Medicinal Foodstuffs. XXV.[1] Hepatoprotective Principle and Structures of Ionone Glucoside, Phenethyl Glycoside, and Flavonol Oligoglycosides from Young Seedpods of Garden Peas, *Pisum sativum* L.

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A new ionone glucoside, pisumionoside, a phenethyl glycoside, sayaendoside, and two acylated flavonol oligoglycosides, pisumflavonosides I and II, were isolated from the young seedpods of garden peas, *Pisum sativum* L., together with quercetin and kaempferol 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosides and quercetin and kaempferol 3-sophorotriosides. The structures of pisumionoside, sayaendoside, and pisumflavonosides I and II were determined on the basis of chemical and physicochemical evidence, respectively. Quercetin 3-sophorotrioside, a principle component, was found to show protective effects on liver injury induced by D-galactosamine and lipopolysaccharide and by carbon tetrachloride in mice.

Key words hepatoprotective principle; pisumionoside; sayaendoside; pisumflavonoside; *Pisum sativum*; garden pea

As a part of our characterization studies on the chemical constituents of medicinal foodstuffs,[1,2] we have characterized bioactive steroid and triterpenoid saponins and diterpene oligoglycosides from Leguminous edible beans [*e.g.*, fenugreek (*Trigonella foenum-graecum*),[3] kidney bean (*Phaseolus vulgaris*),[4] hyacinth bean (*Dolichos lablab*),[5] and sword bean (*Canavalia gladiata*)[6]]. We previously reported the isolation and structure elucidation of two oleanane-type triterpene oligoglycosides, pisumsaponins I and II, and two kaurane-type diterpene oligoglycosides, pisumosides A and B, from green peas, the immature seeds of *Pisum* (*P.*) *sativum* L. (garden peas), together with several bioactive saponins: soyasaponin I, bersimoside I, and dehydrosoyasaponin I.[7] This plant has been widely cultivated from ancient times and consumed as a vegetable. The agricultural species of *P. sativum* are known to be classified into two groups differing in the edible part as immature seed or young seedpod. In a continuing study on *P. sativum*, we have isolated an ionone glucoside, pisumionoside (**1**), a phenethyl glycoside, sayaendoside (**2**), and two flavonol oligoglycosides, pisumflavonosides I (**3**) and II (**4**), from the young seedpods of *P. sativum*. Furthermore, we examined protective effects of the flavonol glycoside constituents on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes. The *in vitro* active component was found to show protective effects on liver injury induced by D-galactosamine and lipopolysaccharide and by carbon tetrachloride in mice. This paper deals with the isolation and structure elucidation of these glycosides as well as the hepatoprotective effect of flavonol oligoglycosides.

Structures of Pisumionoside (1), Sayaendoside (2), and Pisumflavonosides I (3) and II (4) from the Young Seedpods of *P. sativum* The constituents of the young seedpods of *P. sativum* were separated by the procedures shown in Chart 1. Namely, the aqueous methanolic extract from the young seedpods was partitioned into an ethyl-acetate and water mixture to furnish the ethyl-acetate soluble portion and water phase. The water phase was subjected to reversed-phase and normal-phase silica gel column chromatography and finally HPLC to give pisumionoside (**1**, 0.0001%), sayaendoside (**2**, 0.0001%), and pisumflavonosides I (**3**, 0.0013%) and II (**4**, 0.0005%) together with quercetin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**6**, 0.034%),[8] kaempferol 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**7**, 0.0006%),[9] quercetin 3-sophorotrioside (**8**, 0.043%),[8] kaempferol 3-sophorotrioside (**9**, 0.0016%),[9] and L-tryptophan (0.0048%). However, diterpene and triterpene oligoglycosides from the immature seeds of this plant[7] were not isolated from the young seedpods.

Pisumionoside (**1**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −3.4°). The IR spectrum of **1** showed absorption bands due to hydroxyl and olefin functions at 3432, 1671, and 1076 cm^{-1}. In the negative-ion and positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 807 $(2M-H)^-$ and *m/z* 427 $(M+Na)^+$ and the molecular formula $C_{19}H_{32}O_9$ of **1** was determined by high-resolution MS measurement. Acid hydrolysis of **1** with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolidine derivative.[10] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR analytical methods,[11] showed four singlet methyls [δ 1.12, 1.50, 1.71, 2.27 (all s, 12, 13, 11, 10-H_3)], two methylenes [δ 1.92, 2.22 (both m, 2-H_2), 2.25, 2.38 (both m, 4-H_2)], an olefin [δ 6.91, 7.97 (both d, J=16.3 Hz, 7, 8-H)], a methine bearing a hydroxyl group [δ 4.55 (m, 3-H)], two quaternary carbons bearing a hydroxyl group [δ_C 80.5 (6-C), 75.2 (5-C)], and a carbonyl carbon [δ_C 197.7 (9-C)] together with a β-D-glucopyranosyl moiety [δ 5.05 (d, J=7.9 Hz, 1′-H)]. The connectivities of the quaternary carbons and the position of the glucosidic linkage were clarified by an HMBC experiment with **1**. Namely, long-range correlations were observed between the following protons and carbons: 11, 12-H_3 and 1, 2, 6-C, 13-H_3 and 4, 5, 6-C, 10-H_3 and 8, 9-C, 7-H and 6, 9-C, 1′-H and 3-C (Fig. 1). Enzymatic hydrolysis of **1** with naringinase liberated (*E*)-4-(*r*-1′,*t*-2′,*c*-4′-trihydroxy-2′,6′,6′-trimethylcy-

