Characterization of Two Novel Polysaccharides Having Immunological Activities from the Root of *Panax ginseng*

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Two acidic polysaccharides, called ginsenan S-IA and ginsenan S-IIA, were isolated from the root of *Panax ginseng* C. A. MEYER. They were homogeneous on electrophoresis and gel chromatography, and their molecular masses were estimated to be 5.6×10^4 and 1.0×10^5 , respectively. Ginsenan S-IA is composed of L-arabinose:D-galactose:D-galacturonic acid in the molar ratio of 8:8:1, and ginsenan S-IIA is composed of L-arabinose:D-galactose:D-glucose:D-galacturonic acid in the molar ratio of 15:10:2:5, in addition to small amounts of O-acetyl groups. About a half (ginsenan S-IA) and about a quarter (ginsenan S-IIA) of the hexuronic acid residues exist as methyl esters. Reduction of carboxyl groups, methylation analysis, nuclear magnetic resonance and periodate oxidation studies indicated that their structural features include mainly α -1,5-linked L-arabino- β -3,6-branched D-galactan type structural units. Both polysaccharides showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test and pronounced anti-complementary activity. These substances are the first examples having a relatively high content of both α -3,5-branched L-arabinose and β -1,4-linked D-galactose units among the acidic arabinogalactans with activities on phagocytosis and anti-complement.

Keywords polysaccharide structure; immunological activity; *Panax ginseng*; acidic arabinogalactan; ginsenan S-IA; ginsenan S-IIA

We very recently isolated and elucidated the structural features of two acidic polysaccharides from the root of *Panax ginseng* G. A. MEYER having reticuloendothelial system (RES)-potentiating, anti-complementary and alkaline phosphatase-inducing activities, and called them ginsenans PA and PB.¹⁾ The root of this plant is a very important crude drug in China and Japan. Ginsenans PA and PB were obtained as major polysaccharides having immunological activity. The present paper describes the isolation, structural features and immunological activities of these two novel polysaccharides from the water extract of the root of *Panax ginseng*.

Materials and Methods

Isolation of Polysaccharides The material was imported from the Republic of Korea. The sliced roots (600 g) were extracted with hot water (6 l) under stirring for 30 min in a boiling water bath. After suction filtration and centrifugation, the residue was similarly extracted with hot water (3 l). The supernatants were combined and the solution (6.6 l) was added to 1% sodium sulfate (66 ml); 5% cetyltrimethylammonium bromide (CTAB, 990 ml) was then added to the solution. After centrifugation, the supernatant obtained was poured into two volumes of ethanol. The precipitate was treated with 80% ethanol, and after centrifugation, the precipitate was dissolved in water, then dialyzed, concentrated and applied to a column (4 × 28 cm) of diethylaminoethyl (DEAE)-Sephadex A-25 which had been pretreated as described in a previous report.²⁾ After elution with water and 0.1 M acetate buffer (pH 5.0) (400 ml each), the column was successively eluted with 0.2 M and 0.3 M acetate buffer (400 ml each). Fractions of 20 ml were collected and analyzed by the phenol-sulfuric acid method.³⁾ The eluates obtained from tubes 50 to 55 were combined, dialyzed, concentrated and applied to a column (5 × 80 cm) of Sephadex G-25. The column was eluted with water and fractions of 20 ml were collected. The eluates obtained from tubes 30 to 34 were combined, concentrated and lyophilized. The yield of this fraction A was 38 mg. The eluates obtained from tubes 70 to 74 of the DEAE-Sephadex A-25 column chromatography above mentioned provided fr. B after purification by the same gel chromatography as for fr. A. Yield, 31 mg. Fraction A (90 mg) was dissolved in 1/15 M phosphate buffer (pH 7.0) containing 0.15 M NaCl, 1 mM MgCl₂ and 1 mM CaCl₂, and applied to a column (1.5 × 40 cm) of Con A-Sepharose (Pharmacia Co.). The column was equilibrated and eluted with the same buffer at 4°C, and fractions of 10 ml were collected. The eluates obtained from tubes 4 to 13 were combined, dialyzed, concentrated and applied to a column (5 × 80 cm) of Sephadex G-25. The

column was eluted with water and fractions of 20 ml were collected. The eluates obtained from tubes 30 to 36 were combined, concentrated and lyophilized. Ginsenan S-IA (64 mg) was obtained as a white powder. Fraction B (90 mg), in contrast, was purified by affinity chromatography followed by dialysis and gel chromatography using the same columns of Con A-Sepharose and Sephadex G-25. Ginsenan S-IIA (54 mg) was obtained as a white powder.

