Inhibition of IgE Antibody Formation by n-Pentyl α -L-Sorbopyranoside

Naoki INAGAKI, Akihida KODA, Akira YAGI*, Yasushi HARAGUCHI**,
Hiroichi NAGAI, Kanji NODA** and Itsuo NISHIOKA*

Department of Pharmacology, Gifu Pharmaceutical University,
Mitahora-higashi, Gifu 502, Japan

*Faculty of Pharmaceutical Sciences, Kyushu University,
Maidashi, Higashi-ku, Fukuoka 812, Japan

**Research Laboratory, Hisamitsu Pharmaceutical Co., Ltd.,
Tashiro, Tosu, Saga 841, Japan

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Abstract—Effect of n-pentyl glycosides and alkyl α -L-sorbopyranosides on IgE antibody formation in rats and mice were investigated. When n-pentyl α -L-sorbopyranoside was given subcutaneously or orally, the IgE antibody formation in rats and mice was suppressed, while no suppression of hemagglutinin formation was observed. The study on timing of administration indicated that n-pentyl α -L-sorbopyranoside significantly suppressed the secondary IgE antibody response when administered before the secondary immunization.

By the screening of alcoholic or aqueous extracts of twenty traditional Chinese crude drugs which had been widely used for diseases caused by allergies, alcoholic extracts of *Zizyphus Fructus* and *Gentianae scabrae* Radix were found to suppress the IgE antibody formation in rats immunized with dinitrophenylated *Ascaris suum* extract (DNP-As) (1). Alcoholic extract of *Zizyphus Fructus* was less suppressive in hemolytic plaque forming cell (HPFC) formation in spleens of mice immunized with sheep red blood cells (SRBC) than that of *Gentianae scabrae* Radix. Furthermore, alcoholic extract of *Zizyphus Fructus* directly suppressed 48-hr homologous passive cutaneous anaphylaxis (48-hr homologous PCA) in rats sensitized with anti-DNP-As IgE serum. These results suggested that the alcoholic extract of *Zizyphus Fructus* has constituents which suppress the IgE antibody formation without affecting the generation of IgM antibody forming cells and directly suppress the PCA.

Chemical and immunological studies on the alcoholic extract of *Zizyphus Fructus* demonstrated that ethyl α -D-fructofura-

noside, a configurational and conformational isomer of ethyl D-fructosides, was identified as one of the active components which suppress the IgE antibody formation (2). In an earlier experiment on derivatization of alkyl D-fructosides, it was found that n-pentyl β -D-fructopyranoside given either i.p. or p.o. effectively suppressed the IgE antibody formation in both rats and mice without any suppression of hemagglutinin (HA) formation (3).

In order to find out a more specific and more potent inhibitor of IgE antibody formation, the screening of various synthesized alkyl glycosides to IgE antibody and HA formations was undertaken. This paper describes how n-pentyl α -L-sorbopyranoside suppresses the IgE antibody formation in connection with the action of n-pentyl β -D-fructopyranoside (3).

Materials and Methods

Animals: Male and female Wistar rats of 9–10 weeks of age, male and female ddy mice of 7 weeks of age and female BALB/c mice of 7–11 weeks of age were obtained from Shizuoka Laboratory Animal Center (Shizuoka

Japan). They were fed laboratory chow and water ad libitum.

Alkyl glycosides: Alkyl glycosides were prepared in good yields by a modification of the standard method. In a typical experiment, dried L-sorbose (10.0 g) was dissolved in n-pentyl alcohol (490 ml) and kept at 140°C for 2 hr. Then, sulfuric acid (0.2 g) was added to the solution and was allowed to stand for 24 hr at room temperature. The reaction mixture was neutralized with 25% ammonia, the precipitate was filtrated off; and the filtrate was concentrated to a syrup. The syrup containing conformational and configurational isomers was chromatographed over a silica-gel column, using EtOAc saturated with H₂O or CHCl₃-EtOH-H₂O (7:3:1) as a solvent, to give 8.0 g of n-pentyl α -L-sorbose in a pure L-sorbopyranoside form. On a large scale production, however, the yield of n-pentyl α -L-sorbopyranoside decreased to 66%. In the same way, the formation of alkyl glycosides was undertaken to yield a mixture of conformational and configurational isomers, each of which was purified by means of silica-gel chromatography.

