Synthesis of 4-Alkoxyaryl β-D-Glucopyranosides and Their Inhibitory Effects on Histamine Release from Rat Peritoneal Mast Cells Induced by Concanavalin A

Tzer Chuan WANG, Haruhiro FURUKAWA, Yasunori NIHRO, Hisao KAKEGAWA, Hitoshi MATSUMOTO, and Toshio SATOH*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan.
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The inhibitory effects of newly synthesized 4-alkoxyaryl β-D-glucopyranosides on histamine release from rat peritoneal mast cells induced by concanavalin A were examined. A plot of hydrophobicity (*k'*) against inhibitory activity of the compounds showed a distinct maximum, and 4-decyloxy-2,3,6-trimethylphenyl β-D-glucopyranoside was the most potent inhibitor among the tested compounds.

Keywords 4-alkoxyaryl β-D-glucopyranoside; 4-decyloxy-2,3,6-trimethylphenyl β-D-glucopyranoside; histamine release inhibition; β-glucosidase

Concanavalin A (Con A), a plant lectin isolated from *Canavalia ensiformis*,[1] specifically binds to the carbohydrate chains of Fc portion of immunoglobulin E (IgE)[2] located in mast cells and basophils to induce degranulation of the cells and release of chemical mediators such as histamine, leukotrienes and prostaglandins.[3-7] Keller found that high concentrations of glucopyranose, mannopyranose and their methylglycopyranosides inhibited histamine release induced by not only Con A but also antigen–antibody reaction.[8] From this, it was suggested that a similar recognition of carbohydrate chains operates in both histamine release systems. Our previous studies concerning the inhibitory effects of aryl α-D-mannopyranosides on histamine release induced by both antigen–antibody reaction[9] and Con A[10] supported this hypothesis. As type I allergy is mediated by antigen–antibody reaction, we hoped to find novel anti-allergic drugs through studies on Con A-induced histamine release inhibition.

On the other hand, we are also engaged in medicinal chemical studies on tocopheryl glycosides. Among the tocopheryl glycosides studied, glucopyranoside and mannopyranoside exhibited strong inhibitory effects on histamine release induced by antigen–antibody reac-