Glass-Fiber Paper Electrophoresis This was carried out as described previously⁴⁾ on Whatman GF83 glass-fiber paper at 570 V for 70 min with 0.025 M Na₂B₄O₇·10H₂O–0.1 N NaOH (10:1, pH 9.2). Ginsenans S-IA and S-IIA gave a single spot at distances of 91 and 70 mm from the origin toward the cathode, respectively.

Gel Chromatography The sample (2 mg) was dissolved in 0.1 M Tris-HCl buffer (pH 7.0), and applied to a column (2.6 × 96 cm) of Toyopearl HW-55F, pre-equilibrated and developed with the same buffer. Fractions of 5 ml were collected and analyzed by the phenol-sulfuric acid method. Standard pullulans (Shōwa Denkō Co.) having known molecular masses were run on the column to obtain a calibration curve.

Qualitative Analysis of Component Sugars Hydrolysis and cellulose thin-layer chromatography (TLC) of component sugars were performed as described previously.⁵⁾ The configurations of component neutral sugars were identified by gas chromatography (GC) of trimethylsilylated α -methylbenzylamino-alditol derivatives.⁶⁾ GC was carried out on a Shimadzu GC-14A gas chromatograph equipped with a hydrogen flame ionization detector.

Determination of Components Neutral sugars were analyzed by GC after conversion of the hydrolyzate into alditol acetates as described previously.⁷⁾ Galacturonic acid was determined by the *m*-hydroxybiphenyl method.⁸⁾

Determination of O-Acetyl Groups The sample was hydrolyzed with 0.2 N hydrochloric acid and analyzed by GC using propionic acid as an internal standard as described previously.⁹⁾

Determination of O-Methyl Groups in Methyl Esters The sample was dissolved in 0.5 N sodium hydroxide containing ethanol as an internal standard and the solution was left at 20°C for 30 min. The product was directly subjected to GC with a column (3.2 mm i.d. × 2.1 m long spiral glass) packed with 15% PEG 6000 on Shimalite F (40 to 80 mesh) at 80°C with a helium flow of 40 ml per min; *t_R* (min), methanol 4.5; ethanol (internal standard) 5.7.

Nuclear Magnetic Resonance (NMR) NMR spectra were recorded on a JEOL JMN A500 FT NMR spectrometer in heavy water containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard at 70°C.

Reduction of Carboxyl Groups The polysaccharides were reduced with 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate and sodium borohydride as described in a previous report.¹⁰⁾ The

reduction was repeated three times under the same conditions. Yields were 12.5 from 20.6 mg of ginsenoside S-IA and 15.0 from 20.6 mg of ginsenoside S-IIA.

Methylation This was performed with powdered sodium hydroxide and methyl iodide in dimethyl sulfoxide as described previously.¹¹⁾ The yields were 3.0 from 5.0 mg of ginsenoside S-IA, 3.6 from 5.3 mg of the carboxyl-reduced ginsenoside S-IA, 2.5 from 5.0 mg of ginsenoside S-IIA, and 2.0 from 4.8 mg of the carboxyl-reduced ginsenoside S-IIA.

Analysis of the Methylated Products The products were hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated as described previously.¹²⁾ The partially methylated alditol acetates obtained were analyzed by gas chromatography-mass spectrometry (GC-MS) using a fused silica capillary column (0.32 mm i.d. $\times$ 30 m) of SP-2330 (Supelco Co.) with a programmed temperature increase of 4°C per min from 160 to 220°C at a helium flow of 1 ml per min. GC-MS was performed with a JEOL JMS-GX303 mass spectrometer. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GC are listed in Table I.

Periodate Oxidation Each of the polysaccharides (8.0 mg) was dissolved in 0.05 N sodium hydroxide (1.6 ml) and stood at room temperature for 30 min, then the solution was neutralized with 5 M acetic acid. The solution was adjusted to 2 ml with water, then 0.1 M sodium metaperiodate (2 ml) was added. The reaction mixture was kept at 4°C in the dark, and the periodate consumption was measured by a spectrometric method.¹³⁾ The oxidation was completed after 6 d. The reaction mixture was successively treated with ethylene glycol (0.04 ml) at 4°C for 1 h and sodium borohydride (40 mg) at 4°C for 16 h, then adjusted to pH 5.0 by addition of acetic acid. The solution was concentrated and applied to a column (2.6 $\times$ 88 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 9 to 11 were combined, concentrated and lyophilized. Yield, 7.6 mg each.