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected.

Optical rotation was measured in a MeOH solution on a JASCO DIP-SL automatic polarimeter at 22°C.

¹³C nuclear magnetic resonance (NMR) spectra of the 0.5 M CD₃OD solution were recorded at 22°C in a 5 mm spinning tube with the JEOL FX-100 using Me₄Si as the internal standard (25.5 MHz). Fourier transform measurement conditions: spectral width, 47.3 KHz; pulse flipping angle, 45–90°; number of data points, 8192; pulse repetition, 3 sec.

Mass spectral data were recorded on a JEOL-D 100 mass spectrometer with an accelerating potential of 30 eV and a source temperature at 170°C.

Gas liquid chromatography (GLC) analyses were performed on a Shimadzu GC-4BM gas chromatograph equipped with flame ionization detectors, and carried out on permethyl ethers (condition A) and on the trimethylsilyl (TMS) ether (condition B) of

alkyl L-sorbosides with an on column system. Condition A: 5% 1,4-butanediol succinate, Shimalite W (60–80 mesh), 3 mm ϕ ×2 m glass column, column temperature of 175°C, detector temperature at 220°C, carrier gas N₂ at 40 ml/min. Condition B: 2% silicone OV 17, Chromosorb W (AW-DMCS, 60–80 mesh), 3 mm ϕ ×2 m glass column, column temperature of 200°C, carrier gas N₂ at 40 ml/min.

Immunization: Female Wistar rats and BALB/c mice were used for the formation of antibodies. Throughout the experiment, DNP-As was used as an antigen. The preparation of DNP-As was as follows: An extract from *Ascaris suum* prepared by the method of Strejan and Campbell (4) was dinitrophenylated with 2,4-dinitrobenzene sulfonic acid by the method of Eisen et al. (5). The reaction mixture was dialyzed to remove uncoupled reagent, then, lyophilized and stored at –20°C. Protein content of the antigen measured by the micro-biuret method (6) was 21.0%, and the amount of antigen employed in this experiment was expressed as the amount of protein. As adjuvants, killed *Bordetella pertussis* (killed *B. pertussis*), 2×10¹⁰/ml, supplied by Kaken Pharmaceutical Co., Ltd., and aluminium hydroxide gel (alum) were used for rats and mice, respectively. Alum was prepared by the method of Levine and Vaz (7) with slight modifications. Briefly, a mixture of equal volume of 2 N aluminium sulfate and 2 N sodium hydroxide was stirred for 10 min at room temperature. Then, the precipitate was further stirred with a Polytron (Kinematica GmbH) and washed with distilled water until no sulfate ion could be detected in the supernatant. Finally, alum was suspended in 0.02 M borate buffered saline (pH 8.0) in a concentration of 20 mg/ml.

According to the method of Tada and Okumura (8), rats were immunized with 1 mg of DNP-As and 10¹⁰ of killed *B. pertussis* (Day 0). Five days later (Day 5), they were boosted with 0.5 mg of DNP-As alone. On Day 8, rats were sacrificed, and the serum was obtained from each rat.

Mice were immunized i.p. with 10 μ g of DNP-As and 2 mg of alum or 2.5 μ g of DNP-As and 0.5 mg of alum. In some experiments,

mice were immunized secondarily (2nd immunization) in the same way as the primary (1st) immunization. They were bled at appropriate intervals, and sera were separated.

Serum samples obtained from rats and mice were stored at -20°C until the time of assay. Test compounds were dissolved in physiological saline and given s.c. to the animals for 5 days before or after the immunization. In some experiments, test compounds were dissolved in 2% NaHCO_3 solution and administered p.o. Control animals received the vehicle only, except for the non-treated control.

Evaluation of IgE antibody: Serum IgE antibody levels were evaluated by the PCA according to the technique described by Ovary (9). Briefly, 2-fold serially diluted serum samples were injected intradermally into the shaved backs of normal male Wistar rats in duplicate. A twenty-four-hr sensitization period was used for mouse IgE antibody, and a 48-hr sensitization period was used for rat IgE antibody. PCA was elicited by an intravenous injection of 1 ml of saline containing 1 mg of DNP-As and 5 mg of Evans blue dye. Thirty min after the injection, rats were sacrificed, and the PCA titer was measured. The PCA titer recorded is the greatest dilution that gave a positive skin reaction.