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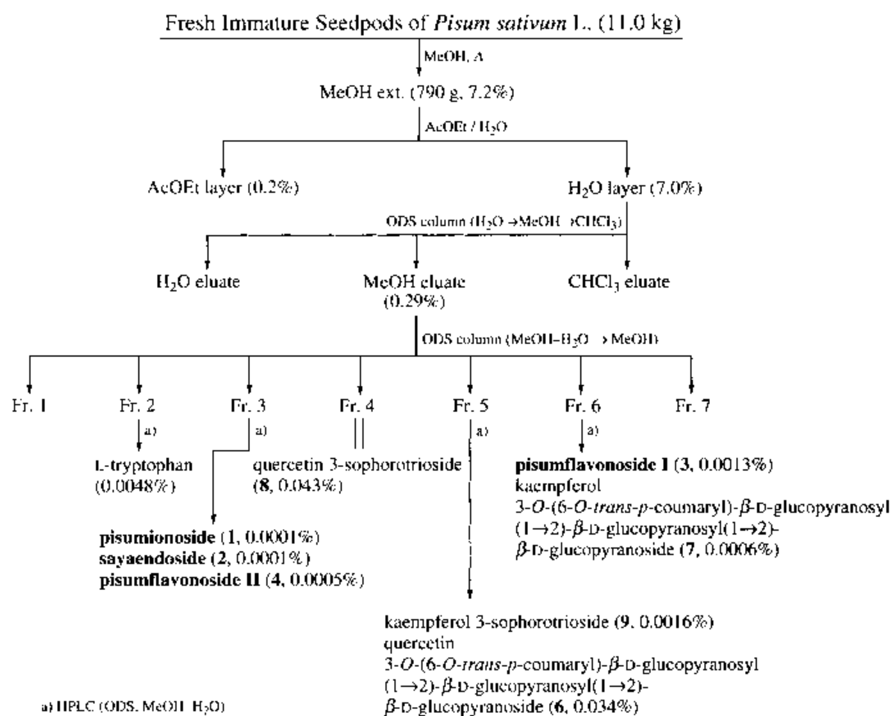

Chart 1

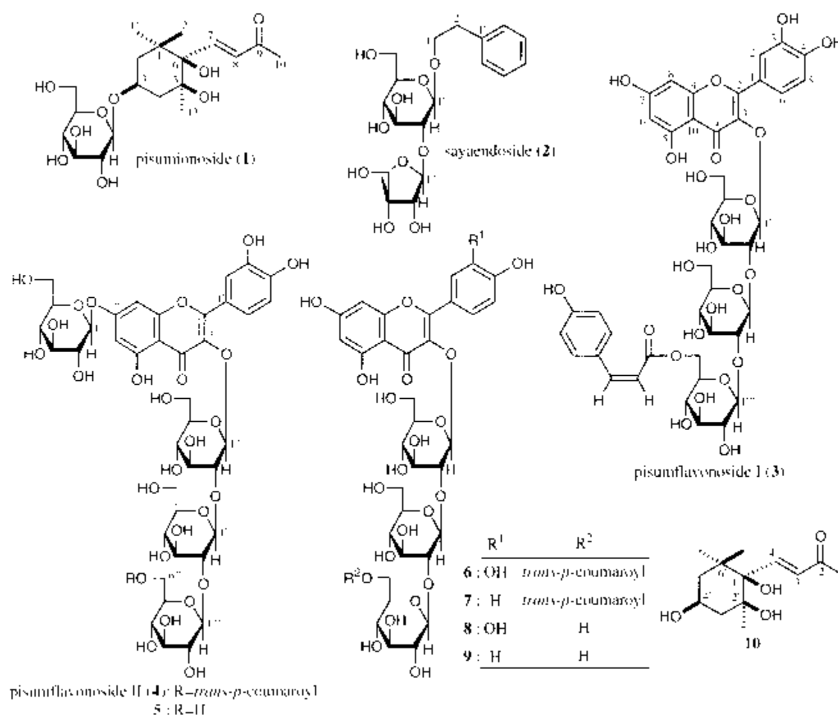

Chart 2

clohexyl)but-3-en-2-one (**10**).[12] On the basis of the above evidence, the structure of pisumionoside (**1**) was determined as shown.

Sayaendoside (**2**) was obtained as a yellow powder and its IR spectrum showed absorption bands at 1684 cm^{-1} due to olefin functions and the strong absorption bands at 3432 and 1075 cm^{-1} suggestive of an oligoglycosidic structure. The negative- and positive-ion FAB-MS of **2** showed quasimolecular ion peaks at *m/z* 415 $(M-H)^-$ and *m/z* 439 $(M+Na)^+$, respectively, and high-resolution MS measurement of the quasimolecular ion peak $(M+Na)^+$ revealed the molecular formula of **2** to be $C_{19}H_{28}O_{10}$. Acid hydrolysis of **2** with 9% hydrochloric acid (HCl)–dry methanol liberated methyl glycoside of glucose and apiose, while D-glucose was obtained by acid hydrolysis with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v).[10] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR

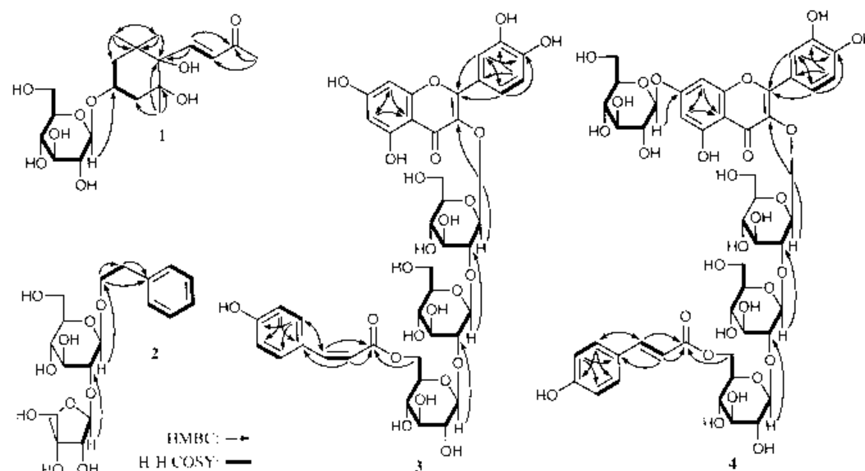

Fig. 1

Table 1. ^{13}C-NMR Data for Pisumionoside (**1**) and Sayaendoside (**2**)

	1		2
C-1	38.8	C-1	70.7
2	41.5	2	36.8
3	74.0	C-1′	139.4
4	36.9	2′,6′	126.7
5	75.2	3′,5′	128.7
6	80.5	4′	129.4
7	131.6	Glc-1″	103.2
8	151.6	2″	77.7
9	197.7	3″	78.9
10	27.0	4″	71.9
11	28.3	5″	78.4
12	28.2	6″	62.8
13	28.0	Api-1‴	110.4
Glc-1′	101.4	2‴	78.0
2′	75.3	3‴	80.8
3′	78.8	4‴	66.5
4′	71.8	5‴	75.6
5′	78.8		
6′	62.8		

125 MHz, pyridine-d_5.

(Table 1) spectra[11] of **2** indicated the presence of a β-D-glucopyranosyl moiety [δ 4.80 (d, J=7.3 Hz, 1″-H)], an β-D-apiofuranosyl moiety [δ 4.25 (m, 4‴-H), 6.42 (br s, 1‴-H)], and a phenethyl moiety [δ 3.09 (ddd, J=6.4, 9.3, 16.3 Hz, 2-H_2), 3.86, 4.22 (both ddd, J=6.4, 9.3, 16.3 Hz, 1-H_2), 7.18 (m, 4′-H), 7.26 (br d, 2′, 3′, 5′, 6′-H)]. In the HMBC experiment of **2**, long-range correlations were observed between the 1″-proton and the 1-carbon and between the 1‴-proton and the 2″-carbon. Consequently, the structure of sayaendoside was determined to be phenethyl 1-*O*-β-D-apiofuranosyl (1→2)-β-D-glucopyranoside (**2**).

Pisumflavonoside I (**3**), isolated as a yellow powder, showed absorption bands at 3410, 1655, 1607, 1076, and 825 cm^{-1} ascribable to hydroxyl, carbonyl, and ester functions in the IR spectrum. In the UV spectrum of **3**, absorption maxima were observed at 258 (log ε 4.4) and 313 (4.4) nm suggestive of a flavonol structure. The molecular formula $C_{42}H_{46}O_{24}$ of **3** was determined from the negative-ion and positive-ion FAB-MS [m/z 933 $(M-H)^-$, m/z 935 $(M+H)^+$, m/z 957 $(M+Na)^+$] and by high-resolution MS measurement. In the negative-ion FAB-MS of **3**, fragment ion peaks were observed at m/z 787 $(M-C_9H_7O_2)^-$, m/z 625 $(M-C_{15}H_{17}O_7)^-$, m/z 463 $(M-C_{21}H_{27}O_{12})^-$, and m/z 301 $(M-C_{27}H_{37}O_{17})^-$, which were derived by cleavage of the glycosidic linkages. Alkaline hydrolysis of **3** with 1% NaOMe-MeOH provided quercetin 3-*O*-sophorotrioside (**8**)[8] and *cis*-*p*-coumaric acid. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 2) spectra[11] of **3** showed signals due to a quercetin 3-*O*-sophorotrioside moiety [δ 4.59 (d, J=8.9 Hz, 1⁗-H), 4.74 (d, J=7.6 Hz, 1‴-H), 5.74 (d, J=7.6 Hz, 1″-H), 6.19, 6.39 (both br s, 6, 8-H), 6.87 (d, J=7.9 Hz, 5′-H), 7.59 (br s, 2′-H), 7.60 (br d, J=*ca.* 8 Hz, 6′-H), 12.69 (br s, 5-OH)] and a *cis*-*p*-coumaric acid moiety [δ 5.89, 6.83 (both d, J=13.2 Hz, 2, 3-H), 6.76, 7.67 (both d, J=8.4 Hz, 3′, 5′, 2′, 6′-H)]. In the HMBC experiment of **3**, a long-range correlation was observed between the 6⁗-protons and the carbonyl carbon of *cis*-*p*-coumaric acid. Furthermore, comparison of the ^{13}C-NMR data for **3** with those for **8** showed an acylation shift around the 6⁗-position. Those findings led us to formulate the structure of pisumflavonoside I to be quercetin 3-*O*-(6-*O*-*cis*-*p*-coumaroyl)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (**3**).

