Synthesis and Physiological Activity of Novel Tocopheryl Glycosides

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Vitamin E glycosides were synthesized and enzymatic hydrolysis was examined for use as potential prodrugs, however, the glycoside bond was found to be stable. On the other hand, among the glycosides synthesized, *dl*-α-tocopherylglucoside (6b) and *dl*-α-tocopherylmannoside (6c) showed strong inhibitory action on histamine release from mast cells. In addition, 6c also showed a suppressive action on IgE antibody formation. Thus, tocopheryl glycoside showed new properties compared to tocopherol (vitamin E). In particular, 6c was shown to be a novel lead compound with excellent manifold anti-allergic activity and anti-inflammatory activity.

Key words tocopheryl glucoside; anti-inflammatory drug; allergy; IgE; pro-drug

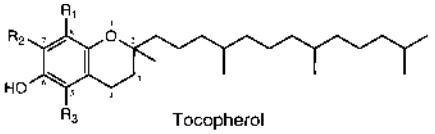
Vitamins are organic compounds that control nutrition in humans and animals in extremely small amounts and are involved in various physiological phenomena in the living body. We have to take vitamins in our diet because they are not produced through biosynthesis. Recently, many vitamins which have a specific physiological activity have been used as medicines. Among them, vitamin E (tocopherol), an oil-soluble vitamin, has been in frequent use in large amounts and for long periods since the 1970s to treat various diseases and has attracted great attention clinically. Clinical information concerning vitamin E is widespread and includes its effects on gynecological internal secretion control against sterility, heart circulation, liver diseases, peripheral blood circulation and thrombosis,[2)] drug poisoning and radiation damage, aging, and carcinogenesis. While vitamin E thus has the potential to be a very useful drug, it has some defects as drug such as poor stability and absorbability and accumulation in fat. Vitamin E naturally occurs in two forms, tocopherol and tocotrienol, each having four homologues, α, β, γ, and δ (Fig. 1).[3)] In the work described herein, we have attempted to synthesize vitamin E derivatives (prodrugs) that possess better stability and absorbability and rapidly emerge as vitamin E in the body, by chemically modifying the phenolic hydroxyl group of tocopherol and have examined the pharmacological activity of the resulting derivatives.

Chemistry Glycolation of α- and δ-homologues was performed according to the Helferich method.[4)] Each tocopherol (**2**, **3**) and each acetylated sugars (**1a**—**c**) was dissolved in nitrobenzene. After adding *p*-toluene sulfonic acid as a catalyst to the resultant solution, the reactants were allowed to react under an N_2 atmosphere during distillation at 90 °C of the solvent at reduced pressure (20 mmHg) to give the intermediate compounds (**4a**—**c**, **5a**—**c**). Deacetylation of **4a**—**c** and **5a**—**c** in dry methanol containing sodium methoxide at reflux yielded the desired compounds (**6a**—**c**, **7a**—**c**) (Chart 1).[5,6)]

Results and Discussion

Enzymatic hydrolysis of the glycoside bond is necessary in the body in order that the synthesized tocopherol derivatives can display the physiological activity of vitamin E. A regeneration test was then conducted. Since the compounds synthesized were hardly soluble in water, enzymatic hydrolysis was examined using water-containing organic solvents and cosolvents. Then, stability of β-galactosidase[7)] in organic solvents containing 50% water was examined. As a result, the enzyme was found to keep 90% of its activity in *tert*-butanol containing 30% water, even after 10 h, as shown in Fig. 2. Hydrolysis of **6a** and **7a** by β-galactosidase was then tested in the mixed solvent. As seen in Fig. 3, the percentage of hydrolysis for **6a** was below 50% even after 8 h, showing that the compound is poorly hydrolyzable. On the contrary, **7a**, δ-tocopherol and α-tocopherol without methyl groups at the 5 and 7 positions, was rapidly hydrolyzed enzymatically. Examination was then carried out on the stability of β-glucosidase. In contrast to β-galactosidase, β-glucosidase lost its activity appreciably in organic solvent containing water and hydrolysis experiments were hence performed on **6b** and **7b** solubilized in Triton X-100 solution. Almost no hydrolysis was observed with **6b** even after 72 h, while **7b** was easily hydrolyzed, as shown in Fig. 4. This was thought to be due to the steric effect of methyl groups at the 5 and 7 positions of α-tocopherol and δ-tocopherol was then expected to be hydrolyzed several hundred times more easily. Contrary to this expectation, however, a preliminary *in vivo* experiment revealed that both the α- and δ-homologues were hardly hydrolyzed enzymatically, suggesting that they can not act as prodrugs of vitamin E.