Evaluation of hemagglutinin (HA): The passive hemagglutination test was carried out by the method of Avrameas et al. (10). Serum samples were serially diluted in microtiter plates, and each portion of the serum (50 μl) was mixed with 50 μl of DNP-As tanned SRBC suspension. After incubation for 2 hr at 37°C , the agglutinations at the bottom of the plates were evaluated. The HA titer recorded is the greatest dilution that gave complete agglutination.

Toxicity test: Toxicity of alkyl glycosides was estimated as follows: Test compounds, in doses of 0.3, 0.5, 1, 2 and 5 g/kg, were given to ddY mice or Wistar rats by various routes. In p.o. administration, animals were fasted for 6 to 8 hr before the administration. After the observation period of 21 days, the number of survivals were counted.

Statistical analysis: Titers given in the

tables are the mean except for the value obtained from pooled sera. Difference between the control group and the experimental group was statistically evaluated by Wilcoxon's *U*-test (11).

Results

Effect of n-pentyl glycosides on antibody formations in mice: Nine kinds of n-pentyl glycosides were synthesized by the method described in the experimental section, and pyranosides of β -D-arabinose, β -D-galactose, α -L-sorbose and β -D-ribofuranose were obtained as a pure form. The physical properties of these compounds are shown in Table 1. To investigate the effect of these compounds on antibody formations, mice were immunized with 10 μg of DNP-As and 2 mg of alum, and these compounds were given at 100 mg/kg for 5 days from the day of immunization. Ten days after the immunization, mice were bled and serum samples were collected. Table 1 shows that n-pentyl α -L-sorbopyranoside given s.c. (Exp. I) reduced the PCA titer and that n-pentyl β -D-ribofuranoside, n-pentyl β -D-galactopyranoside, n-pentyl α , β -L-rhamnopyranoside and n-pentyl α -L-sorbopyranoside dissolved in 2% NaHCO_3 solution and given p.o. (Exp. II) reduced the PCA titer. All these compounds showed almost no reducing effect on the HA titer.

Physical properties of alkyl α -L-sorbopyranosides and their effect on antibody formations: As n-pentyl α -L-sorbopyranoside suppressed the IgE antibody formation without affecting the HA formation, several alkyl α -L-sorbopyranosides were synthesized, and the effect on antibody formations were investigated. ^{13}C NMR spectral data and other physical properties of alkyl α -L-sorbopyranosides synthesized are shown in Tables 2 and 3. The effect of these compounds on antibody formations in rats is shown in Table 3. Rats received these compounds at 100 mg/kg, s.c., for 5 days from Day 0. n-Butyl α -L-sorbopyranoside reduced the PCA titer, but n-pentyl α -L-sorbopyranoside only showed a tendency to do so. These compounds did not reduce the HA titer. The effects of these compounds in mice are shown in Table 4. In two experiments, mice

Table 1. Effect of n-pentyl glycosides on IgE antibody and hemagglutinin (HA) formations in female BALB/c mice

n-Pentyl glycoside	Mp	[α] _D	Yield (%)		Exp. I		Exp. II		
					PCA titer	HA titer	PCA titer	HA titer	
Control				(6)	166	32	(5)	486	32
β -L-Arabinopyranoside	142–3	–240	68	(5)	198	64	(5)	352	64
α , β -D-Xylopyranoside	NT	NT	NT	(5)	179	64	(5)	349	32
β -D-Ribofuranoside	71–2	–52.6	22	(5)	115	32	(5)	90 [†]	16
α , β -D-Glucopyranoside	NT	NT	NT	(5)	154	128	(5)	224	32
β -D-Galactopyranoside	96–7	NT	40	(5)	230	64	(5)	186*	32
α , β -D-Mannopyranoside	NT	NT	NT	(4)	96	32	(5)	461	128
α β -L-Rhamnopyranoside	NT	NT	NT	(4)	352	128	(5)	157*	64
α -L-Sorbo pyranoside	92–3	–70.1	80	(5)	54 [†]	32	(5)	179*	16
β -D-Fructopyranoside	129–30	–123.0	35	(5)	150	32	(5)	262	64