Pisumflavonoside II (**4**) was also obtained as a yellow powder and its IR spectrum showed absorption bands at 3410, 1655, 1605, 1075, and 837 cm^{-1} ascribable to hydroxyl, carbonyl, and ester functions. The UV spectrum of **4** showed absorption maxima at 258 (4.3) and 314 (4.4) nm suggestive of a flavonol structure. The molecular formula $C_{48}H_{56}O_{29}$ of **4** was determined from the negative-ion and positive-ion FAB-MS [m/z 1095 $(M-H)^-$, m/z 1097 $(M+H)^+$] and by high-resolution MS measurement. Alkaline hydrolysis of **4** with 10% aq. KOH–50% aq. 1,4-dioxane liberated *trans*-*p*-coumaric acid and desacyl-pisumflavonoside II (**5**). The molecular formula $C_{39}H_{50}O_{27}$ of **5** was determined by the negative- and positive-ion FAB-MS [m/z 949 $(M-H)^-$, m/z 951 $(M+H)^+$, m/z 973 $(M+Na)^+$] and high-resolution MS measurement. Acid hydrolysis of **5** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) liberated D-glucose, which was identified by GLC analysis of trimethylsilyl thiazolidine derivative.[10] The proton and carbon signals due to the aglycone moiety in the ^{1}H-NMR and ^{13}C-NMR spectra of **5** were superimposable to those of quercetin 3,7-*O*-diglyco-

Table 2. ^{13}C-NMR Data for Pisumflavonosides I (**3**) and II (**4**), Desacyl-pisumflavonoside II (**5**), and Quercetin 3-Sophorotrioside (**8**)

	3	**4**	**5**	**8**		**3**	**4**	**5**	**8**
C-2	155.4	155.8	155.6	155.5	Glc-1″	98.1	98.1	98.0	98.1
3	132.9	133.2	132.9	133.0	2″	83.1	82.9	82.9	83.2
4	177.4	177.5	177.2	177.4	3″	76.1	76.2	75.8	75.9
5	161.2	160.9	161.6	161.2	4″	69.7	69.6	69.7	69.7
6	98.6	99.2	98.5	98.5	5″	76.2	76.2	76.3	76.1
7	164.2	162.7	164.0	163.9	6″	60.6	60.5	60.6	60.9
8	93.4	94.2	93.1	93.3	Glc-1‴	102.1	102.0	102.2	102.3
9	156.2	156.2	156.7	156.2	2‴	83.6	83.1	83.6	83.5
10	103.8	105.6	103.4	103.9	3‴	76.2	76.4	76.1	76.1
1′	121.2	121.0	120.8	121.2	4‴	69.0	69.0	68.7	69.1
2′	116.1	116.4	115.9	116.1	5‴	77.3	77.3	77.2	77.4
3′	144.7	144.8	144.7	144.7	6‴	60.6	60.7	60.3	60.7
4′	148.4	148.6	148.5	148.4	Glc-1⁗	104.4	104.4	104.4	104.4
5′	115.3	115.3	115.7	115.3	2⁗	74.6	74.6	74.4	74.8
6′	121.6	121.6	121.6	121.6	3⁗	75.8	75.8	75.8	75.9
Coumaroyl-1	165.7	166.9			4⁗	69.5	69.7	69.4	69.5
2	115.4	114.0			5⁗	74.2	74.3	75.8	77.3
3	143.5	144.7			6⁗	63.2	63.3	60.5	60.7
1′	125.4	125.1			Glc-1⁗′		99.8	99.6	
2′	132.6	130.2			2⁗′		73.1	72.7	
3′	114.8	115.6			3⁗′		76.4	76.4	
4′	158.7	159.7			4⁗′		69.6	69.7	
5′	114.8	115.6			5⁗′		77.1	77.8	
6′	132.6	130.2			6⁗′		60.6	60.5	

125 MHz, DMSO-d_6.

Table 3. Effects of Flavonol Oligoglycosides (**3**, **4**, **6**—**9**) on D-Galactosamine-Induced Cytotoxicity in Primary Cultured Mouse Hepatocytes

	Inhibition (%)				
Conc. (μM)	1	3	10	30	100
Pisumflavonoside I (**3**)	1.1±0.9	4.1±1.4	8.6±1.6**	9.0±1.4**	12.1±1.5**
Pisumflavonoside II (**4**)	4.5±0.9	5.8±2.6	6.8±1.6*	10.9±1.2**	15.5±1.0**
Quercetin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (**6**)	6.7±1.1*	6.2±0.4*	9.8±1.9*	10.5±1.9**	12.2±1.3**
Kaempferol 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (**7**)	1.5±0.9	1.7±1.7	4.7±2.5	11.1±2.6**	11.3±2.2**
Quercetin 3-sophorotrioside (**8**)	8.0±1.7*	7.3±1.0	18.3±1.5**	19.7±1.3**	27.0±3.6**
Kaempferol 3-sophorotrioside (**9**)	0.9±1.3	0.1±0.4	0.0±0.5	9.9±2.8*	14.5±4.2**

Each value represents the mean±S.E.M. (n=4). Significantly different from the control: * p<0.05, ** p<0.01. Cytotoxicity was not observed.