We expected the tocopherol derivatives to have new physiological activity because they were resistant to enzymatic hy-



Tocopherol	R_1	R_2	R_3	Tocotrienol
α-Tocopherol	CH_3	CH_3	CH_3	α-Tocotrienol
β-Tocopherol	CH_3	H	CH_3	β-Tocotrienol
γ-Tocopherol	CH_3	CH_3	H	γ-Tocotrienol
δ-Tocopherol	CH_3	H	H	δ-Tocotrienol

Fig. 1. Structure of Tocopherol and Tocotrienol

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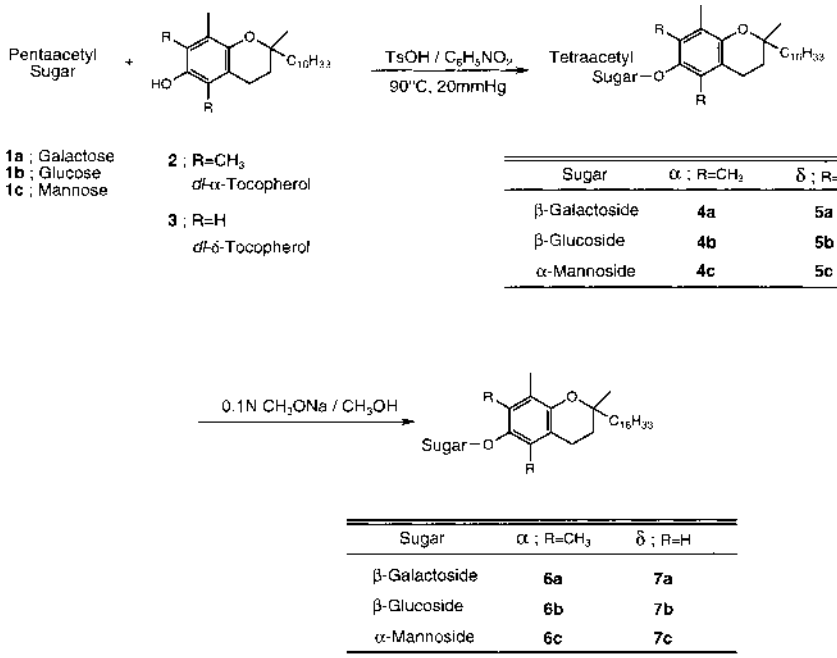
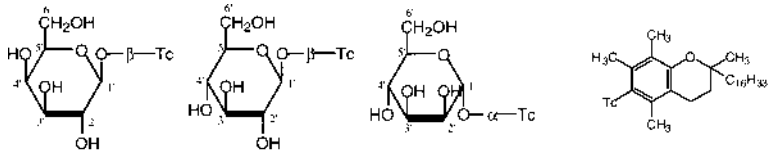


Sugar	α ; R=CH3	δ ; R=H
β-Galactoside	**4a**	**5a**
β-Glucoside	**4b**	**5b**
α-Mannoside	**4c**	**5c**

Sugar	α ; R=CH3	δ ; R=H
β-Galactoside	**6a**	**7a**
β-Glucoside	**6b**	**7b**
α-Mannoside	**6c**	**7c**

Chart 1

Table 1. Physical Properties of Compounds **6a**—**c**



6a **6b** **6c**

dl-α-Tocopherylglycoside	mp (°C)	UV $\lambda_{max}^{CH_3OH}$ nm (logε)	$[\alpha]_D^{24}$ (c=1.0, CH_3OH)	Yield (%)
β-Galactoside (**6a**)	176—177	293 (2.70) 210 (3.30)	10.1°	56
β-Glucoside (**6b**)	140—141	293 (2.63) 210 (3.28)	−1.5°	54
α-Mannoside (**6c**)	128—130	280 (3.35) 213 (4.04)	13.7°	59

Table 2. ^{1}H- and ^{13}C-NMR Data of Compounds **6a**—**c**

Compound	Anomeric proton ^{1}H-NMR δ (ppm)[a]	^{13}C-NMR δ (ppm)[a]					
		C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
6a	4.68 (1H, d, J=7.8 Hz)	104.5	74.9	76.7	69.8	76.5	61.8
6b	4.51 (1H, d, J=7.6 Hz)	102.7	75.7	77.0	70.5	78.5	60.8
6c	4.52 (1H, d, J=3.0 Hz)	106.7	73.0	72.4	68.9	75.0	63.4