Mice were immunized with 10 μ g of DNP-As and 2 mg of alum on Day 0. n-Pentyl glycosides were given 100 mg/kg/day, s.c., in Exp. I and p.o. in Exp. II for 5 days from Day 0. In p.o. administration, n-pentyl glycosides were dissolved in 2% NaHCO₃ solution. Serum was collected from each mouse on Day 10 to estimate antibody titers. PCA titers are represented as the mean, and HA titers are values estimated with pooled sera. Figures in parentheses indicate the number of mice used. * $P \leq 0.05$, [†]: $P \leq 0.01$, NT: Not tested.

were immunized with 10 μ g of DNP-As and 2 mg of alum on Day 0, and serum samples were obtained on Day 10. Test compounds were given 100 mg/kg/day, s.c., for 5 days from Day 0 in both Exp. I and II. In Exp. I, n-butyl α -L-sorbopyranoside reduced the PCA titer without affecting the HA titer, but n-pentyl α -L-sorbopyranoside did not reduce the PCA titer. In Exp. II, n-pentyl and n-hexyl α -L-sorbopyranosides reduced the PCA titer.

Effect of n-pentyl β -D-fructopyranoside, n-butyl α -L-sorbopyranoside and n-pentyl α -L-sorbopyranoside on antibody formations in mice: The possibility was indicated that n-butyl and n-pentyl α -L-sorbopyranosides suppress the IgE antibody formation in rats and mice without suppressing the HA formation. As shown in Table 5, toxicities of these two compounds were very low. Then, further experiments were performed in connection with the action of n-pentyl β -D-fructopyranoside reported previously (3).

First, the timing of administration of these compounds was investigated in mice. Mice were immunized with 2.5 μ g of DNP-As and 0.5 mg of alum, twice, on Days 0 (1st immunization) and 30 (2nd immunization), in the same way. Administrations, 100 mg/kg/

day, s.c., for 5 days, of these compounds were performed as follows: 1) before the 1st immunization (Day -4~0), 2) after the 1st immunization (Day 0~4), 3) before the 2nd immunization (Day 26~30), and 4) after the 2nd immunization (Day 30~34). As shown in Table 6, n-pentyl β -D-fructopyranoside administered after the 1st immunization reduced the PCA titer throughout the experiment. These alkyl glycosides given before the 2nd immunization significantly reduced the PCA titer on Day 37. In the case that these compounds were given after the 1st and before or after the 2nd immunization, the HA titer on Day 37 was significantly increased.

Next, the studies on dose-dependency of these compounds were carried out in mice. Mice were immunized with 2.5 μ g of DNP-As and 0.5 mg of alum, twice, on Days 0 and 36. The three alkyl glycosides were given 10-200 mg/kg/day, s.c., for 5 days from Day 0. As shown in Table 7, n-pentyl α -L-sorbopyranoside given in doses of 20~200 mg/kg reduced the PCA titer throughout the experiment, but a clear dose-dependency was not observed. The HA titer in this experiment was not reduced at any time.

n-Pentyl β -D-fructopyranoside and n-butyl α -L-sorbopyranoside were less effective

Table 2. ¹³C NMR spectral data of alkyl α-L-sorboopyranosides

Alkyl α-L-sorboopyranoside	Chemical shift from TMS (ppm)*							
	C-2	C-3†	C-4†	C-5†	C-6†	C-1†	O-Alkyl	
Methyl	100.9	75.4	73.2	71.0	63.8	62.1	48.5	
Ethyl	101.1	75.4	73.0	71.0	63.7	62.5	56.8	15.8
n-Propyl	100.8	75.3	73.1	70.9	63.6	62.8	62.5	24.0 11.0
n-Butyl	100.9	75.4	73.1	71.0	63.8	62.6	61.0	33.1 20.3 14.2
iso-Butyl	100.7	75.3	73.3	71.0	67.7	63.7	62.6	29.7 20.0 19.7
n-Pentyl	100.8	75.2	73.0	70.8	63.6	62.5	61.2	30.5 29.2 23.3 14.2
iso-Pentyl	100.9	75.3	73.1	70.9	63.8	62.6	59.7	39.8 25.9 23.0 22.9
n-Hexyl	100.8	75.3	73.1	70.9	63.7	62.6	61.3	32.6 30.9 26.8 23.4 14.3

*: Recorded at 25.5 MHz in CD₃OD with Me₄Si as the internal standard. †: These assignments may be reversed.