sides. Namely, the ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[11] of **5** showed signals due to a quercetin moiety [δ 6.42, 6.73 (both d, J=2.1 Hz, 6, 8-H), 6.88 (d, J=8.2 Hz, 5′-H), 7.61 (d, J=2.1 Hz, 2′-H), 7.59 (dd-like, 6′-H), 12.65 (br s, 5-OH)] and four β-D-glucopyranosyl moieties [δ 4.61 (d, J=7.6 Hz, 1⁗-H), 4.76 (d, J=7.9 Hz, 1‴-H), 5.07 (d, J=7.3 Hz, 1⁗′-H), 5.74 (d, J=7.6 Hz, 1″-H)]. The bisdesmoside structure bonding to the 3- and 7-positions of quercetin was identified by a HMBC experiment. Thus, long-range correlations were observed between the 1″-proton and the 3-carbon, between the 1‴-proton and the 2″-carbon, between the 1⁗-proton and the 2‴-carbon, and between the 1⁗′-proton and the 7-carbon. On the basis of this evidence, the structure of **5** was elucidated as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[11] of **4** showed signals assignable to a *trans*-*p*-coumaric acid moiety [δ 6.41, 7.56 (both d, J=16.0 Hz, 2, 3-H), 6.75, 7.54 (both d, J=8.5 Hz, 3′, 5′, 2′, 6′-H)] together with a desacyl-pisumflavonoside II moiety [δ 4.18 (dd, J=5.5 Hz), 4.49 (dd-like) (6⁗-H_2)]. The linked position of the *trans*-*p*-coumaric acid moiety was determined by a HMBC experiment, which showed a long-range correlation between the 6⁗-protons and the carbonyl carbon of *trans*-*p*-coumaric acid. Furthermore, an acylation shift was observed at the 6⁗-position by comparison of the ^{13}C-NMR data for **4** with those for **5**. Consequently, the structure of pisumflavonoside II was determined to be 7-*O*-β-D-glucopyranosyl-quercetin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (**4**).

Hepatoprotective Effects of Flavonol Oligoglycosides In the course of our studies on hepatoprotective constituents from natural medicines, we have characterized new diarylheptanoid glycosides from *Betula platyphylla*,[13] dihydroflavonols from *Hovenia dulcis*,[14] sesquiterpenes from *Curcuma zedoaria*,[15] and triterpene oligoglycosides from *Bupleurum scorzonerifolium*[16] as the active principles. Since we have found that some flavonol oligoglycosides from *Tilia argentea* showed potent *in vitro* and *in vivo* hepatoprotective effects,[17] flavonol oligoglycosides (**3**, **4**, **6**—**9**) were examined by monitoring the inhibition of cytotoxicity induced by

Table 4. Inhibitory Effects of Quercetin 3-Sophorotrioside (**8**) on D-Galactosamine/Lipopolysaccharide-Induced Liver Injury in Mice

	Dose (mg/kg, *p.o.*)	*n*	s-GPT (Karmen Unit)	s-GOT (Karmen Unit)	Mortality
Control	—	11	4064±1031	3776±550	1/12
Quercetin 3-sophorotrioside (**8**)	50	9	2840±740	3046±529	1/10
	100	9	2623±815	2665±507	1/10
	200	8	1474±324*	1825±182*	2/10
Hydrocortisone	20	5	82±11**	133±11**	0/5

Each value represents the mean±S.E.M. Significantly different from the control: * $p<0.05$, ** $p<0.01$.

Table 5. Inhibitory Effect of Quercetin 3-Sophorotrioside (**8**) on CCl_4-Induced Liver Injury in Mice

	Dose (mg/kg, *p.o.*)	*n*	Serum GPT (Karmen unit)	Serum GOT (Karmen unit)
Control	—	12	6339±1097	7329±715
Quercetin 3-sophorotrioside (**8**)	50	9	6183±1567	8096±1522
	100	9	3329±805	5552±925
	200	8	2113±255*	3759±417*
Malotilate	200	8	761±17**	1321±358**

Each value represents the mean±S.E.M. Significantly different from the control: * $p<0.05$, ** $p<0.01$.

D-galactosamine in primary cultured mouse hepatocytes. As shown in Table 3, quercetin 3-sophorotrioside (**8**), a principal constituent, showed the inhibitory effect, but the activities of other compounds were very weak. In an *in vivo* experiment as shown in Tables 4 and 5, **8** was found to inhibit the liver injury induced by D-galactosamine and lipopolysaccharide and by carbon tetrachloride (CCl_4) in mice.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1)]