a) Determined in CD_3OD

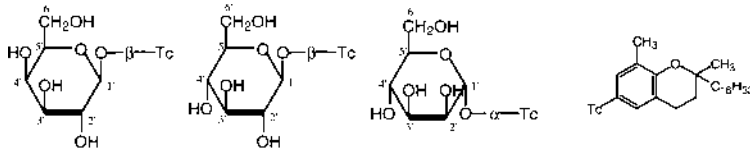
drolysis. Vitamin E is known to have a strong affinity to biomembranes through interaction of its side chains with arachidonic acid in the phospholipids of the membrane.[8—10] Sugar chains in immunoglobulins (IgG, IgM, IgE, IgA, IgD) adhered to mast cells and basophils are also known to play an important role in the recognition and function of the globulins. We then examined the inhibitory action of the tocopherol derivatives on histamine release from mast cells caused by antigen–antibody reaction, in particular the type I allergic reaction in which IgE is involved. As a result, **6b** and **6c** were found to inhibit histamine release (Fig. 5). The inhibiton was concentration-dependent between 10 and 300 μg/ml. The percentage of inhibition for **6b** was 25% at 10 μg/ml, 40% at 30 μg/ml, 55% at 100 μg/ml, and 75% at 300 μg/ml while that for **6c** was 28% at 10 μg/ml, 55% at 30 μg/ml, 72% at 100 μg/ml, and 76% at 300 μg/ml. Regarding the mechanism, the results suggest the possibility that these derivatives prevent the antigen from forming a linkage with IgE antibody because **7c** displayed better inhibition than **7b** and the sugar chain attached to Fc fragment of the δ-chain in IgE is mainly mannose.

Since **6b** and **6c** were found to have antiallergic activity, we then examined their suppressive action on IgE antibody formation. As shown in Table 5, **6c** suppressed IgE formation at doses of 30 and 100 mg/kg against a control, the action being stronger than that of hydrocortisone, a positive control, at 10 mg/kg.

Conclusions

Vitamin E is a compound that exhibits a variety of physio-

Table 3. Physical Properties of Compounds **7a**—**c**



7a **7b** **7c**

dl-δ-Tocopherylglycoside	mp (°C)	UV $\lambda_{max}^{CH_3OH}$ nm (logε)	$[\alpha]_D^{24}$ (c=1.0, CH_3OH)	Yield (%)
β-Galactoside (**7a**)	70—72	289 (3.47) 212 (3.98)	−11.2°	39
β-Glucoside (**7b**)	46—49	282 (3.58) 210 (3.96)	−18.2°	57
α-Mannoside (**7c**)	187—189	281 (3.30) 212 (3.87)	24.5°	46

Table 4. ^{1}H- and ^{13}C-NMR Data of Compounds **7a**—**c**

Compound	Anomeric proton ^{1}H-NMR δ (ppm)[a]	^{13}C-NMR δ (ppm)[a]					
		C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
7a	4.72 (1H, d, J=7.2 Hz)	102.6	75.7	77.1	69.5	76.4	60.2
7b	4.74 (1H, d, J=7.8 Hz)	103.2	75.8	77.2	69.1	78.5	61.2
7c	4.53 (1H, d, J=3.2 Hz)	101.6	73.0	72.3	69.2	76.0	63.0

a) Determined in CD_3OD

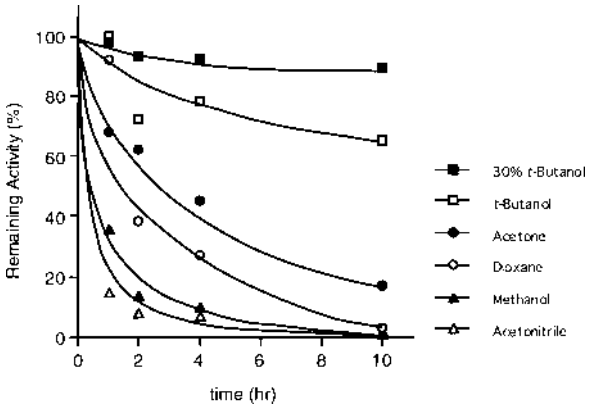


Fig. 2. Stability of β-Galactosidase in 50% Aqueous Organic Solvents at 35 °C

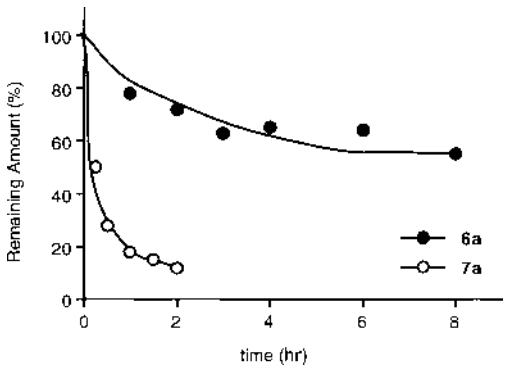


Fig. 3. Hydrolysis of **6a** and **7a** by β-Galactosidase (2560 Units) in 30% *tert*-Butanol at 37 °C

