**), myricanene A 5-*O*-α-L-arabinofuranosyl(1 → 6)-β-D-glucopyranoside (**97**), and myricanene B 5-*O*-α-L-arabinofuranosyl(1 → 6)-β-D-glucopyranoside (**98**), and a triterpene, myricetrione (**102**), were isolated as new compounds. On the other hand, three diarylheptanoids, acerosides B_1 (**116**) and B_2 (**117**) and aeroketoside (**118**), and four aromatic compounds, rhododendroketoside (**126**), (–)-sakuraresinoside (**131**), aceronikol (**132**), and nikoenoside (**134**), were isolated as new compounds from the bark of *A. nikoense*. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence, which included application of the modified Mosher's method [18–21].

Inhibitory effects of the constituents from *M. rubra* and *A. nikoense* on LPS-activated NO production in mouse peritoneal macrophages.



Scheme 3 Chemical constituents from the bark of *M. rubra*



Scheme 4 Chemical constituents from the bark of *A. nikoense*

The constituents from the barks of *M. rubra* and *A. nikoense* and related compounds on NO production from LPS-activated mouse peritoneal macrophages were examined [19, 20]. As shown in Fig. 7, eight biphenyl type diarylheptanoids, (+)-*S*-myricanol (**93a**, IC_{50} = 19 μM), myricanenes A (**97a**, 23 μM) and B (**98a**, ca. 30 μM), myricanane (**98b**, 26 μM), myricanol (**100**, 23 μM), myricanone (**101**, 23 μM), and acerogenins E (**122**, 24 μM) and K (**123**, 25 μM), showed an inhibitory effect on NO production (IC_{50} = 19, ca. 30 μM), whose activities were equivalent to that of N^G-monomethyl-L-arginine (L-NMMA), a nonselective NOS inhibitor, (IC_{50} = 28 μM). However, their glycosides (**93**, **94**, **97**, **98**) showed weak or no activity. Comparison of the inhibitory activities for diphenyl ether type diarylheptanoids, acerogenins A (**120**, 74 μM) and B (**121**, 88 μM) and straight-chain-type diarylheptanoid, (–)-centrolobol (**125**, 73 μM), with those for biphenyl-type diarylheptanoids suggested that biphenyl-type diarylheptanoids had stronger NO production inhibitory activities than those for diphenyl-ether-type and straight-chain-type diarylheptanoids.

In addition, a triterpene [rhoiptelenol (**109**, 24 μM)], and polyphenols [**112** (49 μM), (–)-epigallocatechin (**113**, 65 μM), (–)-epigallocatechin 3-*O*-gallate (**114**, 27 μM), and 3,5-dimethoxy-4-hydroxyphenol 1-*O*-β-D-(6′-*O*-galloyl)glucopyranoside (**115**, 3.0 μM)] from *M. rubra*, were found to inhibit NO production.

Next, the effects of two biphenyl type diarylheptanoid constituents (**100**, **101**) and a polyphenol constituent (**115**) on iNOS induction were examined [19]. iNOS was detected at 130 kDa after a 12-h incubation with LPS by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-Western blotting analysis as shown in Fig. 8. iNOS inductions of LPS-activated macrophages were shown to be suppressed by two biphenyl-type diarylheptanoid constituents (**100**, **101**) in close relationg to their inhibitions of NO. On the other hand, **115** also suppressed the iNOS induction, but the effect was weaker than that on the NO production. These results suggested that **100** and **101** inhibited NO production mainly due to their inhibitory activities against iNOS induction in LPS-activated macrophages, and the inhibitory activity of **115** against iNOS induction was partly involved in its mechanism of action.

Inhibitory effects of the constituents from *M. rubra* and *A. nikoense* on the release of β-hexosaminidase from RBL-2H3 cells.

Histamine, which is released from mast cells stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker in in vitro experiments on immediate allergic reactions. β-Hexosaminidase is also stored in the secretory granules of mast cells and is released concomitantly with histamine when mast cells are immunologically activated [38, 39]. Therefore, it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells. In our search for antiallergic principles from herbal medicines, we examined the effects of constituents from the barks of *M. rubra* and *A. nikoense* and related compounds on the release of β-hexosaminidase induced by dinitrophenylated bovine serum albutine (DNP-BSA) from RBL-2H3 cells sensitized with anti-