Phagocytic Activity This was measured by *in vivo* carbon clearance test as described previously.⁷⁾ The samples and a positive control, zymosan (Tokyo kasei Co.), were each dissolved and suspended in physiological saline and dosed i.p. (20 mg/kg body weight) to male mice (ICR-SPF) once a day.

Anti-complementary Activity This was measured as described in a previous report.¹⁴⁾ Geratin-veronal-buffered saline (pH 7.4) containing 500 μ M Mg^{2+} and 150 μ M Ca^{2+} (GVB²⁺) was prepared, and normal human serum (NHS) was obtained from a healthy adult. Various dilutions of the samples in water were incubated and the residual total hemolytic complement (TCH₅₀) was determined using immunoglobulin M (IgM)-hemolysis-sensitized sheep erythrocytes. NHS was incubated with water and GVB²⁺ to provide a control, and the activities of the samples were expressed as the percentage inhibition of the TCH₅₀ of the control. Plantago-mucilage A from the seed of *Plantago asiatica* L.¹⁵⁾ was used as a positive control.

Results

The hot water extract obtained from the root of *Panax ginseng* was treated with CTAB in the presence of small amounts of sodium sulfate. The supernatant obtained was poured into ethanol. The precipitate was dialyzed and applied to a column chromatography of DEAE-Sephadex A-25, then the stepwise elution with acetate buffer was carried out. The eluates with 0.2 and 0.3 M acetate buffer were separately subjected to affinity chromatography on Con A-Sepharose. A pure polysaccharide designated as ginsenoside S-IA was obtained from the passed-through fraction of the former eluate with a phosphate buffer, followed by dialysis and gel chromatography with Sephadex G-25. Another pure polysaccharide designated as ginsenoside S-IIA was obtained from the eluate with 0.3 M acetate buffer by treatment with chromatographies similar to those of ginsenoside S-IA. The isolation method of the two polysaccharides is summarized in Fig. 1.

Each polysaccharide gave a single spot on glass-fiber paper electrophoresis, and a single peak on gel chromatography with Toyopearl HW-55F. Gel chromatography gave values of 5.6×10^4 and 1.0×10^5 for the molecular masses

Fig. 1. Isolation of Ginsenoside S-IA and Ginsenoside S-IIA

of ginsenoside S-IA and ginsenoside S-IIA, respectively. Ginsenoside S-IA had $[\alpha]_D^{24} -29.5^\circ$ ($c=0.1$, H₂O) and ginsenoside S-IIA had $[\alpha]_D^{24} +8.0^\circ$ ($c=0.1$, H₂O).

Ginsenoside S-IA is composed of L-arabinose, D-galactose and D-galacturonic acid, and ginsenoside S-IIA is composed of L-arabinose, D-galactose, D-glucose and D-galacturonic acid. Quantitative analyses showed that ginsenoside S-IA contained 42.3% arabinose, 50.8% galactose and 6.9% galacturonic acid, and that ginsenoside S-IIA contained 42.0% arabinose, 32.6% galactose, 6.2% glucose and 19.2% galacturonic acid. Their molar ratios were 8:8:1 in ginsenoside S-IA and 15:10:2:5 in ginsenoside S-IIA.

The carbon-13 NMR (¹³C-NMR) spectra of ginsenoside S-IA and ginsenoside S-IIA showed signals at δ 21.67 and 177.40 ppm, and signals at δ 21.68 and 178.08 ppm, suggesting the presence of *O*-acetyl groups. In addition, these ¹³C-NMR spectra showed signals at δ 56.98 and 55.51 ppm suggesting the presence of *O*-methyl groups as carboxylic acid methyl esters. The presence of these groups was confirmed by GC of the hydrolyzates, and the contents of acetyl and methoxyl groups were 3.1% and 0.6% in ginsenoside S-IA, and were 1.1% and 0.8% in ginsenoside S-IIA, respectively. Thus existence of about half of the hexuronic

TABLE I. Methylation Analysis of Ginsenon S-IA, Ginsenon S-IIA and Their Carboxyl-Reduced Derivatives