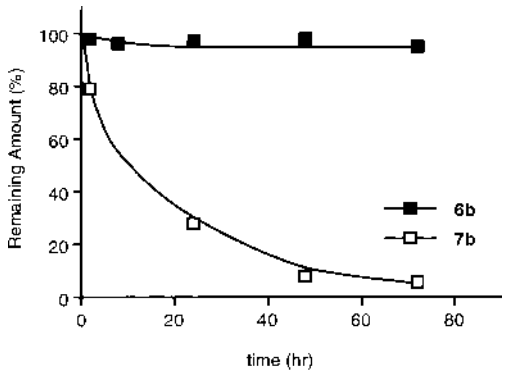


Fig. 4. Hydrolysis of **6b** and **7b** by β-Glucosidase (110 Units) in 0.1 M Acetate Buffer at pH 5.0

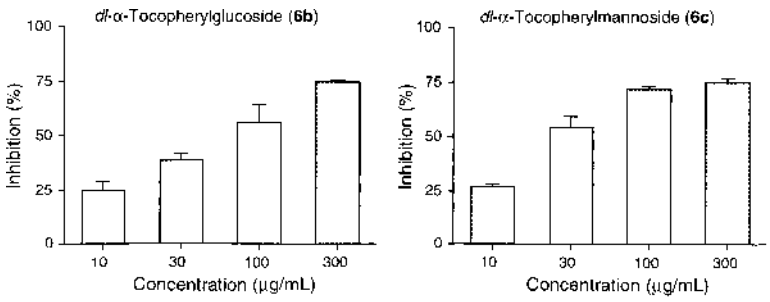


Fig. 5. Inhibition of Histamine Release from Rat Mast Cells Induced by Antigen–Antibody Reaction

Table 5. Suppressive Action of Plasma IgE Antibody Formation

Compound	Plasma IgE level[a]					Average[b]
	1	2	3	4	5	
Control	256	256	512	512	—	384.0±73.9
6c 30 mg/kg (total 11 d)	256	256	64	128	128	166.4±38.4*
6c 100 mg/kg (total 6 d)	128	64	256	128	64	128.0±35.1*
Hydrocortisone 10 mg/kg	512	64	512	256	—	336.0±108.9**

a) Plasma IgE level was expressed at maximum diluted magnification indicated positive (blue spot had more than a diameter of 5 mm). *b*) Average is indicated for plasma IgE level. Results are expressed as the average of 4 or 5 rats. Significantly different from control, * $p<0.05$ ** $p<$NS (*t*-test).

logical activities. However, this vitamin is not a good medicine due to its poor absorbability and bioavailability in the original form. This is because the hydroxyl group at the 6-position, the cause of the instability of vitamin E, is essential its physiological activities. Many derivatives have been synthesized so far to improve the stability of vitamin E, nevertheless, the problem of rather poor absorbability of these derivatives remains since most are ester derivatives that are readily decomposed by esterases in the intestinal tract. This makes the derivatives unsatisfactory as medicines.

We have attempted to obtain vitamin E derivatives as prodrugs that are readily hydrolyzable in the body, through synthesis of vitamin E derivatives with higher water solubility so that the usefulness of this oil-soluble vitamin is increased. Thus, we have synthesized various vitamin E glycosides by introducing a sugar group into the vitamin molecule taking account of the presence of the enzyme glucosidase in the body.

Initially, we speculated that tocopherol glycosides would act as prodrugs because the glycoside bond was expected to undergo hydrolysis to give tocopherol. However, contrary to our expectation, tocopheryl glycoside (**6b**) was resistant to hydrolysis and more than 95% of the glycoside remained unhydrolyzed 70 h after initiation of the reaction (Fig. 4). In contrast, **7b** with no methyl group at the 5- and 7-positions of the tocopherol moiety, was rapidly hydrolyzed, showing a big difference in hydrolyzability between the α- and δ-homologues. This is presumed to result from increased steric hindrance due to the presence of methyl groups at the 5- and 7-positions in **6b**. Tocopheryl glycosides were also found to be hardly affected by the hydrolyzing action of tissue homogenate and serum, though data are not shown. This would suggest that tocopheryl glycosides are not good prodrugs of vitamin E because of their high stability against hydrolysis in the body.

