10. CHEMICAL STUDIES ON THE CONSTITUENTS OF *PERILLA FRUTESCENS*

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INTRODUCTION

The chemical constituents of Perilla concerningvolatile components and pigments have been investigated in detail, as also other compounds having some biological or pharmacological activities, since the plant has been used for various purposes. Its constituents, terpenoids, phenolics, flavonoids, cyanogenic glycosides, and anthocyanins have been reported. In recent years, we have focused on the glycosidic constituents of Perilla, and have isolated about twenty glycosides including nine new glucosides. Additionally, the inhibitory effect of perillosides A and C, and of related monoterpene glucosides on aldose reductase and their structure-activity relationships have also been elucidated.

In this review, we summarize the chemical constituents of Perilla and their biological activities, but the survey is restricted to secondary metabolites. The constituents classified as pigments such as flavonoids and anthocyanins are not discussed here, but will be covered in a later chapter.

CHEMICAL STRUCTURES OF CONSTITUENTS

Since the various beneficial properties ascribed to Perilla are associated with consumption of the leaves and seeds of the plant, their chemical constituents have been thoroughly studied. The constituents have been obtained by steam distillation and extraction with some solvent. The characterization of the volatile components was generally achieved by GC, GC-FT/IR, and GC-MS measurements without purification. The structure elucidation of the compounds, on the other hand, was performed by spectral and chemical evidence, after purification by fine distillation and swelling chromatographic methods such as CC, GC, TLC, and HPLC. Spectroscopical analysis (IR, UV, MS, and NMR, etc.) was used to elucidate the chemical structure of the purified compound. High resolution MS, MS/MS and NMR spectroscopic techniques provided significant information regarding the structural linkages. Accordingly, we present that the constituents of Perilla are separated into volatile and non-volatile components, the latter being further divided into chemical classes, e.g., terpenoids, phenolics, flavonoids, glycosides, and other constituents.

Table 1 Components of the essential oils from the Perilla species

Compounds	*Species**	*Plant organ*	*References*
(-)-perillaldehyde **(1)**, (-)-limonene **(2)** α-pinene, etc.	shiso and ao-iiso		Okuda (1967)
elsholtziaketone **(13)**, naginataketone **(14)**, perillaketone **(15)** (isoamyl-3-furylketone)	egoma		*Ibid.*
citral **(9)**, perillene	lemon-egoma		*Ibid.*
1 (about **50** %), 2, perillyl alcohol **(3)**, pinene, camphene, etc	ao-jiso (commercial oil)	aerial part	Masada (1975)
13, 14, linalool **(6)**, **1**-octen-3-ol, etc.	egoma	aerial part	Fujita *et al.* (1966)
β-caryophyllene **(7)**, elemicin **(10)**, myristicin **(11)**, dillapiole **(12)**, isoegomaketone **(16)**, etc.	several species	aerial part	Ito (1966, 1968)
α-farnesene **(8)**, allofarnesene	ao-jiso	aerial part	Sakai and Hirose (1969)
1 - 3, 6, *trans*-shisool **(4)**, *cis*-shisool **(5)**, etc.	shiso and ao-iiso	aerial part	Fujita *et al.* (1970a, b)
15, 16, etc.	shiso	leaf	Ina and Ogura (1970), Ina and Suzuki (1971)
1, 3, 7, 10, carvone, phenethyl alcohol, etc.	shiso, katamen-jiso	fruit	Kameoka and Nishikawa (1976)
perillaketone **(15)**	Tennessee	aerial part	Wilson *et al.* (1977)
rosefuran, **7, 15**, etc.	Bangladesh	aerial part	Misra and Husain (1987)
Chemotaxonomy **1 – 7, 9 – 16,** etc.	110 samples in Japan	aerial part	Ito (1970)
1, 2, 9 – 17, perillene, etc.	a number of species (genetic studies)	leaf, fruit, cotyledon, calyx	Koezuka *et al.* (1984, 1986a, b, c), Nishizawa *et al.* (1990a, *b*), Yuba *et al.* (1992), Honda (1994)

*shiso, ao-jiso, egoma, lemon-egoma, and katamen-jiso: called in Japanese.

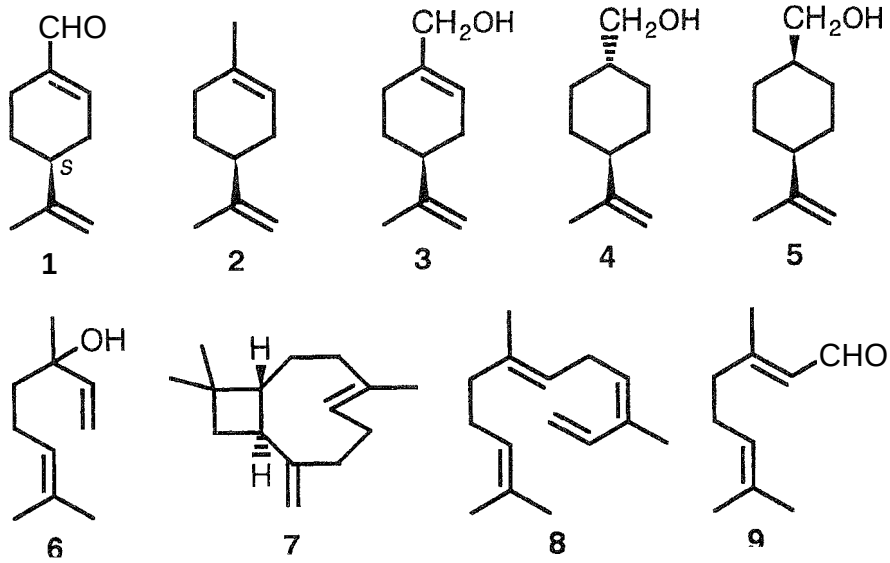

Figure 1 Mono- and sesquiterpenoids in Perilla essential oils

Volatile Components in Essential Oils

The volatile components in Perilla leaves have been obtained as an essential oil by steam distillation. There are many reports on the essential oils obtained from a number of *Perilla* varieties (Table 1). Their volatile compounds, mono- and sesquiterpenes, phenylpropanoids, and furylketones have already been reported, but all of them are not contained in any one subspecies or variety of Perilla. Some typical ones are shown in Figures 1 and 2. Attempts to classify them into the chemotypes of the essential oils have been undergone, as the volatile components in some varieties of Perilla might be characterized by their biosynthesis. It has recently become apparent that the chemotypes of the essential oils can be classified into six types on an individual level, based on the main volatile components (Honda, 1994). These investigations on the chemotypes of the essential oils and on the genetic controls of the volatile compounds will be described in the next chapter. In this section, the chemical structures of the compounds in essential oils are described, even if most of them reported in the past might be obtained from a mixture of different chemotype species.

Commercial Perilla oil (Perilla essential oil), which has a Perilla-like odor, is mainly obtained from green Perilla leaves, called "ao-jiso" in Japanese, by steam distillation, although there are several kinds of Perilla species being cultivated in Japan. Its volatile components are comprised of a rich mixture of mono- and sesquiterpenes. Typical monoterpenes are (-)-perillaldehyde (= (4*S*)-1,8-*p*-menthadien-7-al, (**1**)and (-)-limonene (= (4*S*)-1,8-*p*-menthadiene, (**2**), sesquiterpenes β-caryophyllene (7) and α-farnesene (8) (Masada, 1975). The major compound, perillaldehyde about 50 – 60 % of the essential

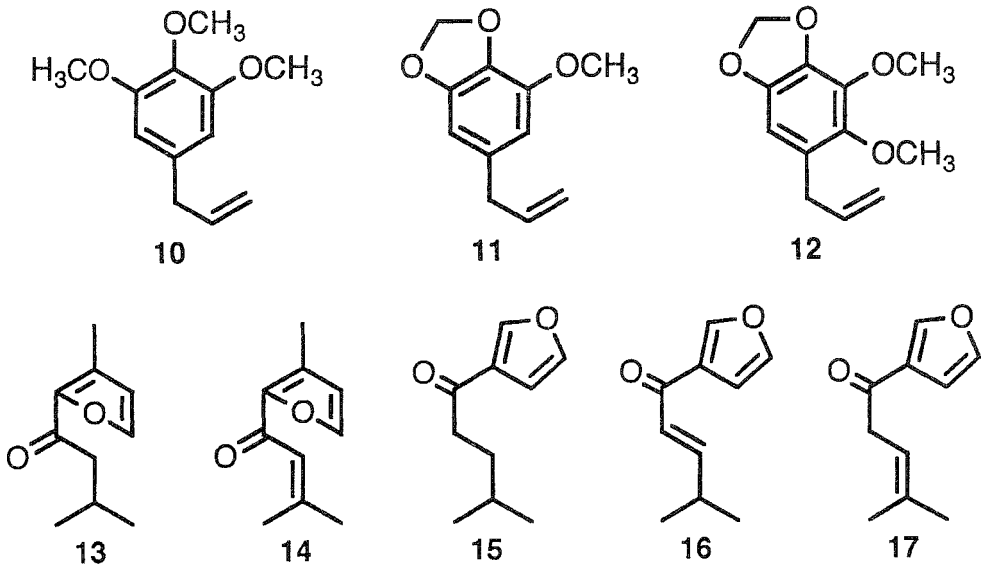

Figure 2 Phenylpropanoids and furylketones in Perilla essential oils

oil, has a powerful fatty-spicy, oily-herbaceous odor and a sweet-herbaceous taste (Arctander, 1969), and it is well-known that its anti-oxime is about two thousand times sweeter than sucrose. Other characteristic compounds having a Perilla-like odor, (-)-perillyl alcohol (**3**), *trans*-shisool (**4**), *cis*-shisool (**5**), and linalool (6) are present in Perilla leaves, which also contain α-pinene, β-pinene, camphene, 3-octanol, 1-octen-3-ol, allofarnesene, β-farnesene, etc., as minor components (Table 1). This type is classified as a perillaldehyde type.