	Relative retention time ^{a)}	Molar ratios			
		Ginsenon S-IA	Ginsenon S-IIA	Ginsenon S-IA	Ginsenon S-IIA
1,4-Ac ₂ -2,3,5-Me ₃ -L-arabinitol	0.68	18	22	18	22
1,4,5-Ac ₃ -2,3-Me ₂ -L-arabinitol	1.15	6	10	6	10
1,3,4,5-Ac ₄ -2-Me-L-arabinitol	1.46	5	4	5	4
1,2,4,5-Ac ₄ -3-Me-L-arabinitol	1.57	—	4	—	4
1,5-Ac ₂ -2,3,4,6-Me ₄ -D-galactitol	1.11	1	1	1	1
1,3,5-Ac ₃ -2,4,6-Me ₃ -D-galactitol	1.40	5	8	5	8
1,4,5-Ac ₃ -2,3,6-Me ₃ -D-galactitol	1.48	4	4	7	10
1,5,6-Ac ₃ -2,3,4-Me ₃ -D-galactitol	1.63	5	2	5	2
1,3,4,5-Ac ₄ -2,6-Me ₂ -D-galactitol	1.68	1	1	1	1
1,4,5,6-Ac ₄ -2,3-Me ₂ -D-galactitol	1.98	1	—	1	—
1,3,5,6-Ac ₄ -2,4-Me ₂ -D-galactitol	2.02	12	12	12	12
1,5-Ac ₂ -2,3,4,6-Me ₄ -D-glucitol	1.00	—	1	—	1
1,4,5-Ac ₃ -2,3,6-Me ₃ -D-glucitol	1.54	—	1	—	1
1,4,5,6-Ac ₄ -2,3-Me ₂ -D-glucitol	1.94	—	1	—	1

a) Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. Abbreviations: Ac = acetyl; Me = methyl (e.g., 1,4-Ac₂-2,3,5-Me₃ = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-).

acid residues in ginsenon S-IA and of about a quarter of the hexuronic acid residues in ginsenon S-IIA as methyl esters is evident.

Further, the ¹³C-NMR spectrum of ginsenon S-IA showed four signals due to anomeric carbons at δ 101.07, 106.06, 110.22 and 111.73 ppm. The first and the second were assigned to the anomeric carbons of α -D-galactopyranosyluronic acid and β -D-galactopyranose, respectively.^{16,17)} The signals at δ 110.22 and 111.73 ppm were assigned to the anomeric carbons of α -L-arabinofuranose.¹⁸⁾ In the case of the ¹³C-NMR spectrum of ginsenon S-IIA, five signals due to anomeric carbons at δ 101.92, 102.75, 106.07, 110.25 and 111.77 ppm were shown. These signals were assigned to the anomeric carbons of α -D-galactopyranosyluronic acid, α -D-glucopyranose, β -D-galactopyranose and α -L-arabinofuranose, respectively.¹⁶⁻¹⁸⁾

The carboxyl groups of hexuronic acid residues in both polysaccharides were reduced to give the corresponding neutral sugar residues.¹⁹⁾ Both ginsenon S-IA and its carboxyl-reduced derivative were methylated with solid sodium hydroxide and methyl iodide in dimethyl sulfoxide.²⁰⁾ Similarly, both ginsenon S-IIA and its carboxyl-reduced derivative were methylated under the same conditions. The four methylated products thus obtained were hydrolyzed, then converted into partially methylated alditol acetates. Analysis by GC-MS gave the results shown in Table I. The results indicate that D-galacturonic acid residues in both polysaccharides produced 2,3,6-tri-*O*-methyl D-galactose in the methylation products from their carboxyl-reduced derivatives.

Both polysaccharides were deacetylated and subjected to periodate oxidation followed by reduction. All hexuronic acid residues in both of them and glucose units in ginsenon S-IIA were completely degraded by this treatment. The product obtained from ginsenon S-IA contained 7.2% arabinose and 29.9% galactose, and the product from ginsenon S-IIA had 8.1% arabinose and 23.5% galactose. These results were consistent with those of methylation analysis.

Based on the accumulated evidence described above, it can be concluded that ginsenon S-IA and ginsenon S-IIA have the structural features shown in Charts 1 and 2,

Chart 1. Component Sugar Residues in the Minimal Unit in the Structure of Ginsenon S-IA

a) Number of residues. Araf, arabinofuranose; Galp, galactopyranose; GalpA, galactopyranosyluronic acid

Chart 2. Component Sugar Residues in the Minimal Unit in the Structure of Ginsenon S-IIA

a) Number of residues. Glcp, glucopyranose.

respectively.