However, if these tocopheryl glycosides have other types of physiological activity, they would presumably retain their activity, whilst many other physiologically active glycosides are known to lose their activity through hydrolysis. In fact, as shown in this paper, tocopheryl glucoside (**6b**) and tocopheryl mannoside (**6c**) exhibited histamine release suppression (Fig. 5) and IgE antibody production inhibition (Table 5). This suggests a direct interaction of tocopheryl glycosides with lipid membrane-binding immunoglobulins in the immunoreaction. These glycosides may also be effective as anti-inflammatory agents because they showed a suppressive action on carrageenin-induced inflammation, an experimental model of inflammation, and hypersensitivity (data not shown). Since there are no reports on histamine release suppression and anti-inflammatory activity for tocopherol (vitamin E), the above new findings are presumed to result from the glycosidation of tocopherol. Finally, the glycosides synthesized in this work (**6b**, **6c**) are crystalline solids unlike the oily vitamin E and this would facilitate handling of the compounds as medicines. Thus, the possibility has been shown that of the compounds **6b** and **6c** may be a new type of immunoregulator.

Experimental

Chemistry Measurements of ^{1}H-NMR, ^{13}C-NMR, and IR spectra were performed using Varian EM 360, GX 400, or Hitachi R-42FT spectrometers in CD_3OH solution, and a Shimadzu IR-27G, respectively. MS and UV spectra were measured on a Shimadzu GC-MS 900B and a Shimadzu UV-300, respectively. Melting point measurements were made on a BÜCHI 510. Optical rotations were measured on a DIP-140.

Synthesis of *dl*-α-Tocopheryltetraacetylgalactoside (4a) *dl*-α-Tocopherol (**2**) (8.00 g, 18.5 mmol) and β-D-galactopyranosepentaacetate (**1a**) (3.30 g, 8.46 mmol) were dissolved in nitrobenzene (5.0 ml) and *p*-toluene sulfonic acid (0.08 g, 0.47 mmol) was added to the resultant solution. The reactants were allowed to react at 90 °C for 5 h under an N_2 atmosphere at 20 mmHg. The reaction mixture was washed three times each with water (100 ml) and saturated NaCl solution (100 ml) after adding benzene (100 ml) and dehydrated with anhydrous Na_2SO_4. The solvent was removed by distillation under reduced pressure and the residue was fractionated by silica gel column chromatography (benzene : ethyl acetate=9 : 1) to give product (**4a**) (2.87 g, 3.78 mmol, 20%).

4a: Yellow oil. IR (KBr): 1760 cm^{-1}. EI-MS *m/z* 760 (M^+).

Synthesis of *dl*-α-Tocopherylgalactoside (6a) *dl*-α-Tocopheryltetraacetylgalactoside (**4a**) (2.87 g, 3.78 mmol) was dissolved in dry CH_3OH (8.0 ml) and 0.1 N CH_3ONa (2.0 ml) was then added to the solution. After refluxing for 5 min, the reaction mixture was neutralized with Amberlite IR-120 (H^+) and decolorized with active charcoal. The solvent was removed by distillation under reduced pressure from the reaction mixture after filtration to yield crude crystals (**6a**) (1.78 g, 3.01 mmol, 80%). Recrystallization from H_2O–ethanol (1 : 4) gave **6a** (1.25 g, 2.11 mmol, 56%).

6a: White prisms. mp 176—177 °C. IR (KBr): 3390, 1160 cm^{-1}. EI-MS *m/z* 592 (M^+).

Synthesis of *dl*-α-Tocopheryltetraacetylglucoside (4b) *dl*-α-Tocopherol (**2**) (9.00 g, 20.93 mmol) and β-D-glucopyranosepentaacetate (**1b**) (3.30 g, 8.46 mmol) were dissolved in nitrobenzene (5.0 ml) and *p*-toluene sulfonic acid (0.07 g, 0.41 mmol) was then added to the solution. The reactants were allowed to react at 90 °C for 5 h under an N_2 atmosphere at 20 mmHg. The reaction mixture was washed three times each with water (100 ml) and saturated salt solution (100 ml) after addition of benzene (100 ml) and dehydrated with anhydrous Na_2SO_4. After the solvent was removed by distillation under reduced pressure, the residue was fractionated by silica gel column chromatography (benzene : ethyl acetate=9 : 1) to give **4b** (2.36 g, 3.11 mmol, 15%).

4b: Yellow oil. IR (KBr): 1700 cm^{-1}. EI-MS *m/z* 760 (M^+).