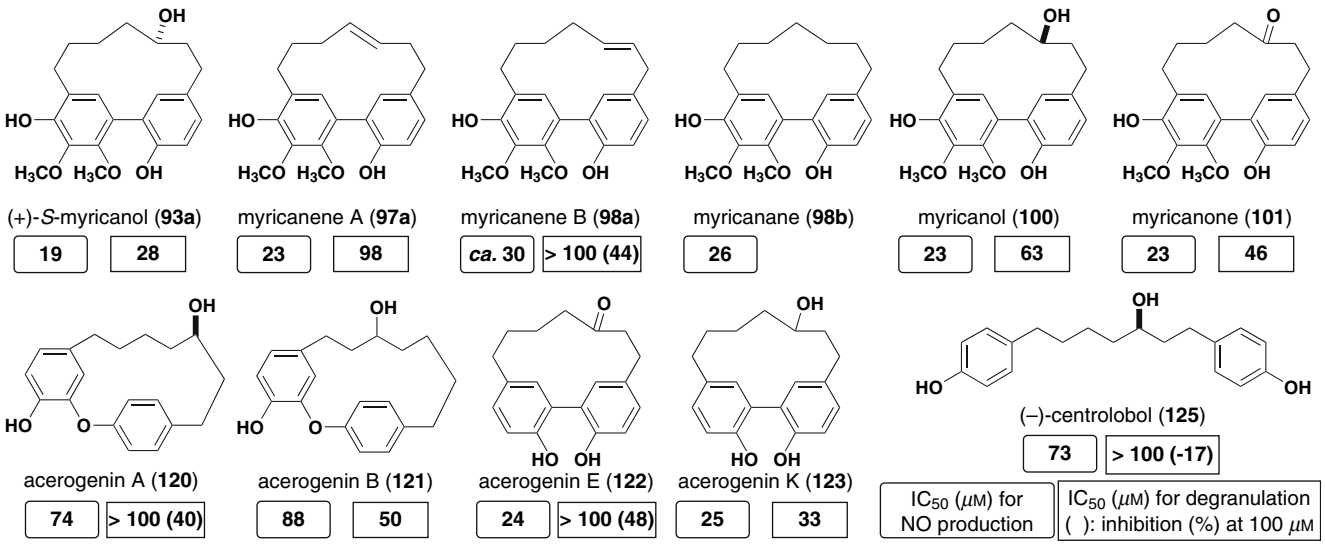


Fig. 7 Inhibitory effects of diarylheptanoid constituents from *M. rubra* and *A. nikoense* and related compounds on LPS-activated NO production in mouse peritoneal macrophages and on the release of β-hexosaminidase from RBL-2H3 cells

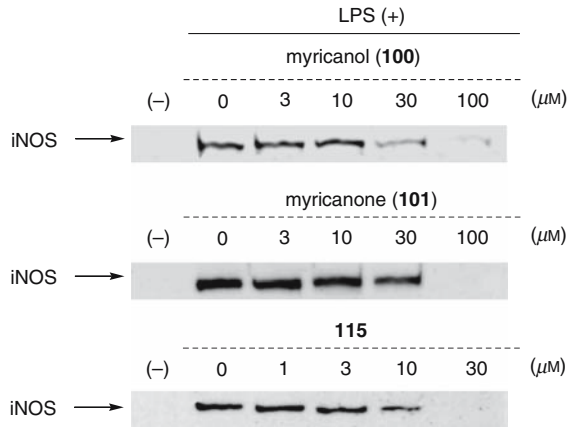


Fig. 8 Effects of biphenyl type diarylheptanoids (**100**, **101**) and **115** on iNOS induction in LPS-activated mouse macrophages

DNP IgE [18, 21]. As shown in Fig. 7, five biphenyl type diarylheptanoids, (+)-*S*-myricanol (**93a**, IC_{50} = 28 μM), myricanene A (**97a**, 98 μM), myricanol (**100**, 63 μM), myricanone (**101**, 46 μM), and acerogenin K (**123**, 33 μM), and a diphenyl ether type diarylheptanoid, acerogenin B (**121**, 50 μM), and a flavonol, myricetin (**84**, 23 μM), showed inhibitory activity, and their activities were stronger than those of antiallergic agent, tranilast (0.49 mM) and ketotifen fumarate (0.22 mM). In addition, the effects of active compounds were examined to clarify whether their effects were due to the inhibition of enzyme activity or of degranulation. The results showed that these active compounds did not affect the enzyme activity of β-hexosaminidase.