Other types of essential oils, in contrast, have little or no Perilla-like odor. They have been classified into five types by Honda (1994). In a type containing phenylpropanoids, elemicin (**10**), myristicin (**11**), and dillapiole (**12**), are present as major compounds. In two other types containing furylketones, either elsholtziaketone (13) and naginataketone (**14**), or perillaketone (**15**), isoegomaketone (**16**), and egomaketone (**17**), are also present in the leaves of the plant. The last type being classified to perillene type, contains perillene together with 9 as a minor component (Nishizawa *et al.*, 1989). The other type having a citrus odor, which is classified as *P. frutescens* Britt. var. *citriodora* (Makino) Ohwi, contains citral (**9**) as a major component. These compounds in the Perilla leaves essential oils are also present in the fruits of the same plant (Kameoka and Nishikawa, 1976).

In addition, biosyntheses of mono- and sesquiterpenes have been investigated by using *Perilla* callus, but it is not discussed here (Suga *et al.*, 1986; Tamura *et al.*, 1989; Nabeta *eta!.*, 1983, 1984, 1993).

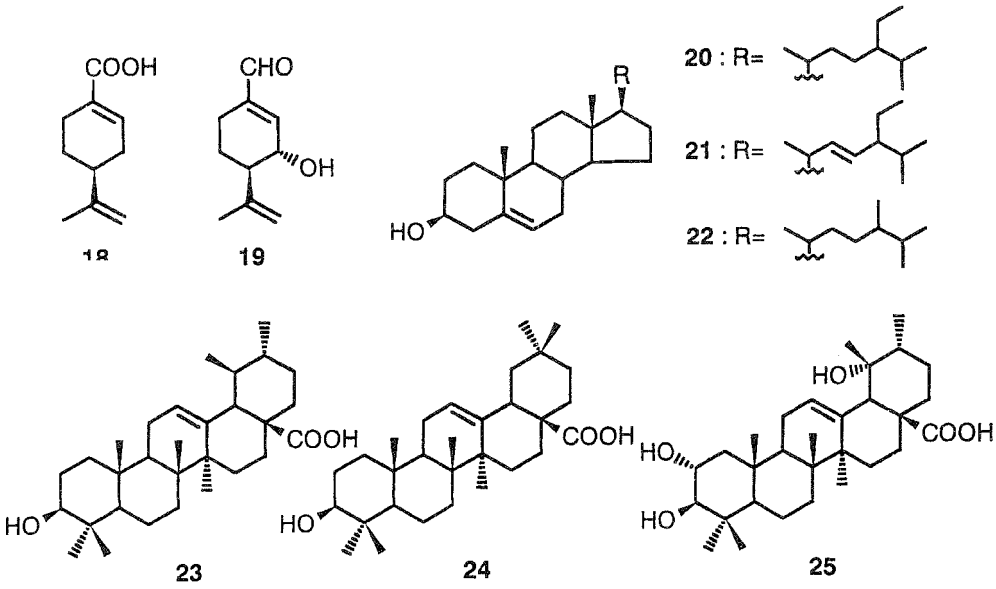

Figure 3 Son-volatile monoterpenes, sterols and triterpenes isolated from *P. frutescens.*

Non-volatile Compounds

The chemical structures of non-volatile compounds are described here. A number of triterpenoids, phenolics, flavonoids, and glycosides, which have various biological activities, have so far been reported. They might be commonly found in some varieties of Perilla in contrast with the volatile components in essential oil.

Terpenoids and Sterols

The structures of non-volatile terpenoids and sterols are shown in Figure 3. As a non-volatile monoterpene, perillic acid (18) is well-known as a autooxidation product of the compound **1** (Okuda, 1967). (3*S*,4*R*)-3-Hydroxy-4-(1-methylethenyl)-1-cyclohexene-1-carboxaldehyde (19) has been isolated from green Perilla by Matsumoto *et al.* (1995).

Besides the widespread sterols, β-sitosterol (**20**), stigmasterol (**21**), and campesterol (22) from both the leaves and seeds of Perilla (Honda *et al.*, 1986; Part *et al.*, 1982; Noda *et al.*, 1975), Perilla leaves also contain triterpenoids, ursolic acid (**23**), oleanolic acid (24) (Koshimizu, 1991), and tormentic acid (25). The latter acid *25* has been newly identified in purple Perilla leaves, called "aka-jiso" or "shiso" in Japanese, together with the acids 23 and 24 including dillapiole as a major volatile compound in our investigation (Fujita *et al.*, 1994). Studies on biosynthesis of triterpenoids have been carried out by using Perilla cell cultures (Tomita *et al.*, 1985, 1994). Higher terpenoids, five carotenoids, β-carotene, lutein, neoxanthin, antheraxanthin, and violaxanthin, have also been identified in green Perilla leaves by Takagi (1985).

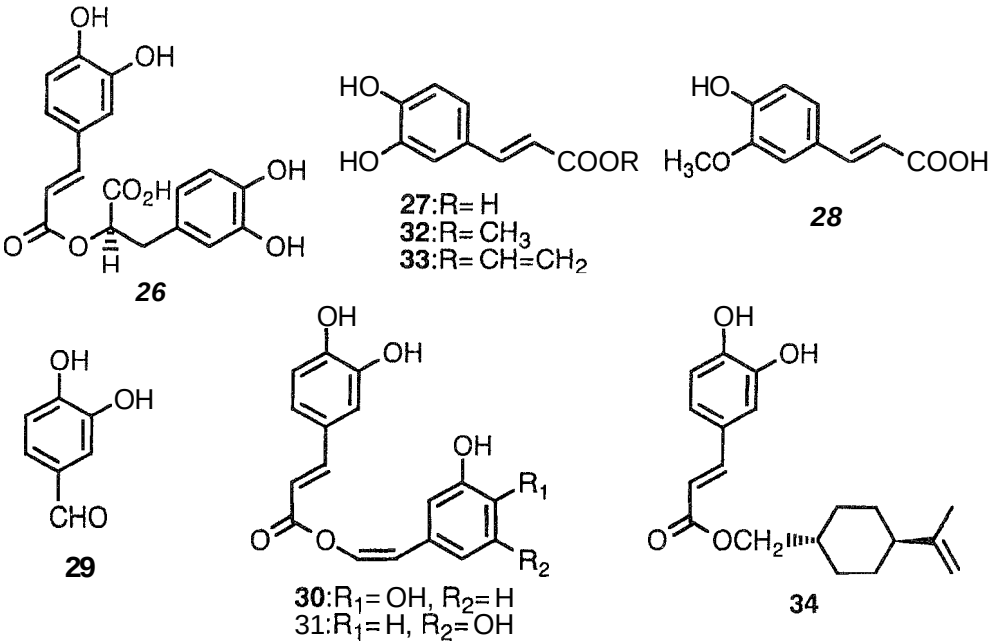

Figure 4 Phenolics and cinnamates from *P. frutescens*

Phenolics, Cinnamates and *Phenylpropanoids*

In common with other members of the Labiatae family, Perilla leaves contain a rich mixture of phenolics and cinnamates (Figure 4). Typical of these are cinnamic acid derivatives, rosmarinic acid (**26**), caffeic acid (**27**), and ferulic acid (28) (Okuda *et al.*, 1986; Aritomi, 1982). Five esters of caffeic acid, 2-(3,4-dihydroxyphenyl)ethenyl caffeate (**30**), 2-(3,5-dihydroxyphenyl)-ethenyl caffeate (31) (Nakanishi *et al.*, 1990), methyl caffeate (**32**), vinyl caffeate (**33**), and 8-*p*-menthen-7-yl caffeate (=*trans*-shisool-3-(3,4-dihydroxyphenyl)-2-propenoate, 34) (Matsumoto *et al.*, 1995), have also been isolated from *Perilla* leaves. As a phenolic, protocatechuic aldehyde (29) is present in Perilla leaves (Fujita et *al.*, 1994). In addition, α- and γ-tocopherol in the leaves of the plant have been identified as an antioxidative substance (Su *et al.*, 1986). Except for the compounds, **10**–12 introduced in section 1, small amounts of a phenylpropanoid, eugenol and other aromatics, benzaldehyde, benzylalcohol, and phenethyl alcohol, which have been mainly characterized by the essential oils described in Table 1, are also present.