Table 3. Physical properties of alkyl α-L-sorboopyranosides and their effect on IgE antibody and hemagglutinin (HA) formations in female Wistar rats

Exp.	Alkyl α-L-sorboopyranoside	Mp	[α] _D	Yield (%)	PCA titer	HA titer
Exp. I	Control				168	1902
	Methyl	123-5	-92.3	99	199	4352
	Ethyl	126-8	-77.0	69	130	4535
	n-Propyl	94-5	-77.3	66	105	6144†
	n-Butyl	46-7	-73.2	67	68*	9344*
Exp. II	Control				344	4242
	iso-Butyl	79.0	-60.2	64	244	7296
	n-Pentyl	91-2	-71.1	66	172	4352
	iso-Pentyl	72.0	-65.6	68	264	7680
	n-Hexyl	77-8	-77.5	42	380	3776

Rats were immunized with 1 mg of DNP-As and 10¹⁰ of killed *B. pertussis* on Day 0 and boosted with 0.5 mg of DNP-As on Day 5. Alkyl α-L-sorboopyranosides were given 100 mg/kg/day, s.c., for 5 days from Day 0. Serum was collected from each rat on Day 8 to estimate antibody titers. Titers are re-presented as the mean of 8 rats. *: P≤0.05, †: P≤0.01.

Table 4. Effect of alkyl α -L-sorbopyranosides on IgE antibody and hemagglutinin (HA) formations in female BALB/c mice

Alkyl α -L-sorbopyranoside		Exp. I			Exp. II	
		PCA titer	HA titer		PCA titer	HA titer
Control	(8)	480	112	(7)	841	567
Methyl	(6)	512	256*	(4)	544	256
Ethyl	(6)	427	160	(4)	800	384
n-Propyl	(6)	373	160	(4)	704	320
n-Butyl	(6)	256*	107	(4)	1024	320
iso-Butyl	(6)	469	117	(4)	800	160
n-Pentyl	(6)	416	139	(4)	416*	384
iso-Pentyl	(6)	448	149	(4)	896	416
n-Hexyl	(6)	448	117	(4)	416*	160

Mice were immunized with 10 μ g of DNP-As and 2 mg of alum on Day 0, and alkyl α -L-sorbopyranosides were given 100 mg/kg/day, s.c., for 5 days from Day 0 in both Exp. I and II. Serum was collected from each mouse on Day 10 to estimate antibody titers. Titers are represented as the mean, and figures in parentheses indicate the number of mice used. *: $P \leq 0.05$.

Table 5. Toxicity of n-butyl and n-pentyl α -L-sorbopyranosides

Route	Sex	Toxic dose (g/kg)			
		n-Butyl α -L-sorbopyranoside		n-Pentyl α -L-sorbopyranoside	
		Rat	Mouse	Rat	Mouse
p.o.	male	>5	>5	>5	5~2
	female	NT	NT	>5	5~2
s.c.	male	>5	>5	>5	2
	female	NT	NT	>5	2
i.p.	male	>5	>1	2	1~0.5
	female	NT	NT	2	1~0.5
i.v.	male	>2	>2	2~1	2~1
	female	NT	NT	2~1	2~1

Each value represents the dose or dose range which gave 50% lethality.

than n-pentyl α -L-sorbopyranoside in this experiment (data not shown).