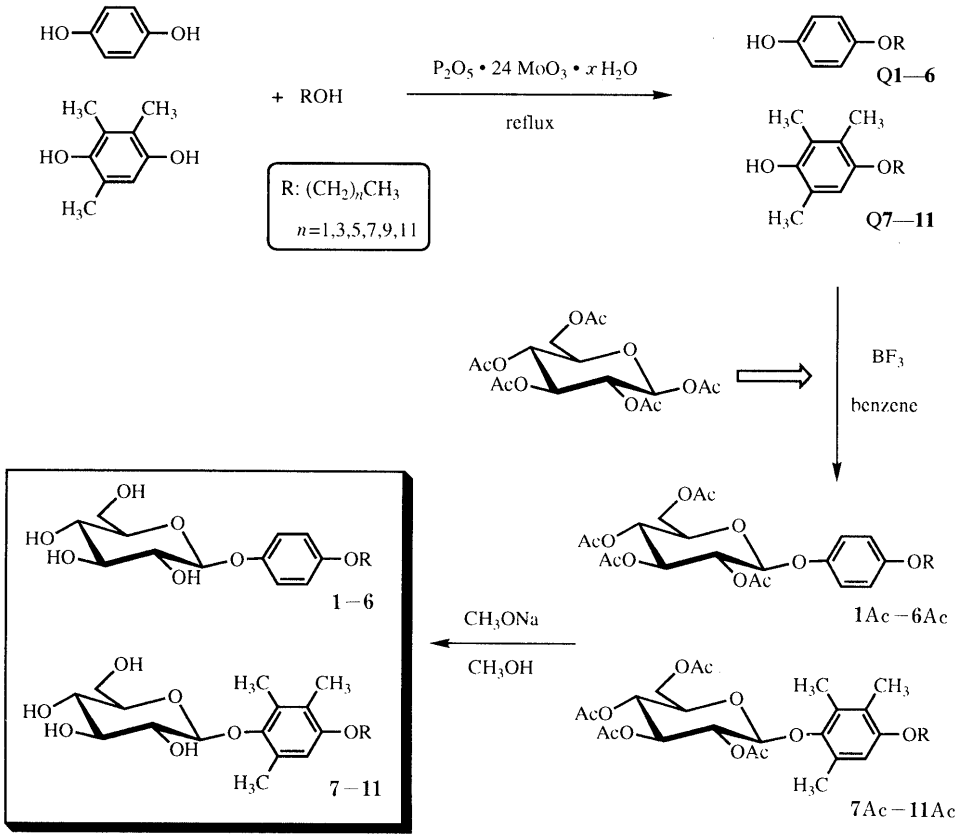

Chart 1

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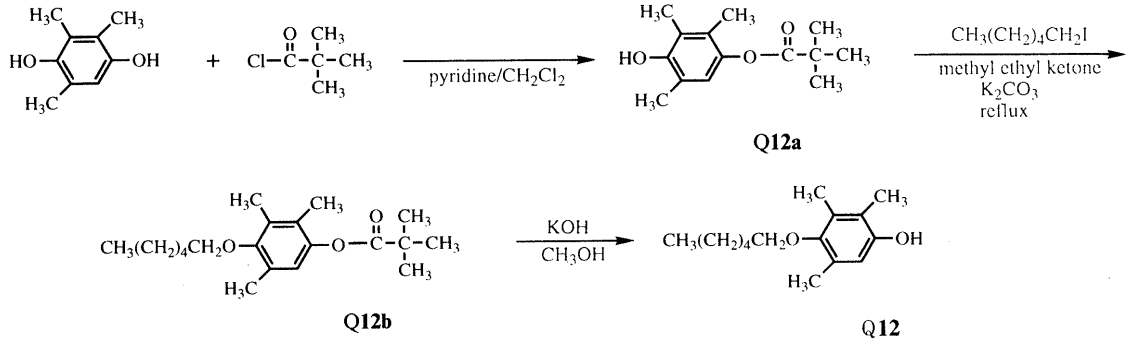
tion.[11] The tocopheryl groups, used as the aglycone, were considered to act as an anchor which binds to the cell membrane.[11] In order to investigate the relationship between the activities of glycopyranosides and the structures of their aglycone moieties, we synthesized a series of 4-alkoxy-2,3,6-trimethylphenyl β-D-glucopyranosides, structurally resembling tocopheryl glucoside, and examined their influence on histamine release from rat peritoneal mast cells induced by Con A.

Synthesis of 4-Alkoxy-2,3,6-trimethylphenyl β-D-Glucopyranosides and 4-Alkoxyphenyl β-D-Glucopyranosides The synthetic route to 4-alkoxyaryl β-D-glucopyranosides (**1**—**11**) is shown in Chart 1. Condensation of pentaacetyl β-D-glucopyranose with 4-alkoxylphenols (**Q1**—**Q11**) in the presence of boron trifluoride in benzene produced 4-alkoxylaryl β-D-glucopyranoside tetraacetates (**1Ac**—**11Ac**), and these compounds were deacetylated with a catalytic amount of sodium methoxide in methanol to afford the 4-alkoxyaryl β-D-glucopyranosides (**1**—**11**).

The 4-alkoxylphenols (**Q1**—**Q11**), used in the preparation of compounds **1**—**11**, were synthesized from hydroquinones and alcohols in the presence of phosphomolybdic acid (Chart 1).[12] As shown in Chart 2, 4-hexyloxy-2,3,5-trimethylphenol (**Q12**) was synthesized through pivaloylation,[13] hexylation and hydrolysis. The signal of α-protons of the hexyl group appeared at 3.66 ppm (2H, t, $J=6.4$ Hz) for this compound, but at 3.87 ppm for compound **Q8**, indicating that the structure of compound **Q8** is 4-hexyloxyl-2,3,6-trimethylphenol. On the basis of these results, the other compounds **Q7** and **Q9**—**Q11** were determined to be 4-alkoxy-2,3,6-trimethylphenols.

The structures of compounds **1**—**11** were determined on the basis of ^{1}H-NMR and ^{13}C-NMR spectral data. The ^{13}C-NMR chemical shifts for phenyl β-D-glucopyranoside and its α-anomer, which are commercially available, were 103.1 and 97.9 ppm, respectively. The signals of the anomeric carbons for compounds **1**—**6** appeared at the same position as that of phenyl β-D-glucopyranoside, showing that these compounds are β-anomers. The ^{13}C-NMR of anomeric carbon signal for 2,6-dimethylphenyl β-D-glucopyranoside appeared at 105.9 ppm, demonstrating that compounds **7**—**11**, having a 2,6-dimethyl group, are β-anomers. On the other hand, those for phenyl β-D-glucopyranoside tetraacetate, 2,6-