Flavonoids and *Anthocyanins*

The characteristic compounds obtained from purple Perilla are introduced here (Figure 5), although there have been many reports of pigments such as flavonoids and anthocyanins from Perilla. Typical flavonoids are apigenin (**35**), luteolin (**36**), scutellarein (**37**), and their glycosides (Ishikura, 1981; Aritomi, 1982; Yoshida *et al.*, 1993), while

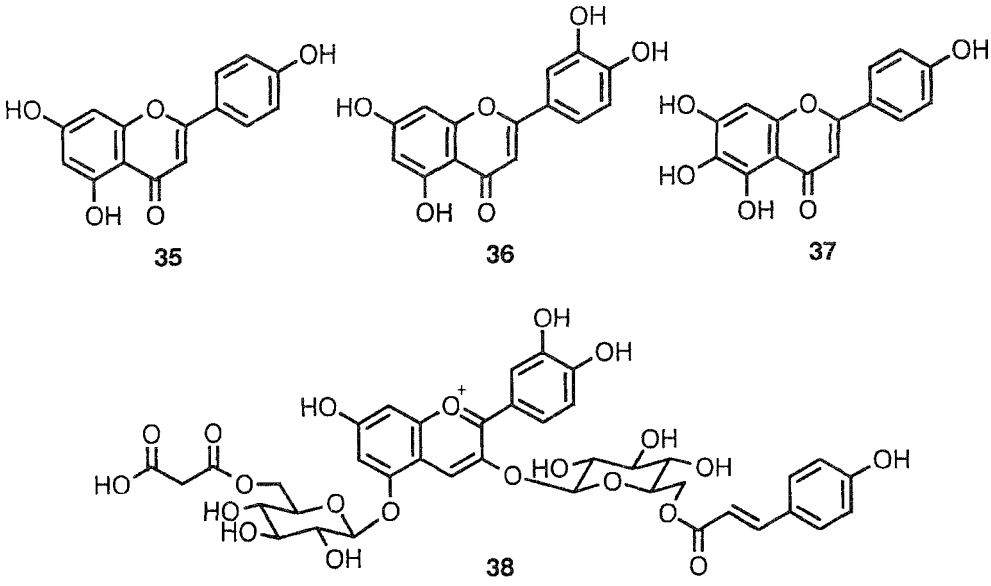

Figure 5 Typical flavonoids and anthocyanin from *P. frutescens.*

typical anthocyanins are acylated glucosides of cyanidin, malonylshisonin (38) and shisonin (Kondo *et al.*, 1989 ; Yoshida *et al.*, 1990). Other pigments will be described in the following chapter.

Genetic study on the anthocyanin production in Perilla leaf and stem has been reported by Koezuka *et al.* (1985a), and production of phenylpropanoids and anthocyanins by callus tissue has also been investigated by Tamura *et al.* (1989).

Glycosides

About twenty glycosides, except for pigments, including nine new glucosides, have been found from green and purple Perilla leaves in our investigation, and they are classified into terpenoids, phenylpropanoids, cyanogenics, jasmonoids, phenylvalericacid and other glycosides, respectively (Figures 6–8).

First, four monoterpene glucosides perillosides A-D (**39**–**42**), along with eugenyl β-D-glucopyranoside (**43**) and benzyl β-D-glucopyranoside (**44**), have been isolated from the methanolic extract of green Perilla leaves including perillaldehyde as a major component in the essential oil (Fujita and Nakayama, 1992, 1993). The structure of perilloside A was characterized as (4*S*)- (-)-perillyl β-D-glucopyranoside **(39)** by means of spectral and chemical methods. In the same manner, perillosides B, C, and D were determined to be β-D-glucopyranosyl (4*S*)-(-)-perillate (**40**), *trans*-shisool-β-D-glucopyranoside (**41**), *cis*-shisool-β-D-glucopyranoside (**42**), respectively. Two known

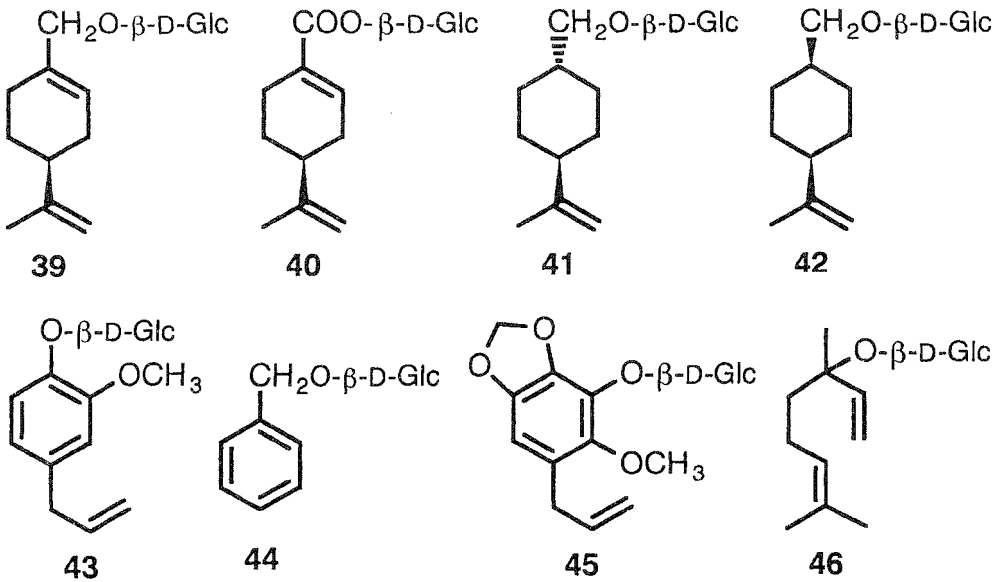

Figure 6 Monoterpenoids and phenylpropanoids glucosides from *P. frutescens*

glucosides, 43 and 44, have already been isolated from *Melissa officinalis* by Mulkens and Kapetanidis (1988) and from *Carica papaya* fruit by Schwab and Schreier (1988), respectively. The corresponding aglycones of these glucosides 39-44 were mentioned above as volatile components. β-Sitosteryl β-D-glucopyranoside was also obtained from the same extract of the green Perilla as another terpenoid glucoside.

Next, phenylpropanoid glucoside perilloside E (45) along with 43, 44, and linalyl β-D-glucopyranoside (46) are present in the methanolic extract of purple Perilla leaves including dillapiole as a major compound in the essential oil (Fujita *et al.*, 1994). The structure of perilloside E, which has a penta-substituted benzene ring consisting of allyl, methylenedioxy, methoxyl and β-D-glucopyranosyloxy moieties, was determined to be 6-methoxy-2,3-methylenedioxy-5-allylphenyl β-D-glucopyranoside (45) by means of long-range ^{13}C-^{1}H correlations of the NMR measurements. The corresponding aglycone of 45, 6-methoxy-2,3-methylenedioxy-5-allylphenol was also found for the first time in plants. Its methylation product is identical to dillapiole. These glucosides 39 46 seem to be closely associated with the volatile constituents in the essential oils, as each aglycone could afford related volatile compound. The result on the analysis of the glucosidic composition in the plant should support the genetic study on the volatile component formation of *Perilla* species.

Two cyanogenic glycosides, prunasin (= (*R*)-2-(β-D-glucopyranosyloxy) phenylacetonitrile, 47) and amygdalin isomer, (*R*)-2-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy) phenylacetonitrile, 48) have been reported by Aritomi *et al.* (1985, 1988). They were hydrolyzed with concentrated hydrochloric acid to (*R*)-mandelic acids.

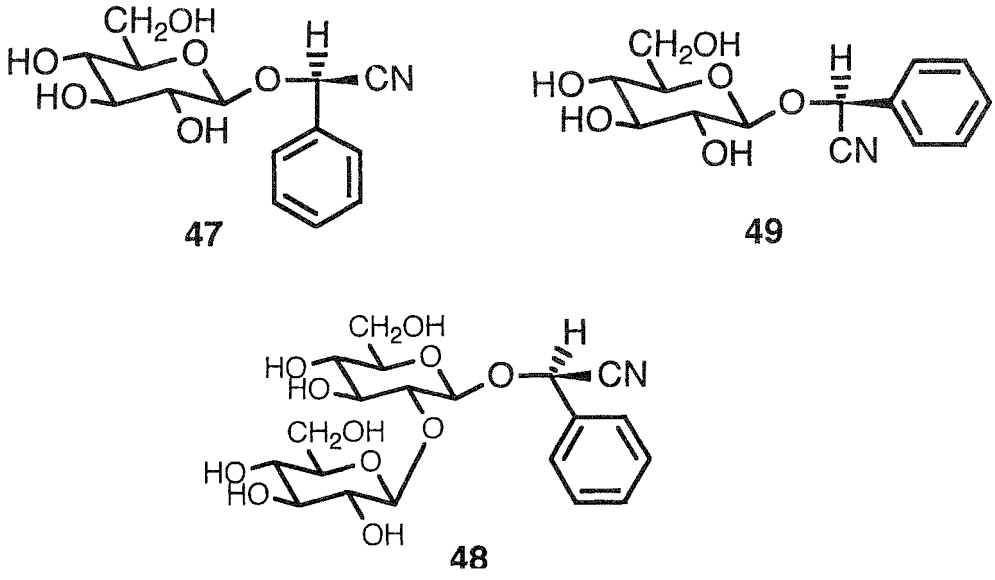

Figure 7 Cyanogenic Glycosides from *P. frutescens.*

Additionally, sambunigrin (= (*S*)-2-(β-**D**-glucopyranosyloxy) phenyl-acetonitrile, **49**), along with **47** and 48, is also present in both green and purple Perilla leaves (Fujita *et al.*, 1994). The chemical and physical properties of **47** and **49** are very similar to each other, since these are diastereoisomers of mandelonitrile glucopyranoside (Schwarzmaier, 1976). However, their 'H- and ^{13}C-NMR spectra are distinguishable by comparison of signals at the anomeric and nitrile methine protons and nitrile carbon as well as two diastereoisomers, dhurrin and taxiphyllin (Nahrstedt *eta!.,* 1993). Since racemization of cyanogenic glucosides have been known to occur under alkaline conditions (Seigler, 1975), more specific studies are required to determine whether only **47** or both conformers exist without racemization.