Discussion

Allergic disorders such as bronchial asthma and hay fever are caused by IgE antibody as classified by Ishizaka et al. (12). This suggests, therefore, a possible therapy for allergic disorders by regulating the IgE antibody level. Several attempts to modulate the IgE antibody response have been made by a number of investigators. Urea-denatured antigens suppress the IgE antibody response not only in the primary response but also in

the ongoing response in mice immunized with native antigens (10, 12–15). It has been shown that modified antigens such as ovalbumin-polyethylene glycol (16), ovalbumin-pullulan (17), DNP-isologous protein (18), DNP-mycobacterium (19), DNP-Ficoll (20), DNP-liposome (21), DNP-polyvinyl alcohol (22), and passively administered antibody (8, 23) suppressed the IgE antibody response. Furthermore, orally administered antigen (24) and aerosolized antigen (25) suppressed the IgE antibody response against the antigen. However, all of these methods for the regulation of the IgE

Table 6. Effect of n-pentyl β -D-fructopyranoside, n-butyl α -L-sorbopyranoside and n-pentyl α -L-sorbopyranoside on IgE antibody and hemagglutinin (HA) formations in female BALB/c mice

Timing of administration and alkyl glycoside	Day 10		Day 20		Day 37	
	PCA titer	HA titer	PCA titer	HA titer	PCA titer	HA titer
Non-treated control	(9)	248	(9)	199	(5)	1178
1) Before the 1st immunization						
n-Pentyl β -D-fructopyranoside	(4)	160	(4)	176	(4)	768
n-Butyl α -L-sorbopyranoside	(6)	256	(6)	277	(6)	8192<
n-Pentyl α -L-sorbopyranoside	(6)	320	(6)	256	(6)	1237
2) After the 1st immunization						
n-Pentyl β -D-fructopyranoside	(6)	107*	(6)	181	(6)	1664†
n-Butyl α -L-sorbopyranoside	(6)	403	(6)	221	(6)	2453
n-Pentyl α -L-sorbopyranoside	(6)	331	(6)	192	(6)	3413
3) Before the 2nd immunization						
n-Pentyl β -D-fructopyranoside			(6)		(6)	8192<†
n-Butyl α -L-sorbopyranoside			(6)		(6)	8192<†
n-Pentyl α -L-sorbopyranoside			(6)		(6)	8192<†
4) After the 2nd immunization						
n-Pentyl β -D-fructopyranoside			(6)		(6)	203†
n-Butyl α -L-sorbopyranoside			(6)		(6)	299†
n-Pentyl α -L-sorbopyranoside			(6)		(6)	213†
n-Pentyl β -D-fructopyranoside			(6)		(6)	2176*
n-Butyl α -L-sorbopyranoside			(6)		(6)	2304
n-Pentyl α -L-sorbopyranoside			(6)		(6)	2560

Mice were immunized with 2.5 μ g of DNP-As and 0.5 mg of alum on Days 0 (1st) and 30 (2nd). Alkyl glycosides were given 100 mg/kg/day, s.c., for 5 days before or after the 1st and/or 2nd immunization. Serum was collected from each mouse on Days 10, 20 and 37 to estimate antibody titers. Titers are represented as the mean, and figures in parentheses indicate the number of mice used. *: $P \leq 0.05$, †: $P \leq 0.01$.

Table 7. Effect of n-pentyl α -L-sorbopyranoside on IgE antibody and hemagglutinin (HA) formations in female BALB/c mice

n-Pentyl α -L-sorbopyranoside (mg/kg)	Day 10		Day 20		Day 43	
	PCA titer	HA titer	PCA titer	HA titer	PCA titer	HA titer
0 (Control)	640	141	138	61	4301	2662
10	282	205	174	96	3789	2355
20	93*	115	74	102	2202	3482
50	179	157	107	51	1741*	1946
100	211	110	53	141	2867	2150
200	173	102	84	96	1792*	2458

Mice were immunized with 2.5 μ g of DNP-As and 0.5 mg of alum on Days 0 and 36. Five doses of n-pentyl α -L-sorbopyranoside were given s.c. for 5 days from Day 0. Serum was collected from each mouse on Days 10, 20 and 43 to estimate antibody titers. Titers are represented as the mean of 5 mice.

*: $P \leq 0.05$.

antibody formation provided only an antigen-specific activity. Recently, Nakashima et al. (26) reported that polysaccharide fractions prepared from a mushroom, *Baikisei Ganoderma applanatum*, suppressed the IgE antibody formation in mice, and this suppression was IgE class-selective and antigen-non-specific.