Isolation of Pisumionoside (1), Sayaendoside (2), and Pisumflavonosides I (3) and II (4), and Four Known Compounds (6—9) The young seedpods of *P. sativum* (11.0 kg, cultivated in Osaka Province, Japan) were cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (790 g, 7.2%), and the MeOH extract (750 g) was partitioned into an AcOEt–H_2O (1 : 1, v/v) mixture. Removal of the solvent from the AcOEt-soluble and H_2O-soluble fractions under reduced pressure yielded the AcOEt extract (20 g, 0.2%) and H_2O extract (730 g, 7.0%). The H_2O extract (700 g) was subjected to reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., 2 kg), H_2O→MeOH→$CHCl_3$] to give the H_2O eluate, the MeOH eluate (29 g, 0.29%), and the $CHCl_3$ eluate. Reversed-phase silica gel column chromatography [1 kg, MeOH–H_2O (30 : 70→50 : 50, v/v)→MeOH] of the MeOH eluate (29 g) gave seven fractions {Fr. 1 (5.0 g), Fr. 2 (2.0 g), Fr. 3 (1.0 g), Fr. 4 [quercetin 3-sophorotrioside (**8**, 4.3 g, 0.043%)], Fr. 5 (1.1 g), Fr. 6 (4.0 g), Fr. 7 (6.2 g)}. Fraction 2 (170 mg) was purified by HPLC [YMC-Pack R&D-ODS-5-A (250×20 mm i.d., YMC Co., Ltd.), MeOH–H_2O (10 : 90, v/v)] to give L-tryptophan (41 mg, 0.0048%). Fraction 3 (1.0 g) was purified by HPLC [MeOH–1% aq. TFA (30 : 70, v/v)] to give **1** (12 mg, 0.0001%), **2** (14 mg, 0.0001%), and **4** (50 mg, 0.0005%). Fraction 5 (209 mg) was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to give kaempferol 3-sophorotrioside (**9**, 30 mg, 0.0016%) and quercetin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**6**, 649 mg, 0.034%). Fraction 6 (1.2 g) was purified by HPLC [MeOH–H_2O (45 : 55, v/v)] to give **3** (38 mg, 0.0013%) and kaempferol 3-*O*-(6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**7**, 18 mg, 0.0006%). The known compounds (**6**—**9**) and L-tryptophan were identified by comparisons of their physical data ([α]$_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[8,9)]

Pisumionoside (**1**): A white powder, $[\alpha]_D^{26}$ −3.4° (c=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{32}O_9Na$ $(M+Na)^+$: 427.1944. Found: 427.1929. IR (KBr): 3432, 1671, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.12, 1.50, 1.71, 2.27 (3H each, all s, 12, 13, 11, 10-H_3), 1.92, 2.22 (1H each, both m, 2-H_2), 2.25, 2.38 (1H, both m, 4-H_2), 4.55 (1H, m, 3-H), 5.05 (1H, d, J=7.9 Hz, 1′-H), 6.91, 7.97 (1H each, both d, J=16.3 Hz, 7, 8-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 807 $(2M-H)^-$. Positive-ion FAB-MS: m/z 427 $(M+Na)^+$.

Sayaendoside (**2**): A yellow powder, $[\alpha]_D^{25}$ −18.6° (c=0.05, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{28}O_{10}Na$ $(M+Na)^+$: 439.1580. Found: 439.1597. IR (KBr): 3432, 1684, 1075, 702 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 3.09 (2H, ddd, J=6.4, 9.3, 16.3 Hz, 2-H_2), 3.86, 4.22 (1H each, both ddd, J=6.4, 9.3, 16.3 Hz, 1-H_2), 4.25 (2H, m, 4‴-H), 4.80 (1H, d, J=7.3 Hz, 1″-H), 6.42 (1H, br s, 1‴-H), 7.18 (1H, m, 4′-H), 7.26 (4H, br d, 2′, 3′, 5′, 6′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 415 $(M-H)^-$. Positive-ion FAB-MS: m/z 439 $(M+Na)^+$, 105 $(M-C_8H_9)^+$.

Pisumflavonoside I (**3**): A yellow powder, $[\alpha]_D^{25}$ −38.2° (c=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{47}O_{24}$ $(M+H)^+$: 935.2457. Found: 935.2470. IR (KBr): 3410, 1655, 1607, 1076, 825 cm^{-1}. UV [MeOH, nm, (log ε)] : 206 (4.7), 258 (4.4), 313 (4.4). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 4.59 (1H, d, J=8.9 Hz, 1⁗-H), 4.74 (1H, d, J=7.6 Hz, 1‴-H), 5.74 (1H, d, J=7.6 Hz, 1″-H), 5.89, 6.83 (1H each, both d, J=13.2 Hz, coumaroyl-2, 3-H), 6.19, 6.39 (1H each, both br s, 6, 8-H), 6.76, 7.67 (2H each, both d, J=8.4 Hz, coumaroyl-3′, 5′, 2′, 6′-H), 6.87 (1H, d, J=7.9 Hz, 5′-H), 7.59 (1H, br s, 2′-H), 7.60 (1H, br d, J=*ca.* 8 Hz, 6′-H), 12.69 (1H, br s, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 933 $(M-H)^-$, 787 $(M-C_9H_7O_2)^-$, 625 $(M-C_{15}H_{17}O_7)^-$, 463 $(M-C_{21}H_{27}O_{12})^-$, 301 $(M-C_{27}H_{37}O_{17})^-$. Positive-ion FAB-MS: m/z 935 $(M+H)^+$, 957 $(M+Na)^+$.

Pisumflavonoside II (**4**): A yellow powder, $[\alpha]_D^{25}$ −59.0° (c=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{48}H_{57}O_{29}$ $(M+H)^+$: 1097.2986. Found: 1097.2999. IR (KBr): 3410, 1655, 1605, 1075, 837 cm^{-1}. UV [MeOH, nm, (log ε)]: 207 (5.0), 258 (4.3), 314 (4.4). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 4.61 (1H, d, J=7.6 Hz, 1⁗-H), 4.76 (1H, d, J=7.9 Hz, 1‴-H), 5.07 (1H, d, J=7.3 Hz, 1⁗′-H), 5.75 (1H, d, J=7.6 Hz, 1″-H), 6.41, 7.56 (1H each, both d, J=16.0 Hz, coumaroyl-2, 3-H), 6.42, 6.73 (1H each, both d, J=2.1 Hz, 6, 8-H), 6.75, 7.54 (2H each, both d, J=8.5 Hz, coumaroyl-3′, 5′, 2′, 6′-H), 6.88 (1H, d, J=8.2 Hz, 5′-H), 7.59 (1H, dd-like, 6′-H), 7.61 (1H, d, J=2.1 Hz, 2′-H), 12.65 (1H, br s, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1095 $(M-H)^-$. Positive-ion FAB-MS: m/z 1097 $(M+H)^+$.