Recently, two jasmonoid glucosides (50 and **51**), a phenylvaleric acid glucoside (**52**), and decenoic acid glucoside (53) are also present in the butanol-soluble fraction obtained from methanolic extract of green Perilla leaves, along with phenolics 26 – 29 and their methyl esters, cyanogenic glucosides **47** – **49** and methyl α-**D**-galactopyranoside (Fujita *et al.*, 1996a, b). The structure of 50 was determined to be 5'-β-**D**-glucopyranosyloxyjasmonic acid, and its absolute configurations at C-1 and C-2 were both assigned to be *R*. The structure of 51 was characterized as 3-β-**D**-glucopyranosyl-3-epi-2-isocucurbic acid. Its absolute configuration at *C-3* position was judged to be R configuration by applying the glucosylation shifts in ^{13}C-NMR spectroscopy. In a similar manner, 52 was elucidated to be 3-β-**D**-glucopyranosyloxy-5-phenylvaleric acid, and its absolute configuration at C-3 position assigned to be R The structure of 53 was determined to be 5-β-**D**-glucopyranosyloxy-(*Z*)-7-decenoic acid.

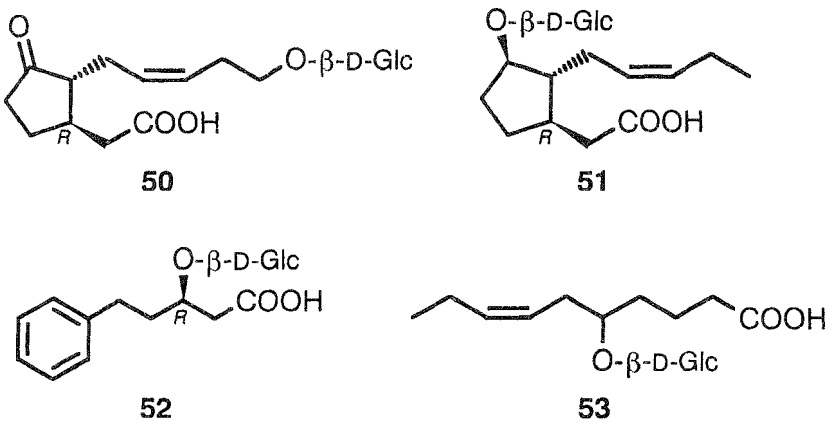

Figure 8 Jasmonoids, phenylvaleric acid, and other glucosides from *P. frutescens*.

Some other glycosides have been isolated from the same plant extract, and the determination of their structures and the absolute configuration at the C-5 position of **53** is now in progress.

Other Constituents

The composition of fatty acids in Perilla seed oil is so characteristic among various edible oils that it is comprised of highly unsaturated fatty acids, mainly α-linolenic acid which is about 50 %. The study of fatty acid distribution in Perilla seed lipids have been reported by Noda and Obata (1975). Recently, it has been shown that the Perilla seed oil (n-3 family), called "egoma oil" (in Japanese), has beneficial effects as compared to common *n*-6 family oils, and it enhances brain activity and nerve systems, and also suppressed the development of cancer, thrombosis and allergic reaction in the animal experiments (Hirano *et al.*, 1991). Palmitic, linoleic, and isooleic acids are also present as lipids or free fatty acids in the chloroform-methanol extract of green Perilla leaves (Nakatsu *et al.*, 1984).

BIOLOGICAL ACTIVITIES OF CONSTITUENTS OF PERILLA

A large number of studies on the biological and pharmacological activities of the constituents of Perilla have been made, but some of these were carried out on crude substances such as an essential oil or a crude extract of the plant. The main biological activities of Perilla components are summarized here.

Volatile Components in Essential Oils

Commercial Perilla oil is widely utilized as a flavoring as are also Perilla leaves and flower stalks, since it can increase the appetite. Therefore, it is not only used in many Japanese

processed foods but has also been reported to have some functional applications for masking fishy odor and as an antimicrobial agent and so forth. Using sensory test on the odor of sardines boiled with its major compound perillaldehyde (**1**), it has been shown that **1** contributed to masking the odor specific to boiled sardines (Kasahara and Nishibori, 1988). The antifungal activity of **1** has been investigated, and **1** showed growth inhibition against dermatophytic fungus, e.g., *Trichophyton violaceum* (Kurita and Koike, 1979). Its activity was synergistically increased by adding salt in an agar media (Kurita *et al.*,1981). Honda *et al.* (1984) have also reported that **1** and citral (9) were found to show a synergistic inhibitory effect on dermatophytic fungal growth. In addition, the compound **1** has unique biological activities such as a sedative and acaricidal. It has been reported that the oral administration of **1** (100 mg/kg) as well as that of an aqueous extract (4.0 g/kg body wt.) of Perilla leaves prolonged sleep induced by hexobarbital-Na in mice (Sugaya *et al.*, 1981). Sedative activity of the combined effect of **1** and stigmasterol (21) has further been discovered by Honda *et al.* (1986) and a significant prolongation of sleep was observed when **1** (2.5 mg/kg) and 21 (5.0 mg/kg) were combined. Insecticidal activity against a tick, namely, acaricide, of **1** has also been reported (Morimoto *et al.*, 1989).

Furthermore, the biological activities of the other components in the Perilla essential oils have been studied. For example, perillaketone (15) was isolated from a Perilla essential oil as an active principle of intestinal propulsion in mice (Koezuka *et al.*, 1985b), and 15 was found to be a potent and lung-selective pulmonary toxicant in mice (Wilson *et al.*, 1977; Garst and Wilson, 1984). Dillapiole (12) was isolated from a Perilla essential oil as an active principle for prolonging hexobarbital-induced sleep in mice (ED_{50}=1.57 mg/kg) (Honda *et al.*, 1988).

Recently, anti-tumor activity of (-)-perillyl alcohol (3) has been investigated. Stark *etal.* (1995) have reported the chemotherapeutic effects of **3** on pancreatic cancer. Gould *etal.* have described that 3 and (+)-limonene inhibited die growth of mammary tumors and induced apoptosis in rat liver tumors (Ren *et al.*, 1994; Shi *eta/.*, 1995; Mills *et al.*, 1995). They have also been shown to inhibit protein prenylation and cell proliferation (Gelb *et al.*, 1994; Crowell *et al.*, 1994).

Triterpenoids

Triterpenoids ursolic acid (23) and oleanolic acid (24) were isolated from Perilla leaves as anti-tumor promoting active substances (Koshimizu *et al.*, 1988, 1991). They were found to show an inhibitory effect on epstein-barr virus activation induced by a 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Ohigashi *et al.*, 1986). The treatment of 23 alone (41 nmol) or both 23 and 24 (41 nmol of each) when applied continuously before each TPA-treatment (4.1 nmol) remarkably delayed the formation of papillomas in mouse skin as compared with the control only with TPA (Tokuda *et al.*, 1986). Moreover, tormentic acid (**25**), which was obtained from other several plants (Takahashi *et al.*, 1974; Numata *et al.*, 1989), was found to show a hypoglycemic activity on rats (Villar *eta!.*, 1986). They investigated the effect of 25 on its activity by using normoglycemic, hyperglycemic, and streptozotocin diabetic rats as compared with glibenclamide, and then showed that these results suggested that 25 acts by increasing insulin secretion

from the islets of Langerhans (Ivorra *et al.*, 1988). Antimicrobial activity of 25, and also 23 and 24, against *Streptococcus mutans* has been reported by Isobe *et al.* (1989).