In order to find a new type of agent which suppresses the IgE antibody formation, extracts from twenty traditional Chinese crude drugs which had been used for allergic disorders were examined, and alcoholic extract of *Zizyphus Fructus* was found to suppress the IgE antibody formation in rats immunized with DNP-As without affecting the generation of IgM antibody forming cells (1). Previous experiments indicated that ethyl α -D-fructofuranoside was one of the active components of the alcoholic extract of *Zizyphus Fructus* (2), and n-pentyl β -D-fructopyranoside, one of the derivatives of ethyl α -D-fructofuranoside, revealed a potent effect (3).

Further studies of n-pentyl glycosides and alkyl α -L-sorbosides on the IgE antibody formation in rats and mice immunized with DNP-As were undertaken to find an antigen-independent and more potent drug.

The purity of each n-pentyl glycoside separated on chromatography was examined by means of gas chromatographical analyses of the trimethylsilyl derivatives. The ring size and anomeric nature were assigned to be glycosides by means of gas chromatography

(27), mass spectrometry (28), ^1H NMR spectrometry (29) and ^{13}C NMR spectroscopy (30). As a potent tool, ^{13}C NMR spectroscopy was effectively applied to the conformational and configurational analyses. Based on the spectral examination, assignments of glycosidic and configurational linkages in each glycoside were accomplished. As shown in Table 2, the ^{13}C NMR spectral examination on C-2 in the alkyl α -L-sorbosides indicated that the synthesized alkyl L-sorbosides had an α -L-sorbopyranoside form. On gas chromatographical analyses, it was confirmed that the purity of each alkyl α -L-sorbopyranoside was 98~99%.

Among n-pentyl glycosides, n-pentyl α -L-sorbopyranoside given s.c. and p.o. suppressed the IgE antibody formation significantly in mice immunized with DNP-As (Table 1). However, the HA formation, estimated with pooled sera, was not suppressed. In p.o. administration, n-pentyl glycosides were dissolved in 2% NaHCO_3 solution to prevent n-pentyl glycosides from degradation by gastric juice (3). In the present results, it was confirmed that n-pentyl α -L-sorbopyranoside was effective when administered p.o. In these experiments, n-pentyl β -L-fructopyranoside was less suppressive than n-pentyl α -L-sorbopyranoside. Besides, n-pentyl α -L-sorbopyranoside had the highest yield. Therefore, the structure-activity relationships of alkyl α -L-sorbopyranosides were examined. In

synthesized alkyl α -L-sorbopyranosides, n-butyl and n-pentyl α -L-sorbopyranosides were found to suppress the IgE antibody formation in both rats and mice, while no suppressive effect of these compounds on the HA formation was observed (Tables 3 and 4). These results suggest that the n-butyl or n-pentyl residue in the α -L-sorbopyranoside ring is necessary for eliciting the suppressing effect.

In all the experiments discussed above, alkyl glycosides were given to the animals after the immunization. Since timing of administration is an important factor which has an effect on the immunization, it was examined using n-butyl and n-pentyl α -L-sorbopyranosides and n-pentyl β -D-fructopyranoside. As shown in Table 6, when these compounds were administered before the 2nd immunization, the secondary IgE antibody response of each group was suppressed significantly, although the HA formation was significantly enhanced. Though the mechanism of the significant enhancement of HA formation observed only in this experiment is not clear, it is sure that these compounds are not suppressive for the HA formation. Next, the dose-dependencies of these compounds were examined. As shown in Table 7, n-pentyl α -L-sorbopyranoside suppressed the IgE antibody formation in doses of 20~200 mg/kg, but a clear dose-dependent action was not observed. n-Butyl α -L-sorbopyranoside and n-pentyl β -D-fructopyranoside, however, were less suppressive than n-pentyl α -L-sorbopyranoside in this experiment.

The toxicity of n-pentyl α -L-sorbopyranoside is very low (Table 5), and the effect is expected to be antigen-independent, because this compound has no relation with any antigenic substance. Accordingly, the possibility of n-pentyl α -L-sorbopyranoside as a new type of antiallergic agent is fully expected. Both n-pentyl α -L-sorbopyranoside and n-pentyl β -D-fructopyranoside having a ketohexopyranose conformation showed a similar suppression of the IgE antibody formation. The n-pentyl residue in a glycoside form of ketohexopyranose might participate in the suppression of the IgE antibody formation.

Further experiments on mechanisms of the action of n-pentyl α -L-sorbopyranoside are in progress.

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