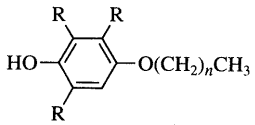


Q12a

Q12b **Q12**

Chart 2

TABLE I. Chemical and Physical Data for 4-Alkoxyphenols **Q1**—**Q11**



Compound	R	*n*	Yield (%)	mp (°C)	^{1}H-NMR chemical shifts measured for $CDCl_3$ solution
Q1	H	1	78	63—65	1.39 (3H, t, $J=6.8$ Hz), 3.97 (2H, q, $J=7.0$ Hz), 4.67 (1H, b), 6.77 (4H, s)
Q2	H	3	73	63—64	0.90 (3H, t, $J=6.0$ Hz), 1.18—1.82 (4H, m), 3.90 (2H, t, $J=6.4$ Hz), 6.77 (4H, s)
Q3	H	5	70	42.5—43	0.90 (3H, t, $J=6.0$ Hz), 1.33—1.92 (8H, m), 3.89 (2H, t, $J=6.2$ Hz), 6.76 (4H, s)
Q4	H	7	60	58.5—59	0.88 (3H, t, $J=6.2$ Hz), 1.31—1.96 (12H, m), 3.89 (2H, t, $J=6.2$ Hz), 4.55 (1H, s), 6.76 (4H, s)
Q5	H	9	49	68.5—69	0.88 (3H, t, $J=5.7$ Hz), 1.02—1.75 (16H, m), 3.89 (2H, t, $J=6.2$ Hz), 4.49 (1H, s), 6.76 (4H, s)
Q6	H	11	55	77—78	0.88 (3H, t, $J=6.2$ Hz), 1.26—1.75 (20H, m), 3.89 (2H, t, $J=6.4$ Hz), 4.55 (1H, s), 6.76 (4H, s)
Q7	CH_3	3	73	65.5—66.5	0.90 (3H, t, $J=6.6$ Hz), 1.30—1.90 (4H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.87 (2H, t, $J=6.2$ Hz), 4.24 (1H, s), 6.51 (1H, s)
Q8	CH_3	5	64	72.5—73	0.90 (3H, t, $J=6.5$ Hz), 1.33—1.92 (8H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.87 (2H, t, $J=5.9$ Hz), 4.24 (1H, s), 6.51 (1H, s)
Q9	CH_3	7	54	70—71	0.89 (3H, t, $J=6.2$ Hz), 1.10—1.76 (12H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.86 (2H, t, $J=6.2$ Hz), 4.24 (1H, s), 6.51 (1H, s)
Q10	CH_3	9	42	76—77	0.88 (3H, t, $J=5.1$ Hz), 1.05—1.94 (16H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.86 (2H, t, $J=6.4$ Hz), 4.24 (1H, s), 6.51 (1H, s)
Q11	CH_3	11	55	81—83	0.88 (3H, t, $J=6.4$ Hz), 1.26—1.97 (20H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.86 (2H, t, $J=6.2$ Hz), 4.24 (1H, s), 6.51 (1H, s)

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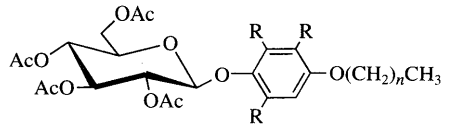
dimethylphenyl β-D-glucopyranoside tetraacetates, and compounds **3**Ac and **8**Ac were 99.2, 101.5, 100.4 and 102.0 ppm, respectively. The similar ^{13}C-NMR chemical shifts indicate that compounds **3**Ac and **8**Ac are β-anomers. Phenyl β-D-glucopyranoside tetraacetates, 2,6-dimethylphenyl β-D-glucopyranoside tetraacetate and 2,6-dimethylphenyl β-D-glucopyranoside were prepared in this laboratory and their structures were confirmed by measurements of melting point and optical rotation.[14,15] Compounds **1**Ac, **2**Ac, **4**Ac, **5**Ac—**7**Ac and **9**Ac—**11**Ac were deacetylated to produce β-D-glucopyranosides, like compounds **3**Ac and **8**Ac, suggesting that these compounds are β-anomers.