Synthesis of *dl*-α-Tocopherylglucoside (6b) *dl*-α-Tocopheryltetraacetylglucoside (**4b**) (2.36 g, 3.11 mmol) was dissolved in dry CH_3OH (8.0 ml) and the solution was refluxed for 5 min after addition of 0.1 N CH_3ONa (2.0 ml). The reaction mixture was neutralized with Amberlite IR-120 (H^+) and decolorized with active charcoal. After filtration, the solvent was removed by distillation under reduced pressure to give **6b** (1.60 g, 2.70 mmol, 78%) as crude crystals. Recrystallization from H_2O–ethanol (3 : 7) yielded **6b** (1.10 g, 1.86 mmol, 54%).

6b: White prisms. mp 140—141 °C. IR (KBr): 3390, 1160 cm^{-1}. EI-MS *m/z* 592 (M^+)

Synthesis of *dl*-α-Tocopheryltetraacetylmannoside (4c) *dl*-α-Tocopherol (**2**) (10.00 g, 23.25 mmol) and β-D-mannopyranosepentaacetate (**1c**) (3.30 g, 8.46 mmol) were dissolved in nitrobenzene (5.0 ml) and *p*-toluene sulfonic acid (0.075 g, 0.44 mmol) was added to the solution. The reactants were allowed to react at 90 °C for 5 h under an N_2 atmosphere at 20 mmHg. After addition of benzene (100 ml), the reaction mixture was washed three times each with H_2O (100 ml) and saturated NaCl solution (100 ml) and dehydrated with anhydrous Na_2SO_4. The solvent was removed by distillation under reduced pressure and the residue was fractionated by silica gel column chromatography (benzene : ethyl acetate=9 : 1) to give **4c** (2.40 g, 3.16

mmol, 14%).

4c: Yellow oil. IR (KBr): 1756 cm^{-1}. EI-MS *m/z* 760 (M^+).

Synthesis of *dl*-δ-Tocopherylmannoside (6c) *dl*-α-Tocopheryltetraacetylmannoside (**4c**) (2,40 g, 3.16 mmol) was dissolved in dry CH_3OH (8.0 ml) and the solution was refluxed for 5 min after addition of 0.1 N CH_3ONa (2.0 ml). The reaction mixture was neutralized with Amberlite IR-120 (H^+) and decolorized with active charcoal. The solvent was removed by distillation under reduced pressure after filtration to give crude crystals of **6c** (1.23 g, 2.08 mmol, 66%). Recrystallization from acetone yielded **6c** (1.10 g, 1.86 mmol, 59%).

6c: White prisms. mp 128—130 °C. IR (KBr): 3390, 1160 cm^{-1}. EI-MS *m/z* 592 (M^+).

Synthesis of *dl*-α-Tocopheryltetraacetylgalactoside (5a) *dl*-α-Tocopherol (**3**) (10.00 g, 24.9 mmol) and β-D-galactopyranosepentaacetate (**1a**) (3.30 g, 8.50 mmol) were dissolved in nitrobenzene (5.0 ml) and *p*-toluene sulfonic acid (0.076 g, 0.45 mmol) was added to the solution. The reactants were allowed to react at 90 °C for 5 h under an N_2 atmosphere at 20 mmHg. After addition of benzene (100 ml), the reaction mixture was washed three times each with H_2O (100 ml) and saturated NaCl solution (100 ml) and dehydrated with anhydrous Na_2SO_4. The solvent was removed by distillation under reduced pressure and the residue was fractionated by silica gel column chromatography (benzene : ethyl acetate=9 : 1) to give **5a** (3.70 g, 5.05 mmol, 20%).

5a: Yellow oil. IR (KBr): 1760 cm^{-1}. EI-MS *m/z* 732 (M^+).

Synthesis of *dl*-α-Tocopherylgalactoside (7a) *dl*-α-Tocopheryltetraacetylgalactoside (**5a**) (3.70 g, 5.05 mmol) was dissolved in dry CH_3OH (8.0 ml) and the solution was refluxed for 5 min after adding 0.1 N CH_3ONa (3.0 ml). The reaction mixture was neutralized with Amberlite IR-120 (H^+) and decolorized with active charcoal. After filtration, the solvent was removed by distillation under reduced pressure to give **7a** as crude crystals (1.60 g, 2.84 mmol, 56%). Recrystallization from H_2O–ethanol (3 : 7) yielded **7a** (1.10 g, 1.95 mmol, 39%).

7a: White prisms. mp 140—141 °C. IR (KBr): 3390, 1169 cm^{-1}. EI-MS *m/z* 564 (M^+).