Acid Hydrolysis of 1, 2, and 5 A solution of **1**, **2**, and **5** (3 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH- form) and residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis (trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose from **1**, **2**, and **5**; GLC conditions: column: Supeluco STBTM-1, 30 m×0.25 mm (i.d.) capillary column, column temperature: 230 °C, He flow rate: 15 ml/min, t_R: 24.2 min.

Enzymatic Hydrolysis of 1 A solution of **1** (4.4 mg) in 0.1 M acetate buffer (pH 4.0, 2.0 ml) was treated with naringinase (Sigma Chemical Co., 5.0 mg) and was stirred at 40 °C for 20 h. After adding EtOH to the reaction mixture, the solvent was removed *in vacuo*. The crude product was purified by reversed-phase (1.0 g, H_2O→MeOH) and normal-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (15 : 3 : 1, lower layer, v/v)]

to give (*E*)-4-(*r*-1′,*t*-2′,*c*-4′-trihydroxy-2′,6′,6′-trimethylcyclohexyl)but-3-en-2-one (**10**, 2.4 mg), which was identified by comparison with reported values[12] ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR).

Methanolysis of 2 A solution of **2** (3 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the residue was dissolved in pyridine (0.01 ml) and the solution was treated with BSTFA (0.02 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i) and methyl apioside (ii). GLC conditions: CBR-M25-025, 25 m×0.25 mm (i.d.) capillary column; column temperature 140—280 °C; He flow rate 15 ml/min; t_R: i, 17.8 min, 18.2 min ii, 14.3 min, 15.3 min.

Alkaline Treatment of 3 A solution of **3** (5.0 mg) in 1% NaOMe–MeOH (1.0 ml) was stirred at room temperature (24 °C) for 3 h. After reaction, the reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer, v/v)] to give quercetin 3-sophorotrioside (**8**, 3.2 mg) and *cis-p*-coumaric acid (0.1 mg), which were identified by comparison with authentic samples (^{1}H-NMR, IR, MS).

Alkaline Treatment of 4 A solution of **4** (10.0 mg) in 10% aq. KOH–50% aq. 1,4-dioxane (1 : 1, v/v, 3.0 ml) was stirred at room temperature (24 °C) for 30 min. After reaction, the reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer, v/v)] to give desacyl-pisumflavonoside II (**5**, 3.2 mg) and *trans-p*-coumaric acid (0.2 mg), which was identified by comparison with authentic samples (^{1}H-NMR, IR, MS).

Desacyl-pisumflavonoside II (**5**): A yellow powder, $[\alpha]_D^{25}$ −82.4° (c=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{39}H_{51}O_{27}$ $(M+H)^+$: 951.2618. Found: 951.2603. IR (KBr): 3410, 1644, 1605, 1071, 830 cm^{-1}. UV [MeOH, nm, (log ε)]: 207 (4.9), 256 (4.3), 314 (4.2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.59 (1H, d, J=7.6 Hz, 1⁗-H), 4.74 (1H, d, J=7.9 Hz, 1‴-H), 5.07 (1H, d, J=7.1 Hz, 1′′′′′-H), 5.74 (1H, d, J=7.5 Hz, 1″-H), 6.42 (1H, d, J=2.1 Hz, 6-H), 6.73 (1H, d, J=2.1 Hz, 8-H), 6.88 (1H, d, J=8.2 Hz, 5′-H), 7.61 (1H, d, J=2.1 Hz, 2′-H), 7.59 (1H, dd-like, 6′-H), 12.65 (1H, br s, 5-OH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 949 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 951 $(M+H)^+$, 973 $(M+Na)^+$.

D-Galactosamine-Induced Hepatocytotoxicity Hepatocytes were isolated from male ddY mice (35—38 g) by collagenase perfusion method. The cell suspension of 4×10^4 cells in 100 μl William's E medium containing fetal calf serum (10%), penicillin (100 units/ml), and streptomycin (100 μg/ml) was inoculated in a 96-well tissue culture plate, and precultured for 4 h at 37 °C under a 5% CO_2 atmosphere. The medium was exchanged with a fresh medium containing D-galactosamine (1 mM) and a test sample (1—100 μM), and the hepatocytes were cultured for 44 h. The medium was exchanged with 100 μl of the fresh medium, and 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg/ml in phosphate-buffered saline) solution was added to the medium. After 4 h culture, the medium was removed, and 100 μl of isopropanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density (O.D.) of the formazan solution was measured by microplate reader at 570 nm.

D-Galactosamine/Lipopolysaccharide-Induced Liver Injury The method described by Tiegs *et al.*[18] was modified and used for the experiment. Male ddY mice weighing 25—30 g were used. After 20 h of fasting, a mixture of D-galactosamine hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and lipopolysaccharide (from *Salmonella enteritidis*, Sigma Chemical Co., St. Louis, MO, U.S.A.) was injected intraperitoneally at a dose of 350 mg/kg and 10 μg/kg to produce liver injury. Each test sample was administered orally 1 h before D-galactosamine/lipopolysaccharide injection. Blood samples were collected 10 h after this, and serum GPT and GOT levels were determined. Hydrocortisone (Sigma Chemical Co.) was used as a reference compound.