Inhibitory Effects of 4-Alkoxyaryl β-D-Glucopyranosides on Con A-Induced Histamine Release The inhibitory effects of 4-alkoxyaryl β-D-glucopyranosides (**1**—**11**) on histamine release from rat peritoneal mast cells induced by 400 μg/ml of Con A are shown in Table VII. Among the compounds tested, compound **10** exhibited the most potent inhibitory effect. The activity of this compound was far stronger than that of *dl*-α-tocopheryl β-D-glucopyranoside or azelastin, which is a potent anti-allergic drug. The correlation between hydrophobicity and inhibitory effect for the compounds tested was examined. The results are shown in Fig. 1. The hydrophobicity was expressed as the capacity ratio (k') on HPLC using a $_5C_{18}$ reversed-phase column. For compounds **7**—**10**, the inhibitory potency increased in proportion to the hydrophobicity. However, compound **11** showed a low potency. Under the HPLC conditions used to evaluate k' for the compounds tested, the retention time for *dl*-α-tocopheryl β-D-glucopyranoside was too long to be determined, apparently indicating that the hydrophobicity of

TABLE II. Physical Properties of 4-Alkoxyphenyl β-D-Glucopyranoside Tetraacetates **1**Ac—**11**Ac



Compound	R	*n*	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ a)
1Ac	H	1	95	111—112	−16.2
2Ac	H	3	74	114—115	−16.0
3Ac	H	5	56	132—135	−12.5
4Ac	H	7	52	92—95	−11.1
5Ac	H	9	50	95—97	−11.1
6Ac	H	11	62	98—99	−10.8
7Ac	CH_3	3	65	120—128 b)	−16.7
8Ac	CH_3	5	42	129—130	−16.4
9Ac	CH_3	7	70	95—98	−15.2
10Ac	CH_3	9	32	94—95	−14.6
11Ac	CH_3	11	53	67—68	−13.8

a) Measured in chloroform. b) Liquid crystal.

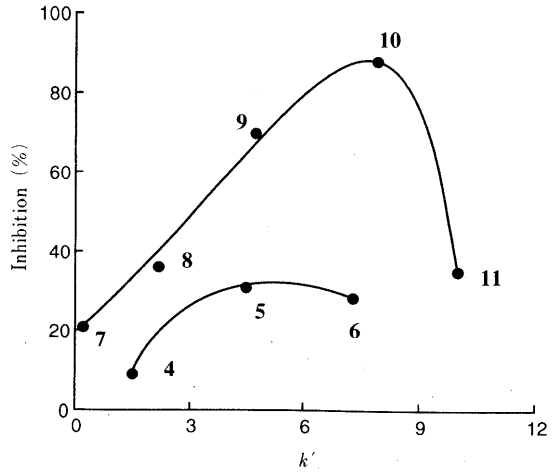


Fig. 1. Relationship between Hydrophobicities (k') of 4-Alkoxyphenyl β-D-Glucopyranosides and Their Inhibitory Effects on Histamine Release from Rat Peritoneal mast Cells Induced by Con A (400 μg/ml)

TABLE III. ^{1}H-NMR Data for 4-Alkoxyphenyl β-D-Glucopyranoside Tetraacetates **1**Ac—**11**Ac in $CDCl_3$