Synthesis of *dl*-δ-Tocopheryltetraacetylglucoside (5b) *dl*-δ-Tocopherol (**3**) (8.00 g, 20.00 mmol) and β-D-glucopyranosepentaacetate (**1b**) (3.30 g, 8.46 mmol) were dissolved in nitrobenzene (5.0 ml) and *p*-toluene sulfonic acid (0.075 g, 0.44 mmol) was added to the solution. The reactants were allowed to react at 90 °C for 5 h under an N_2 atmosphere at 20mmHg. After addition of benzene (100 ml), the reaction mixture was washed three times each with H_2O (100 ml) and saturated NaCl solution (100 ml) and dehydrated with anhydrous Na_2SO_4. After removal of the solvent by distillation under reduced pressure, the residue was fractionated by silica gel column chromatography (benzene : ethyl acetate=8 : 1) to give **5b** (3.40 g, 4.64 mmol, 23%).

5b: Yellow oil. IR (KBr): 1770 cm^{-1}. EI-MS *m/z* 732 (M^+).

Synthesis of *dl*-α-Tocopherylglucoside (7b) *dl*-α-Tocopheryltetraacetylglucoside (**5b**) (3.40 g, 4.64 mmol) was dissolved in dry CH_3OH (8.0 ml) and the solution was refluxed for 5 min after addition of 0.1 N CH_3ONa (3.0 ml). The reaction mixture was neutralized with Amberlite IR-120 (H^+) and decolorized with active charcoal. After filtration, the solvent was removed by distillation under reduced pressure to give **7b** as crude crystals (2.20 g, 3.90 mmol, 84%). Recrystallization from acetone yielded **7b** (1.50 g, 2.66 mmol, 57%).

7b: White prisms. mp 46—49 °C. IR (KBr): 3390, 1160 cm^{-1}. EI-MS *m/z* 564 (M^+).

Synthesis of *dl*-δ-Tocopheryltetraacetylmannoside (5c) *dl*-δ-Tocopherol (**3**) (10.00 g, 25.00 mmol) and β-D-mannopyranosepentaacetate (**1c**) (3.30 g, 8.86 mmol) were dissolved in nitrobenzene (5.0 ml) and *p*-toluene sulfonic acid (0.08 g, 0.50 mmol) was added to the solution. The reactants were allowed to react at 90 °C for 5 h under an N_2 atmosphere at 20 mmHg. After addition of benzene (100 ml), the reaction mixture was washed three times each with H_2O (100 ml) and saturated NaCl solution (100 ml) and dehydrated with anhydrous Na_2SO_4. After removal of the solvent by distillation under reduced pressure, the residue was fractionated by silica gel column chromatography (benzene : ethyl acetate=9 : 1) to give **5c** (3.80 g, 5.19 mmol, 21%).

5c: Yellow oil. IR (KBr): 1760 cm^{-1}. EI-MS *m/z* 732 (M^+).

Synthesis of *dl*-δ-Tocopherylmannoside (7c) *dl*-δ-Tocopheryltetraacetylmannoside (**5c**) (3.80 g, 5.19 mmol) was dissolved in dry CH_3OH (8.0 ml) and the solution was refluxed for 5 min after addition of 0.1 N CH_3ONa (3.0 ml). The reaction mixture was neutralized with Amberlite IR-120 (H^+) and decolorized with active charcoal. After filtration, the solvent was removed by distillation under reduced pressure to give **7c** as crude crystals (2.10 g, 3.72 mmol, 72%). Recrystallization from acetone yielded **7c** (1.36 g, 2.31 mmol, 46%).

7c: White prisms. mp 187—189 °C. IR (KBr): 3390, 1160 cm^{-1}. EI-MS *m/z* 564 (M^+).

Pharmacology. Materials Male Wistar rats, male Sprague Dawley (SD) rats and male C57BL/6Ncrj mice were obtained from Charles River Japan, Inc. L-α-Phosphatidil-L-serine, egg albumin, λ-carrageenin were obtained from Sigma Chemical Co. Bovine albumin, trichloroacetic acid, (TCA) *o*-phthalaldehyde (OPA) triton X-100, olive oil, indomethacin, cortisone acetate, methotrexate were obtained from Wako Pure Chemical Industries, Ltd. Hydrocortizon, picril chloride, diphenhydramin hydrochloride were obtained from Nacalai Tesque, Inc. Pertussis vaccine was obtained from Chiba Serum Research Laboratory.

Enzymatic Hydrolysis by Galactosidase Sample solutions were prepared by dissolving *dl*-α-tocopherylgalactoside (**6a**) (100 mg) and *dl*-δ-tocopherylgalactoside (**7b**) (93.5 mg) in *tert*-butanol. Enzyme solution was made by dissolving 800 mg of β-galactosidase[7] in 140 ml of 0.1 M acetate buffer (pH 5.0). An aliquot (6 ml) of sample solution and that of enzyme solution (14 ml) were mixed to give a 30% *tert*-butanol solution (20 ml). The mixed test solution was incubated in a water bath at 37 °C. The reaction was stopped at the given time intervals by immersing the test solution in boiling water for 3 min. The reaction product was extracted with benzene (20 ml) and the amount determined by HPLC after removal of the solvent by distillation under reduced pressure.