Phenolics and Cinnamates

Phenolic compounds are generally known to have an antioxidative activity. So in common with other members of the Labiatae family, the Perilla extract shows an antioxidative activity because it includes a rich mixture of phenolics, e.g., tocopherols, rosmarinic acid (26) and caffeic acid (27) in it (Su *etal.* 1986). Three caffeates, methyl caffeate (32), vinyl caffeate (33), and *p*-menth-8-en-7-yl caffeate (34) have also been isolated as antioxidative compounds (Matsumoto *et al.*, 1995). Furthermore, two caffeates, 2-(3,4-dihydroxyphenyl)ethenyl caffeate (30) and 2-(3,5-dihydroxyphenyl)ethenyl caffeate (31) have been isolated as inhibitors of xanthine oxidase (Nakanishi *et al.*, 1990). Recently, some phenolics 26, 27, and protocatechuic aldehyde (29) were found to show an inhibitory effect on tumor necrosis factor production (Kosuna *et al.*, 1995). In addition, 26 was isolated from Perilla leaves as a tannic active substance (Okuda *etal.*, 1986), while it was isolated as an active substance on an anti-histamine release activity (IC_{50}=18μM) against compound 48/80 from *Ehretia philippinensis* (Simpol *et al.*, 1994). The anti-inflammatory activity of 26 determined by the inhibition of malondialdehyde formation in human platelets (Gracza *etal.* 1985), and the effect of 26 as a skin conditioner (Fukushima *etal.*, 1988) have been reported.

Flavonoids and Anthocyanins

Flavonoids and anthocyanins are generally known to have an antioxidative activity as well as phenolics. Additionally, there are many reports on several enzymatic inhibitory effects associated with flavonoids obtained from a number of plants and foods. Anthocyanins obtained from purple Perilla are also important as natural colorants which are relatively stable among other natural colorants. The commercial production of anthocyanin pigments by using cell cultures induced from Perilla has been investigated (Koda *et al.*, 1992).

Glycosides

Monoterpene glucosides, perillosides A **(39)** and C (41) obtained from green Perilla leaves, were found to be inhibitors of aldose reductase, and the effect of related glucosides and their tetraacetates on aldose reductase has also been elucidated (Fujita *et al.*, 1995).

Aldose reductase (EC 1.1.1.21, abbr. AR) is a key enzyme of the polyol pathway, which catalyzes the reduction of hexoses to sugar alcohols (Gabbay, 1973). It is expected that AR inhibitors will play an important role in the management of diabetic complications such as cataract, retinopathy, neuropathy, and nephropathy (Kinoshita *et al.*, 1981; Sakamoto and Hotta 1983). We observed the inhibitory effects of glucosides derived from Perilla and related monoterpene glucosides (Figure 9), which were prepared by modified Koenigs-Knorr method (Paulsen *eta!.*, 1985; Schwab *et al.*, 1990), against rat lens AR (RLAR) and human recombinant AR (HRAR) in order to elucidate the relationship

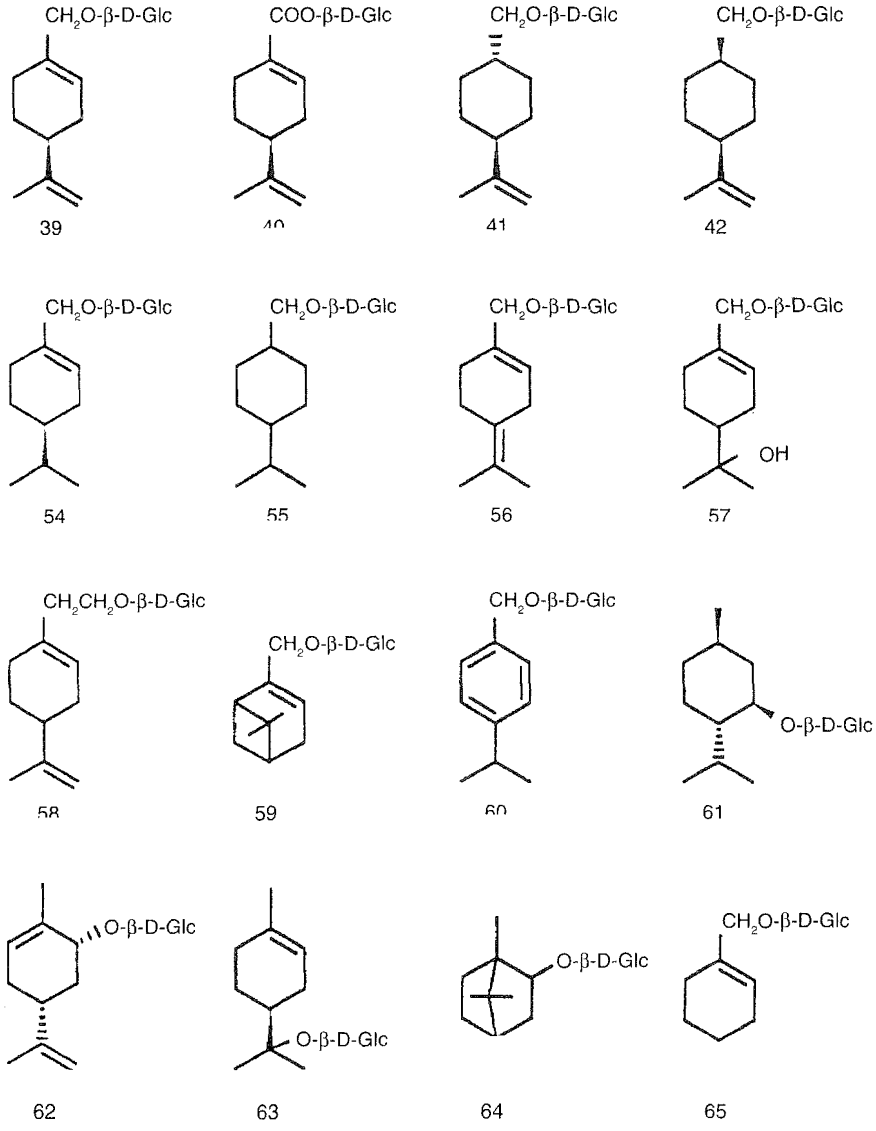

Figure *9* Structures of synthesized monoterpene glucosides

between the structure and inhibitory activity. AR assays using partially purified RLAR and purchased HRAR were conducted according to the method by Hayman *et al.* (1965).

The inhibitory effects of monoterpene glucosides including perillosides on RLAR are shown in Table 2. Perilloside A **(39)** and C (**41**), and their homologues (-)-phellandryl β-**D**-glucopyranoside (**54**) and 1, 4(8)-*p*-menthadien-7-yl β-**D**-glucopyranoside (**56**), and another type, (-)-menthyl β-D-glucopyranoside **(61)** were quite inhibitory to RLAR,

Table 2 Inhibitory effect of glucosides and their tetraacetates on RLAR

	Glc (%)		*Glc $(OAc)_4$ (%)*	
Glucoside	*0.1 mM*	*0.01 mM*	*0.1 mM*	*0.01 mM*
Perilloside A (39)	54.5	6.7	87.1	39.6
Perilloside B (40)	14.4	11.1	30.7	25.5
Perilloside C (41)	46.4	26.1	67.0	30.7
Perilloside D (42)	28.2	14.8	45.2	13.7
(-)-Phellandryl β-D-Glc (54)	43.6	15.7	65.4	10.5
p-Menthan-7-yl β-D-Glc (55)	26.8	13.9	62.3	40.7
1,4(8)-*p*-Menthadien-7-yl β-D-Glc (56)	41.9	32.9	42.3	9.1
1-*p*-Menthene-7,8-diol 7-β-D-Glc (57)	14.8	3.8	26.3	9.5
Homoperillyl β-D-Glc (58)	35.6	17.4	16.2	0
Myrtenyl β-D-Glc (59)	16.3	8.7	37.7	10.5
Cuminyl β-D-Glc (60)	9.5	5.5	-	-
(-)-Menthyl β-D-Glc (61)	38.7	11.6	-	4.2
(-)-*cis*-Carvyl β-D-Glc (62)	9.1	0	38.5	16.2
(-)-α-Terpinyl β-D-Glc (63)	14.2	0	20.8	17.5
Bornyl β-D-Glc (64)	10.4	8.7	33.5	23.8
1-Cyclohexenylmethyl β-D-Glc (65)	2.0	0	62.3	36.6
(x-Type glucoside				
(-)-Perillyl α-D-Glc (66)	20.1	9.1	37.6	7.1
(-)-Phellandryl α-D-Glc (67)	31.7	16.4	65.4	18.7

-: Not measured

where all of the corresponding aglycones, alcohols and acid were inactive in this assay. Their tetraacetates demonstrated an approximately one order higher activity than the corresponding glucosides. The α-D-type glucosides, (-)-perillyl α-D-glucopyranoside (66) and (-)- phellandryl α-D-glucopyranoside (67) and their tetraacetates had a lower inhibitory activity in each case. In the same manner, the inhibitory effects of the glucosides 39, 41, and 54, and their tetraacetates on HRAR were screened and then their inhibitory tendencies were similar to those of the case of RLAR, although the activities were somewhat weaker at the same concentration. These results may suggest as follows; (1) the *p*-menthane skeleton having a glucosyloxy moiety at the C-7 position, e.g., perilloside A (39) is essential for the appearance of inhibitory action; (2) double bonds in the *p*-menthane skeleton increased the activity; (3) equatorial substituents of 39, 41, and 54, when the compounds have a stable chair form, were better than axial ones as in 42 and 55; (4) acetylation of the glucosides resulted in a one order higher activity than the original glucosides; (5) the glucosidic (3-linkage with aglycone was preferred to the α-linkage, the former favoring a planar structure; (6) an ether-type glucoside was better than an ester-type one.