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References

1. Kodansha scientific (2005) In: Kitagawa I, Yoshikawa M (eds.) The handbook of medicinal food pharmaceutical science. Kodansha scientific, Tokyo, pp 3–8
2. Matsuda H, Ninomiya K, Morikawa T, Yoshikawa M (1998) Inhibitory effect and action mechanism of sesquiterpenes from zedoariae rhizoma on D-galactosamine/lipopolysaccharide-induced liver injury. Bioorg Med Chem Lett 8:339–344
3. Yoshikawa M, Murakami T, Morikawa T, Matsuda H (1998) Absolute stereostructures of carabrane-type sesquiterpenes, curcumenone, 4*S*-dihydrocurcumenone, and curcarabranols A and B: vasorelaxtant activity of zedoary sesquiterpenes. Chem Pharm Bull 46:1186–1188
4. Matsuda H, Morikawa T, Ninomiya K, Yoshikawa M (2001) Hepatoprotective constituents from zedoariae rhizoma: absolute stereostructures of three new carabrane-type sesquiterpenes, curcumenolactones A, B, and C. Bioorg Med Chem 9:909–916
5. Matsuda H, Morikawa T, Toguchida I, Ninomiya K, Yoshikawa M (2001) Inhibitors of nitric oxide production and new sesquiterpenes, 4-epicurcumenol, neocurcumenol, gajutsulactones A and B, and zedoarolides A and B from zedoariae rhizoma. Heterocycles 55:841–846
6. Matsuda H, Morikawa T, Ninomiya K, Yoshikawa M (2001) Absolute stereostructures of carabrane-type sesquiterpene and vasorelaxtant-active sesquiterpenes from zedoariae rhizoma. Tetrahedron 57:8443–8453
7. Matsuda H, Morikawa T, Toguchida I, Ninomiya K, Yoshikawa M (2001) Medicinal foodstuffs. XXVIII. Inhibitors of nitric oxide production and new sesquiteropenes, zedoarofuran, 4-epicurcumenol, neocurcumenol, gajutsulactones A and B, and zedoarolides A and B, from zedoariae rhizoma. Chem Pharm Bull 49:1558–1566
8. Morikawa T, Matsuda H, Ninomiya K, Yoshikawa M (2002) Medicinal foodstuffs. XXIX. Potent protective effects of sesquiterpenes and curcumin from zedoariae rhizoma on liver injury induced by D-galactosamine/lipopolysaccharide or tumor necrosis factor-α. Biol Pharm Bull 25:627–631
9. Yoshikawa M, Morikawa T, Matsuda H, Tanabe G, Muraoka O (2002) Absolute stereostructure of potent α-glucosidase inhibitor, salacinol, with unique thiosugar sulfonium sulfate inner salt structure from *Salacia reticulata*. Bioorg Med Chem 10:1547–1554
10. Yoshikawa M, Pongpiriyadacha Y, Kishi A, Kageura T, Wang T, Morikawa T, Matsuda H (2003) Biological activities of *Salacia chinensis* originating in Thailand: the quality evaluation guided by α-glucosidase inhibitory activity (in Japanese). Yakugaku Zasshi 123:871–880
11. Morikawa T, Kishi A, Pongpiriyadacha Y, Matsuda H, Yoshikawa M (2003) Structures of new fridelane-type triterpenes and aldose reductase inhibitors from *Salacia chinensis*. J Nat Prod 66:1191–1196
12. Kishi A, Morikawa T, Matsuda H, Yoshikawa M (2003) Structures of new fridelane-type and norfriedelane-type triterpenes and polyacylated eudesmane-type sesquiterpene from *Salacia chinensis* Linn. (*S. prinoides* DC., Hippocrateaceae) and radical scavenging activities of principal constituents. Chem Pharm Bull 51:1051–1055
13. Matsuda H, Morikawa T, Yoshikawa M (2002) Antidiabetogenic constituents from several natural medicines. Pure Appl Chem 74:1301–1308
14. Yoshikawa M, Morikawa T, Murakami T, Toguchida I, Harima S, Matsuda H (1999) Medicinal flowers. I. Aldose reductase inhibitors and three new eudesmane-type sesquiterpenes, kikkanols A, B, and C, from the flowers of *Chrysanthemum indicum* L. Chem Pharm Bull 47:340–345
15. Yoshikawa M, Morikawa T, Toguchida I, Harima S, Matsuda H (2000) Medicinal flowers. II. Inhibitors of nitric oxide production and absolute stereostructures of five germacrane-type sesquiterpenes, kikkanols D, D monoacetate, E, F, and F monoacetate, from the flowers of *Chrysanthemum indicum* L. Chem Pharm Bull 48:651–656
16. Matsuda H, Morikawa T, Toguchida I, Harima S, Yoshikawa M (2002) Medicinal flowers. VI. Absolute stereostructures of two new flavanone glycosides and a phenylbutanoid glycoside from the flowers of *Chrysanthemum indicum* L.: their inhibitory activities for rat lens aldose reductase. Chem Pharm Bull 50:972–975