The effects of ginsenon S-IA and ginsenon S-IIA on the RES were demonstrated by a modification⁷⁾ of the *in vivo* carbon clearance test²¹⁾ using zymosan as a positive control. As shown in Fig. 2, the phagocytic indices were markedly increased, suggesting activation of the RES by i.p. injection of these polysaccharides.

The anti-complementary activities of ginsenon S-IA and ginsenon S-IIA are shown in Fig. 3. Both polysaccharides showed remarkable activities on almost the same high level.

Fig. 2. Effects of Ginsenan S-IA and Ginsenan S-IIA on Phagocytosis. Significantly different from the control, a) $p < 0.05$; b) $p < 0.01$.

Fig. 3. Anti-complementary Activity of Ginsenan S-IA and Ginsenan S-IIA

Ginsenan S-IA, —○—; ginsenan S-IIA, —□—; Plantago-mucilage A, —▲—. Each point represents the mean ($n=3$).

Discussion

We earlier isolated two immunologically active polysaccharides, called ginsenan PA and ginsenan PB, from the CTAB-precipitate fraction of the hot water extract of ginseng.¹⁾ Structural studies indicated that they have both α -1,5-linked L-arabino- β -3,6-branched D-galactan and α -1,2-linked L-rhamno- α -1,4-linked D-galacturonan moieties with terminal β -D-glucuronic acid units.

During our studies on the immunologically active polysaccharides in crude drugs, 23 substances have so far been isolated and characterized as RES-activating polysaccharides. Among them, acidic arabino-3,6-galactan is a representative group. In addition to ginsenan PA and ginsenan PB, we have already obtained saposchnikovan A,⁷⁾ MVS-IIIA, -IVA and -VI,²²⁻²⁴⁾ ukonans A, B and C,²⁵⁻²⁷⁾ glycyrrhizans UA, UB and GA,^{18,28)} eucommian A,²⁹⁾ AMon-S,³⁰⁾ AMem-P,³¹⁾ and cnidirhan AG³²⁾ as examples of RES-active polysaccharides belonging to this group.

Both ginsenan PA and ginsenan PB possess intermediate L-rhamnose and terminal D-glucuronic acid units. However, neither ginsenan S-IA nor ginsenan S-IIA has these structural units. The main parts of both ginsenan S-IA and ginsenan S-IIA are occupied by 1,5-linked arabino-3,6-galactan moieties. In contrast to the typical arabino-3,6-galactan, ginsenan S-IA and ginsenan S-IIA have relatively high content of α -3,5-branched L-arabinose and β -1,4-linked D-galactose units in common. These substances are the first example having such 3,5-branched arabinose residues among the acidic arabino-3,6-galactans with RES-potentiating activity obtained by us.

Ginsenan S-IA and ginsenan S-IIA showed remarkable

anti-complementary activity on almost the same level. On the other hand, the RES-potentiating activity of ginsenan S-IIA is distinctly superior to that of ginsenan S-IA. The former possesses α -2,5-branched L-arabinose units to the same degree as α -3,5-branched units. Thus the former has a more complicated branching manner in its arabinosyl residues than the latter. In addition, the presence of α -4,6-branched D-glucan moieties in ginsenan S-IIA may contribute to the elevation of this activity as an example of ukonan C.³³⁾ Further studies of the relationship between the biological activities and structural features of the two polysaccharides are in progress.