Kinetic studies were conducted on the compound 39 and tetraacetylperilloside A in order to determine the type of inhibition and inhibition constant (Ki). The Ki values of 39 for the substrates of glyceraldehyde and NADPH, were calculated at 1.4×10^{-4} M and 4.3×10^{-4} M from the Lineweaver-Burk plots, respectively. The apparent type of

enzyme inhibition by **39** was competitive with respect to glyceraldehyde, while non-competitive to NADPH. On the other hand, the Ki values of tetraacetylperilloside A for the substrates of glyceraldehyde and NADPH, were calculated at 2.5×10^{-5} M and 4.0×10^{-5} M, respectively. Its type of inhibition was non-competitive with the both glyceraldehyde and NADPH. In addition, kinetic study of **39** and its tetraacetate with HRAR was conducted, and their type of inhibition were same as those with RLAR. In this case, the Ki values of **39** and tetraacetylperilloside **A** exhibited 4.3×10^{-4} M and 1.1×10^{-4} M for glyceraldehyde, respectively.

This was the first report of monoterpene glucosides such as perillosides as AR inhibitors, although many flavonoids, carboxylic acids, and tannins have historically been examined as AR-inhibitory drugs (Okuda *et al.*, 1984; Kador *et al.*, 1983, 1985; Raskin and Rosenstock, 1987). The inhibitor binding should result from a combination of hydrophobic binding and a reversible charge-transfer reaction. In this experiment, the introduction of acetyl groups as lipophilic substituents into monoterpene glucosides increased the magnitude of the inhibition of AR. Furthermore, the (3-form of monoterpene glucosides showed a higher inhibition than the α-form. These results indicate that ARs have stereochemical requirements for binding, as well as lipophilic binding regions and a charge-transfer pocket. More specific studies are required to elucidate the stereochemical requirements for binding to ARs. However, the present results should contribute to the design of more effective AR inhibitors.

In recent years, we have become interested in biological activities such as the plant growth regulatory activity of jasmonoids (Yamane, 1994). The jasmonoid glucosides **50** and 51 obtained from the green Perilla were stereoisomers of β-D-glucopyranosyltuberonic acid (= 5'-(3-D-glucopyranosyloxy-2-epijasmonic acid) and (1R)-3-β-D-glucopyranosylcucurbic acid, respectively. The former have been isolated as a tuber-inducing substance from potato leaves (Yoshihara *et al.*, 1989), and the later have been isolated as a plant growth inhibitor from *Cucurbita pepo* L. (Fukui *et al.*, 1977). Although these jasmonoid glucosides have been a little reported, a number of jasmonoids have been isolated from plants and microorganisms as a plant growth regulator. The related glucosides **52** and **53** have also been obtained from Perilla leaves. Consequently, these glucosides **50–53** from Perilla are expected to play an important role in plant as well as other jasmonoids.

CONCLUSION

There are a large number of studies on the constituents of Perilla, since the plant has been used for various purposes, e.g., as a garnish, flavoring, and natural colorant. These results suggest that the constituents of Perilla have various beneficial properties compared with other edible plants. Perilla essential oil is not only widely utilized as a flavoring but also used in many processed foods to increase appetite. Therefore, many volatile compounds in Perilla oil have been investigated, and some of their components have been reported to show an antimicrobial, sedative, and anti-tumor activities. A number of non-volatile compounds, on the other hand, terpenoids, phenolics, flavonoids, and glycosides have been isolated from Perilla extract, and have also been described to have some biological activities, for example, an anti-tumor promoting, hypoglycemic,

antioxidative, anti-inflammatory, and aldose reductase inhibitory activities. Recently, the effect of the Perilla water extract on anti-allergic activity has been noted, as has its effectiveness against some allergic symptoms caused by pollen, foods, etc., although the active principle has not been fully identified. If the beneficial properties ascribed to Perilla are made clearer by biological and chemical evidence, the plant will arouse even greater interest.

REFERENCES

Arctander, S. (1969) *Perfume and Flavor Chemicals*, vols. I and II, Montclair, New Jersey, pp. 937.

Aritomi, M. (1982) Chemical studies on edible plant. I. Phenolic constituents of *Perilla frutescens*. *Kaseigaku Zasshi*, **33,** 353–359.

Aritomi, M., Kumori, T. and Kawasaki, T. (1985) Cyanogenic glycosides in leaves of *Perilla frutescens* var. *acuta*. *Phytochemistry*, **24,** 2438–2439.

Aritomi, M. (1988) Chemical studies on the constituents of edible plants. VI. Cyanogenecity and practical use of *Perilla frutescens* var. *acuta* as a food colorant. *Nippon Kasei Gakkaishi*, **39,** 817–822.

Crowell, EL., Ren, Z., Lin, S., Vedejs, E. and Gould, M.N. (1994) Structure-activity relationships among monoterpene inhibitors of protein isoprenylation and cell proliferation. *Biochem. Pharmacol.*, **47,** 1405–1415.

Fujita, T. and Nakayama, M. (1992) Perilloside A, a monoterpene glucoside from *Perilla frutescens*. *Phytochemistry*, **31,** 3265–3267.

Fujita, T. and Nakayama, M. (1993) Monoterpene glucosides and other constituents from *Perilla frutescens*. *Phytochemistry*, **34,** 1545–1548.

Fujita, T., Funayoshi, A. and Nakayama, M. (1994) A phenylpropanoid glucoside from *Perilla frutescens*. *Phytochemistry*, **37,** 543–546.

Fujita, T., Ohira, K., Miyatake, K., Nakano, Y. and Nakayama, M. (1995) Inhibitory effect of perillosides A and C, and related monoterpene glucosides on aldose reductase and their structure-activity relationships. *Chem. Pharm. Bull.*, **43,** 920–926.

Fujita, T., Terato, K. and Nakayama, M. (1996a) Two jasmonoid glucosides and a phenylvaleric acid glucoside from *Perilla frutescens*. *Biosci. Biotech. Biochem.*, **60,** 732–735.

Fujita, T., Terato, K. and Nakayama, M. (1996b) Isolation and structure elucidation of the glucosidic constituents from *Perilla frutescens*. *Koen Yoshisyu – Nippon Nogeikagaku Kaishi*, **70,** pp. 238 (in Japanese).

Fujita, Y., Mizohata, H. and Iwamura, J. (1966) Essential oil of *Perilla frutescens*. IX. *Nippon Kagaku Kaishi*, **87,** 1361–1363 (in Japanese).

Fujita, Y., Fujita, S. and Hayama, Y. (1970a) *trans*-Shisool (*trans*-8-*p*-menthen-7-ol) isolated from the essential oils of *Perilla acuta* Nakai and *P. acuta* f. *viridis* Nakai. *Bull. Chem. Soc. Jpn.*, **43,** 2637–2638.

Fujita, Y., Fujita, S. and Hayama, Y. (1970b) Miscellaneous contributions to the essential oils of the plants from various territories. XXIV. Essential oils of *Perilla acuta* (Thunb.) Nakai and *P. acuta* f. *viridis* (Makino) Nakai. *Nippon Nogeikagaku Kaishi*, **44,** 428-432 (in Japanese).

Fukui, H., Koshimizu, K., Yamazaki, Y, and Usuda, S. (1977) Structures of plant growth inhibitors in seeds of *Cucurbita pepo* L. *Agric. Biol. Chew.*, **41,** 189–194.

Fukushima, M., Yagisawa, T., Kinoshita, K. and Sano, H. (1988) Cosmetics containing rosmarinic acid and/or its salts as skin conditioners. Jpn. Kokai Tokkyo Koho JP63-162611 (in Japanese) (*Chem. Abstr.*, 110: P218817h).

Gabbay, K.H. (1973) The sorbitol pathway and the complications of diabetes. *N. Engl. J. Med.*, **288**, 831–836.

Garst, J.E. and Wilson, B.J. (1984) Synthesis and analysis of various 3-fury1 ketones from *Perilla frutescens*. *J. Agric. Food Chem.*, **32,** 1083–1087.

Gelb, M.H., Tamanoi, F., Yokoyama, K., Ghomashchi, F., Esson, K. and Gould, M.N. (1995) The inhibition of protein prenyltransferases by oxygenated metabolites of limonene and perillyl alcohol. *Cancer Lett.*, **91,** 169–175.

Gracza, L., Koch, H. and Loeffler, E. (1985) Biochemical-pharmacological investigations of medicinal agents of plant origin. I. Isolation of rosmarinic acid from *Symphytum officinale* L. and its anti-inflammatory activity in an *in vitro* model. *Arch, Pharm.* (Weinheim), **318,** 1090–1095.

Hayman, S. and Kinoshita, J.H. (1965) Isolation and properties of lens aldose reductase. **J.** *Biol. Chew.*, **240,** 877–882.