17. Matsuda H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. Chem Pharm Bull 50:788–795
18. Matsuda H, Morikawa T, Tao J, Ueda K, Yoshikawa M (2002) Bioactive constituents of Chinese natural medicines. VII. Inhibitors of degranulation in RBL-2H3 cells and absolute stereostructures of three new diarylheptanoid glycosides from the bark of *Myrica rubra*. Chem Pharm Bull 50:208–215
19. Tao J, Morikawa T, Toguchida I, Ando S, Matsuda H, Yoshikawa M (2002) Inhibitors of nitric oxide production from the bark of *Myrica rubra*: structures of new biphenyl type diarylheptanoid glycosides and taraxerane type triterpene. Bioorg Med Chem 10:4005–4012
20. Morikawa T, Tao J, Toguchida I, Matsuda H, Yoshikawa M (2003) Structures of new cyclic diarylheptanoids and inhibitors of nitric oxide production from Japanese folk medicine *Acer nikoense*. J Nat Prod 66:86–91
21. Morikawa T, Tao J, Ueda K, Matsuda H, Yoshikawa M (2003) Medicinal foodstuffs. XXXI. Structures of new aromatic constituents and inhibitors of degranulation in RBL-2H3 cells from a Japanese folk medicine, the stem bark of *Acer nikoense*. Chem Pharm Bull 51:62–67
22. Beecham AF (1972) CD of α,β-unsaturated lactones. Tetrahedron 28:5543–5554
23. Toubiana R, Toubiana MJ, Tori K, Kuriyama K (1974) Absolute configuration and conformation of confertolide, a germacranolide isolated from *Vernonia conferta*. Tetrahedron Lett 19:1753–1756
24. Ohtani I, Kusumi T, Kashman Y, Kakisawa H (1991) High-field FT NMR application of Mosher's method. The absolute configuration of marine terpenoids. J Am Chem Soc 113:4092–4096
25. Freudenberg MA, Galanos C (1991) Tumor necrosis factor alpha mediates lethal activity of killed gram-negative and gram-positive bacteria in D-galactosamine-treated mice. Infect Immun 59:2110–2115
26. Josephs MD, Bahjat FR, Fukuzuka K, Ksontini R, Solorzano CC, Edwards CK III, Tannahill CL, MacKay SL, Copeland EM III, Moldawer LL (2000) Lipopolysaccharide and D-galactosamine-induced hepatic injury is mediated by TNF-α and not by Fas ligand. Am J Physiol Regul Integr Comp Physiol 278:R1196–R1201
27. Chan MM (1995) Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem Pharmacol 49:1551–1556
28. Kilbourn RG, Griffith OW (1992) Overproduction of nitric oxide in cytokine-mediated and septic shock. J Natl Cancer Inst 84:827–831
29. Hagiwara S, Mitsui M, Karaki H (1993) Effects of felodipine, nifedipine and varapamil on cytosolic Ca^{2+} and contraction in vascular smooth muscle. Eur J Pharmacol 234:1–7
30. Matsuda H, Yoshikawa M, Morikawa T, Tanabe G, Muraoka O (2005) Antidiabetogenic constituents from *Salacia* species. J Trad Med 22(Suppl 1):145–153
31. Terashima H, Hama K, Yamamoto R, Tsuboshima M, Kikkawa R, Hatanake I, Shigeta Y (1984) Effects of a new aldose reductase inhibitor on various tissues in vitro. J Pharmacol Exp Ther 229:226–230
32. Yoshikawa M, Shimada H, Nishida N, Li Y, Toguchida I, Yamahara J, Matsuda H (1998) Antidiabetic principles of natural medicines. II. Aldose reductase and α-glucosidase inhibitors from Brazilian natural medicine, the leaves of *Myrcia multiflora* DC. (Myrtaceae): structures of myrciacitrins I and II and myrciaphenones A and B. Chem Pharm Bull 46:113–119
33. Matsuda H, Nishida N, Yoshikawa M (2002) Antidiabetic principles of natural medicindes. V. Aldose reductase inhibitors from *Myrcia multiflora* DC. (2): structures of myciacitrins III, IV, and V. Chem Pharm Bull 50:429–431
34. Salerno L, Sorrenti V, Di Giacomo C, Romeo G, Siracusa MA (2002) Progress in the development of selective nitric oxide synthase (NOS) inhibitors. Curr Pharm Des 8:177–200
35. Titheradge MA (1999) Nitric oxide in septic shock. Biochem Biophys Acta 1411:437–455
36. Nussler AK, Billiar TR (1993) Inflammation, immunoregulation, and inducible nitric oxide synthase. J Leukoc Biol 54:171–178
37. Natarajan K, Singh S, Burke TR Jr (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB. Proc Natl Acad Sci USA 93:9090–9095
38. Schwartz LB, Lewis RA, Seldin D, Austen KF (1981) Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. J Immunol 126:1290–1294
39. Marquardt DL, Wasserman SI (1983) Modulation of rat serosal mast cell biochemistry by in vivo dexamethasone administration. J Immunol 131:934–939