References

- 1) M. Tomoda, K. Takeda, N. Shimizu, R. Gonda, N. Ohara, K. Takada, K. Hirabayashi, *Biol. Pharm. Bull.*, **16**, 22 (1993).
- 2) N. Shimizu, M. Tomoda, *Chem. Pharm. Bull.*, **31**, 499 (1983).
- 3) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem.*, **28**, 350 (1956).
- 4) M. Tomoda, Y. Yoshida, H. Tanaka, M. Uno, *Chem. Pharm. Bull.*, **19**, 2173 (1971).
- 5) M. Tomoda, S. Kaneko, M. Ebashi, T. Nagakura, *Chem. Pharm. Bull.*, **25**, 1357 (1977).
- 6) R. Oshima, J. Kumanotani, C. Watanabe, *J. Chromatogr.*, **259**, 159 (1983).
- 7) N. Shimizu, M. Tomoda, R. Gonda, H. Kanari, N. Takanashi, N. Takahashi, *Chem. Pharm. Bull.*, **37**, 1329 (1989).
- 8) N. Blumenkrantz, G. Asboe-Hansen, *Anal. Biochem.*, **54**, 484 (1973).
- 9) M. Tomoda, N. Shimizu, K. Shimada, M. Suga, *Chem. Pharm. Bull.*, **33**, 16 (1985).
- 10) M. Tomoda, M. Ichikawa, *Chem. Pharm. Bull.*, **35**, 2360 (1987).
- 11) N. Shimizu, H. Asahara, M. Tomoda, R. Gonda, N. Ohara, *Chem. Pharm. Bull.*, **39**, 2630 (1991).
- 12) M. Tomoda, K. Shimada, Y. Saito, M. Sugi, *Chem. Pharm. Bull.*, **28**, 2933 (1980).
- 13) G. O. Aspinall, R. J. Ferrier, *Chem. Ind. (London)*, **1957**, 1216.
- 14) H. Yamada, T. Nagai, J.-C. Cyong, Y. Otsuka, M. Tomoda, N. Shimizu, K. Shimada, *Carbohydr. Res.*, **144**, 101 (1985).
- 15) M. Tomoda, N. Shimizu, K. Shimada, R. Gonda, H. Sakabe, *Chem. Pharm. Bull.*, **32**, 2182 (1984).
- 16) N. Shimizu, M. Tomoda, *Chem. Pharm. Bull.*, **33**, 5539 (1985).
- 17) K. Bock, C. Pedersen, H. Pedersen, "Advances in Carbohydrate Chemistry and Biochemistry," Vol. 42, ed. by R. S. Tipson, D. Horton, Academic Press Inc., London, 1984, pp. 193-214.
- 18) N. Shimizu, M. Tomoda, M. Satoh, R. Gonda, N. Ohara, *Chem. Pharm. Bull.*, **39**, 2082 (1991).
- 19) R. L. Taylor, H. E. Conrad, *Biochemistry*, **11**, 1383 (1972).
- 20) I. Ciucanu, F. Kerek, *Carbohydr. Res.*, **131**, 209 (1984).
- 21) G. Biozzi, B. Benacerraf, B. N. Halpern, *Br. J. Exp. Pathol.*, **34**, 441 (1953).
- 22) M. Tomoda, M. Kanari, R. Gonda, N. Shimizu, *Phytochemistry*, **28**, 2609 (1989).
- 23) R. Gonda, M. Tomoda, N. Shimizu, M. Kanari, *Planta Medica*, **56**, 73 (1990).
- 24) R. Gonda, M. Tomoda, M. Kanari, N. Shimizu, H. Yamada, *Chem. Pharm. Bull.*, **38**, 2771 (1990).
- 25) M. Tomoda, R. Gonda, N. Shimizu, M. Kanari, M. Kimura, *Phytochemistry*, **29**, 1083 (1990).
- 26) R. Gonda, M. Tomoda, N. Shimizu, M. Kanari, *Chem. Pharm. Bull.*, **38**, 482 (1990).
- 27) R. Gonda, M. Tomoda, *Chem. Pharm. Bull.*, **39**, 441 (1991).
- 28) M. Tomoda, N. Shimizu, M. Kanari, R. Gonda, S. Arai, Y. Okuda, *Chem. Pharm. Bull.*, **38**, 1667 (1990).
- 29) R. Gonda, M. Tomoda, N. Shimizu, M. Kanari, *Chem. Pharm. Bull.*, **38**, 1966 (1990).
- 30) N. Shimizu, M. Tomoda, M. Kanari, R. Gonda, *Chem. Pharm. Bull.*, **39**, 2969 (1991).
- 31) M. Tomoda, N. Shimizu, N. Ohara, R. Gonda, S. Ishii, H. Otsuki, *Phytochemistry*, **31**, 63 (1992).
- 32) M. Tomoda, N. Ohara, R. Gonda, N. Shimizu, K. Takada, Y. Satoh, S. Shirai, *Chem. Pharm. Bull.*, **40**, 3025 (1992).
- 33) R. Gonda, M. Tomoda, N. Ohara, K. Takada, *Biol. Pharm. Bull.*, **16**, 235 (1993).