CCl_4-Induced Liver Injury Male ddY mice weighing 20—25 g were used. After 20 h of fasting, a mixture of 10 (v/v) % CCl_4 in olive oil was injected subcutaneously at a dose of 5 ml/kg to produce CCl_4-induced liver injury. Each test sample was given orally 1 h before CCl_4 injection. Blood samples were collected from infraorbital plexus 20 h after the injection, and serum GOT and GPT were determined by Reitman-Frankel method (S.TA-test Wako, Wako Pure Chemical Industries, Ltd.). Malotilate (Daiichi Pharmaceutical Industry, Tokyo, Japan) was used as a reference compound.

Statistical Analysis Values were expressed as means±S.E.M. One-way analysis of variance following Dunnett's test for multiple comparison analysis were used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

References and Notes

1) Part XXIV: Murakami T., Kishi A., Matsuda H., Hattori M., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 845—848 (2001).
2) *a*) Matsuda H., Kageura T., Toguchida I., Ueda H., Morikawa T., Yoshikawa M., *Life Sci.*, **66**, 2151—2157 (2000); *b*) Matsuda H., Li Y., Yoshikawa M., *ibid.*, **66**, PL41—46 (2000); *c*) *Idem*, *ibid.*, **66**, 2233—2238 (2000); *d*) *Idem*, *ibid.*, **67**, 2921—2927 (2000); *e*) Yoshikawa M., Shimoda H., Uemura T., Morikawa T., Kawahara Y., Matsuda H., *Bioorg. Med. Chem.*, **8**, 2071—2077 (2000); *f*) Li Y., Matsuda H., Wen S., Yamahara J., Yoshikawa M., *Eur. J. Pharmacol.*, **387**, 337—342 (2000); *g*) Li Y., Matsuda H., Yamahara J., Yoshikawa M., *ibid.*, 392, 71—77 (2000); *h*) Yoshikawa M., Uemura T., Shimoda H., Kishi A., Kawahara Y., Matsuda H., *Chem. Pharm. Bull.*, **48**, 1039—1044 (2000); *i*) Matsuda H., Murakami T., Nishida N., Kageura T., Yoshikawa M., *ibid.*, **48**, 1429—1435 (2000); *j*) Murakami T., Emoto A., Matsuda H., Yoshikawa M., *ibid.*, **49**, 54—63 (2001).
3) *a*) Yoshikawa M., Murakami T., Komatsu H., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 81—87 (1997); *b*) Yoshikawa M., Murakami T., Komatsu H., Yamahara J., Matsuda H., *Heterocycles*, **47**, 397—405 (1998); *c*) Murakami T., Kishi A., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **48**, 994—1000 (2000); *d*) Oda K., Matsuda H., Murakami T., Katayama S., Ohgitani T., Yoshikawa M., *Biol. Chem.*, **381**, 67—74 (2000).
4) Yoshikawa M., Shimada H., Komatsu H., Sakurama T., Nishida N., Yamahara J., Shimoda H., Matsuda H., Tani T., *Chem. Pharm. Bull.*, **45**, 877—882 (1997).
5) *a*) Komatsu H., Murakami T., Matsuda H., Yoshikawa M., *Heterocycles*, **48**, 703—710 (1998); *b*) Yoshikawa M., Murakami T., Komatsu H., Matsuda H., *Chem. Pharm. Bull.*, **46**, 812—816 (1998).
6) Murakami T., Kohno K., Kishi A., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **48**, 1673—1680 (2000).
7) Murakami T., Kohno K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 73—77 (2001).
8) Federrico F., Elvira E., Ramon C., Maria A. J., Francisco A. T., *Phytochemistry*, **39**, 1443—1446 (1995).
9) Furuya M., Galston A. W., *Phytochemistry*, **4**, 285—296 (1965).
10) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843—1845 (1986).
11) The ^{1}H- and ^{13}C-NMR spectra of **1**—**5** were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple bond connectivity (HMBC) experiments, and difference nuclear Overhauser effect (dif NOE).
12) Tan S. T., Wilkins A. L., Holland P. T., *Aust. J. Chem.*, **42**, 1799—1804 (1989).
13) Matsuda H., Ishikado A., Nishida N., Ninomiya K., Fujiwara H., Kobayashi Y., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **8**, 2939—2944 (1998).
14) Yoshikawa M., Murakami T., Ueda T., Yoshizumi S., Ninomiya K., Murakami N., Matsuda H., Saito M., Fujii W., Tanaka T., Yamahara J., *Yakugaku Zasshi*, **117**, 108—118 (1997).
15) Matsuda H., Ninomiya K., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **8**, 339—344 (1998).
16) *a*) Matsuda H., Murakami T., Ninomiya K., Inadzuki M., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **7**, 2193—2198 (1997); *b*) Yoshikawa M., Murakami T., Hirano K., Inadzuki M., Ninomiya K., Matsuda H., *Teterahedron Lett.*, **38**, 7395—7398 (1997).
17) Matsuda H., Uemura T., Shimoda H., Ninomiya K., Yoshikawa M., presented at 42nd Symposium on the Chemistry of Natural Products, Nov., 2000, Symposium Paper, pp. 469—474.
18) Tiegs G., Wolter M., Wendel A., *Biochem. Pharmacol.*, **38**, 627—631 (1989).