Hirano, J., Isoda, Y. and Nishizawa, Y. (1991) Utilization of ^- plant oils perilla and flaxseed oils. *Yukagaku*, **40,** 942–950 (in Japanese).

Honda, G., Koga, K., Koezuka, Y. and Tabata, M. (1984) Antidermatophytic compounds of *Perilla frutescens* Britton var. *crispa* Decne. *Shoyakugaku Zasshi*, 38, 127–130 (in Japanese).

Honda, G., Koezuka, Y., Kamisako, W. and Tabata, M. (1986) Isolation of sedative principles from *Perilla frutescens*. *Chem. Pharm. Bull.*, **34,** 1672–1677.

Honda, G., Koezuka, Y. and Tabata, M. (1988) Isolation of dillapiole from a chemotype *Perilla frutescens* as an active principle for prolonging hexobarbital-induced sleep. *Chem. Pharm. Bull.*, **36**, 3153–3155.

Honda, G. (1994) Genetics of essential oil components of *Perilla frutescens*. *Farumashia*, **30,** 486–490 (in Japanese).

Ina, K. and Ogura, I. (1970) Studies on the components of perilla essential oil. I. Neutral essential oil. *Nippon Nogeikagaku Kaishi*, **44,** 209–212 (in Japanese).

Ina, K. and Suzuki, I. (1971) Studies on the components of perilla essential oil. II. Furan derivatives in neutral essential oil. *Nippon Nogeikagaku Kaishi*, **45,** 113–117 (in Japanese).

Ishikura, N. (1981) Anthocyanins and flavones in leaves and seeds of *Perilla* plant. *Agric. Biol. Chem.*, **45,** 1855–1860.

Isobe, T., Noda, Y., Ohsaki, A., Sakanaka, S., Kim, M. and Taniguchi, M. (1989) Studies on the constituents of *Leucoseptrum stellipillum*. *Yakugaku Zasshi*, **109,** 175–178 (in Japanese).

Ito, H. (1966) Studies on the Folium Perillae. IV. *Perilla* spp. containing myristicin and dillapiole as the main components of the essential oils. *Shoyakugaku Zasshi*, **20,** 73–75 (in Japanese).

Ito, PI. (1968) Studies on the Folium Perillae. V. *Perilla* spp. containing elemicin as the main components of the essential oils. *Shoyakugaku Zasshi*, **22,** 151–152 (in Japanese).

Ito, H. (1970) Studies on Folium Perillae. VI. Constituent of essential oils and evaluation of genus *Perilla*. *Yakugaku Zasshi*, **90,** 883–892 (in Japanese).

Ivorra, M.D., Paya, M. and Villar, A. (1988) Hypoglycemic and insulin release effects of tormentic acid: A new hypoglycemic natural product. *Planta Medico*, **1988**, 282–286.

Kador, P.F. and Sharpless, N.E. (1983) Pharmacophor requirements of the aldose reductase inhibitor site. *Mol. Pharmacol.*, **24,** 521–531.

Kador, P.F., Itinoshita, J.H. and Sharpless, N.E. (1985) Aldose reductase inhibitors: A potential new class of agents for the pharmacological control of certain diabetic complications./. *Med. Chew.*, 28, 841–849.

Kameoka, H. and Nishikawa, K. (1976) The composition of the essential oil from *Perilla frutescens* L. Brit. var. *acuta* Thunb. Kudo and *Perilla frutescens* L. Brit. var. *acuta* Thunb. Kudo f. *discolor* Makino. *Nippon Nogeikagaku Kaishi*, **50,** 345-349 (in Japanese).

Kasahara, K. and Nishibori, K. (1988) Suppressing effect of perilla for odor of sardine. *Nippon Suisan Gakkaishi*, **54,** 315–317 (in Japanese).

Kinoshita, J.H., Kador, P. and Catiles, M. (1981) Aldose reductase in diabetic cataracts. *J. Am. Med. Assoc.*, 246, 257–261.

Koda, T., Ichi, T., Yoshimitu, M., Nihongi, Y. and Sekiya, J. (1992) Production of perilla pigment in cell cultures of *Perilla frutescens*. *Nippon Shokuhin Kogyo Gakkaishi*, 39, 839–844 (in Japanese).

Koezuka, Y., Honda, G. and Tabata, M. (1984) Essential oil types of the local varieties and their F_1 hybrids of *Perilla frutescens*. *Shoyakugaku Zasshi*, 38, 238–242 (in Japanese).

Koezuka, Y., Honda, G., Sakamoto, S. and Tabata, M. (1985a) Genetic control of anthocyanin production in *Perilla frutescens*. *Shoyakugaku Zasshi*, 39,228–231.

Koezuka, Y., Honda, G. and Tabata, M. (1985b) An intestinal propulsion promoting substance from *Perilla frutescens* and its mechanism of action. *Planta Medica*, 1985, 480–482.

Koezuka, Y., Honda, G. and Tabata, M. (1986a) Genetic control of the chemical composition of volatile oils in *Perilla frutescens*. *Phytochemistry*, 25, 859–863.

Koezuka, Y., Honda, G. and Tabata, M. (1986b) Genetic control of phenylpropanoids in *Perilla frutescens*. *Phytochemistry*, 25, 2085–2087.

Koezuka, Y., Honda, G. and Tabata, M. (1986c) Genetic control of isoegomaketone formation in *Perilla frutescens*. *Phytochemistry*, 25, 2656–2657.

Kondo, T., Tamura, H., Yoshida, K. and Goto, T. (1989) Structure of malonylshisonin, a genuine pigment in purple leaves of *Perilla ocimoides* L. var. *crispa* Benth. *Agric. Biol. Chem.*, 53,797–800.

Koshimizu, K., Ohigashi, H., Tokuda, H., Kondo, A. and Yamaguchi, K. (1988) Screening of edible plants against possible anti-tumor promoting activity. *Cancer Letters*, 39, 247–257.

Koshimizu, K. (1991) Anti-tumor promoting effect of the constituents in foods. *Kagaku to Seibutsu*, 29, 598–603 (in Japanese).

Kosuna, K., Shirai, J. and Kosaka, H. (1995) Anti-inflammatory effect of the constituents from perilla extract. *Fragrance J.*, 1995, 90–94 (in Japanese).

Kurita, N., Miyaji, M., Kurane, R., Takahara, Y. and Ichimura, K. (1979) Anti-fungal activity and molecular orbital energies of aldehyde compounds from oils of higher plants. *Agric. Biol. Chem.*, 43, 2365–2371.

Kurita, N. and Koike, S. (1981) Synergistic antimicrobial effect of perilla and NaCl. *Nippon Nogeikagaku Kaishi*, 55, 43–46 (in Japanese).

Masada, Y. (1975) *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, Hirokawa Publishing Co., Tokyo, pp. 27–30.

Matsumoto, R., Yamaguchi, H., Chiba, K. and Tada, M. (1995) Antioxidative compounds and novel monoterpenoid from *Perilla frutescens*. *Koen Yoshisyu – Koryo, Terupen oyobi Seiyu Kagaku ni Kansuru Toronkai*, 39th, pp. 199–201 (in Japanese).

Mills, J.J., Chari, R.S., Boyer, I.J., Gould, M.N. and Jirtle, R.L. (1995) Induction of apoptosis in liver tumors by the monoterpene perillyl alcohol. *Cancer Res.*, 55, 979–983.

Misra, L. N. and Husain, A. (1987) The essential oil of *Perilla ocimoides*: A rich source of rosefuran. *Planta Medica*, 53, 379–380.

Morimoto, Y., Takaoka, K. and Watanabe, T. (1989) Acaricidal effect of essential oils. Jpn. Kokai Tokkyo Koho JP01-19004 (in Japanese).

Mulkens, A. and Kapetanidis, I. (1988) Eugenylglucoside, a new natural phenylpropanoid heteroside from *Melissa officinalis*. *J. Nat. Prod.*, 51, 496–498.

Nabeta, K., Ohnishi, Y., Hirose, T. and Sugisawa, H. (1983) Monoterpene biosynthesis by callus tissues and suspension cells from *Perilla* species. *Phytochemistry*, 22, 423–425.

Nabeta, K., Oda, T., Fujimura, T. and Sugisawa, H. (1984) Biosynthesis of cuparene from mevalonic acid-6,6,6-$^2H_3$ by *in vitro* callus culture of *Perilla* sp. *Agric. Biol. Chew.*, *48*, 3141–3143.

Nabeta, K., Kawakita, K., Yada, Y. and Okuyama, H. (1993) Biosynthesis of sesquiterpenes from deuterated mevalonates in *Perilla* callus. *Biosci. Biotech. Biochem.*, 57, 792–798.

Nahrstedt, A., Lechtenberg, M., Brinker, A., Seigler, D.S. and Hegnauer, R. (1993) 4-Hydroxymandelonitrile glucosides, dhurrin in *Suckleya suckleyana* and taxiphyllin in *Girgensohnia oppositiflora* (Chenopodiaceae). *Phytochemistry*, 33, 847–850.