Enzymatic Hydrolysis by Glucosidase Seventy-five micromoles of *dl*-α-tocopherylgalactoside (**6a**) and *dl*-δ-tocopherylgalactoside (**7b**) were dissolved in ethanol with 250 mg of Triton X-100. After removing solvent by distillation under reduced pressure, the residue was dissolved in 5 ml of 0.1 M acetate buffer (pH 5.0) to give sample solutions. Enzyme solution was prepared by dissolving 250 mg of β-glucosidase in 5 ml of 0.1 M acetate buffer (pH 5.0). Aliquots of sample solution (0.1 ml), enzyme solution (0.4 ml) and 0.1 M acetate buffer (pH 5.0) (0.5 ml) were mixed to make the total volume 1.0 ml. This test solution was incubated at 37 °C in a water bath. The reaction was stopped at given time intervals by adding 3 ml of ethanol–acetone (1 : 1). The amount of the product was determined by HPLC after centrifuging the reaction mixture for 15 min at 550×***g***.

HPLC Analysis A 20 μl aliquot was injected into a reverse-phase column (μBondspack C18, φ3/8″×1′, Waters). A pump (ALC/GPC 200, Waters) was used to feed 95% methanol (mobile phase) into the column at a flow rate of 1 ml/min. Column effluent was monitored by measuring the absorbance at 290 nm with a UV detector (UV 100-IV, Jasco).

Histamine Release Inhibiting Action[11] The animals used were Wistar male rats (*ca*. 250 g). Locke solution (10 ml) containing bovine serum albumin (100 μg/ml) was intraperitoneally injected into rat. After massage, disembowelment was performed on the animal to withdraw a cell suspension from the peritoneal cavity. The Locke solution used was prepared from 150 mM NaCl, 5.5 mM KCl, 2.1 mM $CaCl_2 \cdot 2H_2O$, 0.7 mM glucose, 1.2 mM $MgSO_4$, and 0.1 mM phosphate buffer (pH 7.2) to give a concentration of 2.0 v/v%. After centrifugation of the cell suspension at 4 °C for 5 min at 55×***g***, the sediment was washed with a proper volume of iced Locke solution, followed by redispersion of the washed cells in the solution to make the final concentration 5×10^4 cells/ml. To 0.3 ml of the redispersed cell suspension were added 1.3 ml of Locke solution and L-α-phosphatidyl-L-serine to make the final phospholipid concentration 30 μg/ml. Then, the suspension was preincubated at 37 °C for 5 min after addtion of 20 μl of a test solution in ethanol. The control was 20 μl of ethanol. The preincubated cell suspension was incubated for 10 min after addition of 20 μl of an ovalbumin solution in physiological saline as a separating agent. The reaction was stopped by ice-cooling and the suspension was centrifuged for 10 min at 950×***g***. Treatment with 100% TCA was then performed on the supernatant to precipitate the protein and the precipitate was separated by centrifugation. After addition of 1 N NaOH to the clear supernatant, the amount of released histamine (P_S) in the supernatant and that of unreleased histamine (P_R) were determined with a fluorophotometer using OPA as the coloring agent and the percentage inhibition was calculated using the analytical data.[12]

$$\text{histamine release (\%)} = \frac{P_S}{P_S + P_R} \times 100$$

$$\text{inhibition (\%)} = 100 - \left(\frac{\text{histamine release (sample) (\%)}}{\text{histamine release (control) (\%)}} \right) \times 100$$

IgE Formation Suppressing Action[13] The animals used were SD male rats (180—220 g). Ovalbumin was used as the antigen (1 mg/animal) while

$Al(OH)_3$ and pertissius vaccine[14,15] were used as the adjuvants (20mg and 0.6 ml/animal, respectively). Sensitization was made by intracutaneously injecting 0.6 ml of a mixture of the antigen and the adjuvant into the paws of each animal. Paw edema was measured 24 h after injection by the volume method and the treated animals were divided in groups with an equal average swelling volume. The positive control was hydrocortisone. Each test compound was dissolved in physiological saline containing 10% Nikkol and the test solution was intraperitoneally injected daily into the animal for 11 d starting on the day of grouping. To the **6c** administered group (100 mg/kg), the injection was done every 2 d for 6 d. The amount of IgE was measured by the passive cutaneous anaphylaxis (PCA) method[16] on the 15th day.

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