Nakanishi, T., Nishi, M., Inada, A., Obata, H., Tanabe, N., Abe, S. and Wakashiro, M. (1990) Two new potent inhibitors of xanthine oxidase from leaves of *Perilla frutescens* Britton var. *acuta* Kudo. *Chem. Pharm. Bull.*, 38, 1772–1774.

Nakatsu, S., Tomita, K., Nakatsuru, I. and Matsuda, K. (1984) On the lipids in vegetables. I. Fatty acid composition of lipids from vegetables. Bull. Fac. Agric. Miyazaki Univ., 31, 21–32 (in Japanese).

Nishizawa, A., Honda, G. and Tabata, M. (1989) Determination of final steps in biosyntheses of essential oil components in *Perilla frutescens*. *Planta Medica*, 55, 251–253.

Nishizawa, A., Honda, G. and Tabata, M. (1990a) Characteristic incorporation of fatty acids into lower terpenoids in cotyledons of *Perilla frutescens*. *Chew. Pharm. Bull.*, 38, 1317–1319.

Nishizawa, A., Honda, G. and Tabata, M. (1990b) Genetic control of perillene accumulation in *Perilla frutescens*. *Phytochemistry*, **29**, 2873–2875.

Noda, M. and Obata, T. (1975) Fatty acid distribution in perilla seed lipids. *Nippon Nogeikagaku Kaishi*, 49, 251–256 (in Japanese).

Numata, A., Yang, P., Takahashi, C., Fujiki, R., Nabae, M. and Fujita, E. (1989) Cytotoxic triterpenes from a Chinese medicine, Goreishi. *Chew. Pharm. Bull.*, 37, 648–651.

Ohigashi, H., Takamura, H., Koshimizu, K., Tokuda, H. and Ito, Y. (1986) Search for possible antitumor promoters by inhibition of 12-0-tetradecanoylpl~orbol-13-acetate-induced Epstein-barr virus activation; Ursolic acid and oleanolic acid from an anti-inflammatory chinese medicinal plant, *Glechoma hederaceae* L. *Cancer letters*, 30, 143–151.

Okuda, J., Miwa, I., Inagaki, K., Horie, T. and Nakayama, M. (1984) Inhibition of aldose reductase by 3',4'-dihydroxyflavones. *Chew. Pharm. Bull.*, **32**, 767–772.

Okuda, O. (1967) *Koryo Kagaku Souran*, Hirokawa Publishing Co., Tokyo, pp. 342-344 (in Japanese).

Okuda, T., Hatano, T., Agata, 1. and Nishibe, S. (1986) The components of tannic activities in Labiatae plants. I. Rosmarinic acid from Labiatae plants in Japan. *Yakugaku Zasshi*, 106, 1108–1111 (in Japanese).

Part, H.S., Kim, J. G. and Cho, M.J. (1982) Chemical compositions of *Perilla frutescens* Britton var. *crispa* Decaisne cultivated in different area of Korea. II. Sterol compositions. *Hanguk Nonghwa Hakhoe* Chi, 25, 14–20 (*Chem. Abstr.*, **97**:78761w) (in Korean).

Paulsen, H., Le-Nguyen, B., Sinnwell, V., Heemann, V. and Seehofer, F. (1985) Synthese von glycosiden von mono-, sesqui- und diterpenalkoholen. *Liebigs Ann. Chew.*, 1985, 1513–1536.

Raskin, P. and Rosenstock, J. (1987) Aldose reductase inhibitors and diabetic complications. *Am. J. Med.*, 83, 298–306.

Ren, Z. and Gould, M.N. (1994) Inhibition of ubiquinone and cholesterol synthesis by the monoterpene perillyl alcohol. *Cancer Lett.*, 76, 185–190.

Sakai, T. and Hirose, Y. (1969) Farnesenes isolated from the volatile oil of *Perilla frutescens* f. *viridis* Makino. *Bull. Chem. Soc. Jpn.*, 42, 3615.

Sakamoto, N. and Hotta, N. (1983) Inhibitors of aldose reductase and their clinical applications. *Farumashia*, 19, 43–47 (in Japanese).

Schwab, W. and Schreier, P. (1988) Aryl β-D-glucosides from *Carica papaya* fruit. *Phytochemistry*, 27, 1813–1816.

Schwab, W., Scheller, G. and Schreier, P. (1990) Glycosidically bound aroma components from sour cherry. *Phytochemistry*, 29,607–612.

Schwarzmaier, U. (1976) Notiz zur Konfigurationsbestimmung bei freien mandelsaurenitril-glycosiden. *Chew. Ber.*, 109, 3250–3251.

Seigler, D.S. (1975) Isolation and characterization of naturally occurring cyanogenic compounds. *Phytochemistry*, 14, 9–29.

Shi, W. and Gould, M.N. (1995) Induction of differentiation in neuro-2A cells by the monoterpene perillyl alcohol. *Cancer Lett.*, 95, 1–6.

Simpol, L.R., Otsuka, H., Ohtani, K., Kasai, R. and Yamasaki, K. (1994) Nitrile glucosides and rosmarinic acid, the histamine inhibitor from *Ehretia philippinensis*. *Phytochemistry*, 36, 91–95.

Stark, M.J., Burke, Y.D., McKinzie, J.H., Ayoubi, A.S. and Crowell, P.L. (1995) Chemotherapy of pancreatic cancer with the monoterpene perillyl alcohol. *Cancer Lett.*, 96, 15–21.

Su, J.-D., Osawa, T. and Namiki, M. (1986) Screening for antioxidative activity of crude drugs. *Agric. Biol. Chew.*, **50**, 199–203.

Suga, T., Hirata, T., Aoki, T. and Shishibori, T. (1986) Interconversion and cyclization of acyclic allylic pyrophosphates in the biosynthesis of cyclic monoterpenoids in higher plants. *Phytochemistry*, **25**, 2769–2775.

Sugaya, A., Tsuda, T. and Obuchi, T. (1981) Pharmacological studies of Perillae Herba. I. Neuropharmacological action of water extract and perillaldehyde. *Yakugaku Zasshi*, **101**, 642–648.

Takagi, S. (1985) Determination of green leaf carotenoids by HPLC. *Agric. Biol. Chem.*, **49**, 1211–1213.

Takahashi, K., Kawaguchi, S., Nishimura, K., Kubota, K., Tanabe, Y. and Takani, M. (1974) Studies on constituents of medicinal plants. XIII. Constituents of the pericarps of the capsules of *Euscaphis japonica* Pax. (I). *Chem. Pharm. Bull.*, *22*, 650–653.

Tamura, H., Fujiwara, M. and Sugisawa, H. (1989) Production of phenylpropanoids from cultured callus tissue of the leaves of akachirimen-shiso (*Perilla* sp.). *Agric. Biol. Chem.*, 53, 1971–1973.

Tokuda, H., Ohigashi, H., Koshimizu, K. and Ito, Y. (1986) Inhibitory effects of ursolic and oleanolic acid on skin tumor promotion by 12-0-tetradecanoylphorbol-13-acetate. *Cancer Letters*, **33**, 279–285.

Tomita, Y., Arata, M. and Ikeshiro, Y. (1985) Biosynthesis of ursolic acid in cell cultures of *Perilla frutescens* Britt. var. *acuta* Kudo: Mechanism of D- and E-ring formation. *J. Chew, Soc., Chem. Commun.*, 1985, 1087–1088.

Tomita, Y. and Ikeshiro, Y. (1994) Biosynthesis of ursolic acid in cell cultures of *Perilla frutescens*. *Phytochemistry*, **35**, 121–123.

Villar, A., Paya, M., Hortiguela, M.D. and Cortes, D. (1986) Tormentic acid, a new hypoglycemic agent from *Poterium ancistroides*. *Planta Medica*, 1986, 43–45.

Wilson, B.J., Garst, J.E., Linnabary, R.D. and Channell, R.B. (1977) Perilla Ketone: A potent lung toxin from the mint plant, *Perilla frutescens* Britton. *Science*, 197, 573–574.

Yamane, H. (1994) *Plant Hormone Handbook*, Takahashi, N. and Masuda, Y., Eds., Baifukan, Tokyo, pp. 279–292 (in Japanese).

Yoshida, K., Kondo, T., Kameda, K. and Goto, T. (1990) Structure of anthocyanins isolated from purple leaves of *Perilla ocimoides* L. var. *crispa* Bent11 and their isomerization by irradiation of light. *Agric. Biol. Chew.*, **54**, 1745–1751.

Yoshida, K., Kameda, K. and Kondo, T. (1993) Diglucuronoflavones from purple leaves of *Perilla ocimoides*. *Phytochemistry*, 33, 917–919.

Yoshihara, T., Omer, E.A., Koshino, H., Sakamura, S., Kikuta, Y. and Koda, Y. (1989) Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). *Agric. Biol. Chem.*, 53, 2835–2837.

Yuba, A., Honda, G., Mizukoshi, T. and Tabata, M. (1992) Organ-specific expression of a genetic factor inducing monoterpene synthesis in the calyx of *Perilla frutescens*. *Shoyakugaku Zasshi*, 46, 257–260.