Compound	Glucopyranosyl moiety	Aglycone and acetyl groups
1Ac	4.10—4.43 (2H, m), 4.9 (1H, d, J=7.8 Hz), 4.94—5.31 (4H, m)	6.83 (2H, d, J=6.2 Hz), 6.90 (2H, d, J=6.2 Hz), 3.95 (2H, t, J=7.0 Hz), 2.08 (3H, s), 2.07 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.39 (3H, t, J=7.0 Hz)
2Ac	4.10—4.43 (2H, m), 4.93 (1H, d, J=7.9 Hz), 4.94—5.31 (4H, m)	6.84 (2H, d, J=6.4 Hz), 6.90 (2H, d, J=6.4 Hz), 3.92 (2H, t, J=6.2 Hz), 2.08 (3H, s), 2.07 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.25—1.93 (4H, m), 0.97 (3H, t, J=6.6 Hz)
3Ac	4.13 (2H, m), 4.95 (1H, d, J=6.3 Hz), 5.00—5.32 (4H, m)	6.84 (2H, d, J=6.4 Hz), 6.90 (2H, d, J=6.4 Hz), 3.90 (2H, t, J=6.4 Hz), 2.07 (6H, s), 2.03 (3H, s), 2.02 (3H, s), 1.20—1.90 (8H, m), 0.90 (3H, t, J=6.6 Hz)
4Ac	4.23 (2H, m), 4.95 (1H, d, J=7.8 Hz), 4.04—5.38 (4H, m)	6.84 (2H, d, J=6.3 Hz), 6.90 (2H, d, J=6.3 Hz), 3.90 (2H, t, J=6.4 Hz), 2.07 (6H, s), 2.04 (3H, s), 2.03 (3H, s), 1.07—1.89 (12H, m), 0.88 (3H, t, J=5.7 Hz)
5Ac	4.04—4.40 (2H, m), 4.93 (1H, d, J=7.9 Hz), 4.97—5.38 (4H, m)	6.84 (2H, d, J=6.3 Hz), 6.89 (2H, d, J=6.3 Hz), 3.90 (2H, t, J=6.2 Hz), 2.08 (6H, s), 2.04 (6H, s), 1.15—1.90 (16H, m), 0.88 (3H, t, J=5.9 Hz)
6Ac	4.05—4.40 (2H, m), 4.93 (1H, d, J=7.9 Hz), 4.97—5.30 (4H, m)	6.84 (2H, d, J=6.4 Hz), 6.90 (2H, d, J=6.4 Hz), 3.90 (2H, t, J=6.4 Hz), 2.08 (6H, s), 2.04 (6H, s), 1.00—1.90 (20H, m), 0.88 (3H, t, J=5.9 Hz)
7Ac	3.40—3.60 (1H, m), 4.09—4.30 (2H, m), 4.74 (1H, d, J=7.5 Hz), 5.16—5.34 (3H, m)	6.50 (1H, s), 3.90 (2H, t, J=6.2 Hz), 2.23 (3H, s), 2.16 (3H, s), 2.12 (3H, s), 2.03 (6H, m), 2.02 (6H, m), 1.20—1.97 (4H, m), 0.97 (3H, t, J=6.6 Hz)
8Ac	3.45—3.63 (1H, m), 4.09—4.33 (2H, m), 4.75 (1H, d, J=7.4 Hz), 5.16—5.33 (3H, m)	6.49 (1H, s), 3.88 (2H, t, J=6.2 Hz), 2.23 (3H, s), 2.16 (3H, s), 2.11 (3H, s), 2.03 (6H, m), 2.02 (6H, m), 1.20—1.96 (8H, m), 0.90 (3H, t, J=6.0 Hz)
9Ac	3.44—3.65 (1H, m), 4.09—4.32 (2H, m), 4.73 (1H, d, J=7.5 Hz), 5.15—5.37 (3H, m)	6.50 (1H, s), 3.88 (2H, t, J=6.2 Hz), 2.23 (3H, s), 2.16 (3H, s), 2.11 (3H, s), 2.03 (6H, m), 2.02 (6H, m), 1.10—1.90 (12H, m), 0.89 (3H, t, J=5.5 Hz)
10Ac	3.40—3.65 (1H, m), 4.09—4.32 (2H, m), 4.72 (1H, d, J=7.5 Hz), 5.15—5.35 (3H, m)	6.50 (1H, s), 3.88 (2H, t, J=6.2 Hz), 2.23 (3H, s), 2.16 (3H, s), 2.11 (3H, s), 2.03 (6H, m), 2.02 (6H, m), 1.05—1.85 (16H, m), 0.88 (3H, t, J=6.0 Hz)
11Ac	3.45—3.65 (1H, m), 4.09—4.32 (2H, m), 4.72 (1H, d, J=7.5 Hz), 5.15—5.40 (3H, m)	6.49 (1H, s), 3.89 (2H, t, J=6.2 Hz), 2.23 (3H, s), 2.16 (3H, s), 2.11 (3H, s), 2.03 (12H, m), 1.05—1.90 (20H, m), 0.88 (3H, t, J=6.0 Hz)

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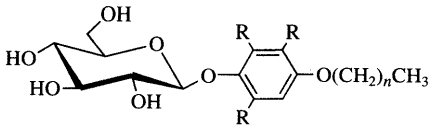
TABLE IV. Chemical and Physical Data for 4-Alkoxyphenyl β-D-Glucopyranosides **1**—**11**



Compound	R	*n*	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ [a]	Analysis (%) Calcd C	Calcd H	Found C	Found H
1	H	1	>97	169—171	−57.0	55.99	6.71	55.80	6.64
2	H	3	>97	150—152	−51.0	58.53	7.37	58.70	7.30
3	H	5	>97	132—135	−46.5	60.66	7.92	60.31	7.88
4 [b]	H	7	>97	110—150 [c]	−40.3	59.68	8.51	60.62	8.15
5	H	9	>97	102—106	−36.9	61.38	8.90	62.51	8.91
6	H	11	>97	200—201	−31.0	65.43	9.15	64.80	9.49
7	CH_3	3	>97	188—191	−5.5	61.61	8.16	60.84	8.39
8 [d]	CH_3	5	>97	184—186	−4.7	61.90	8.66	62.00	8.51
9 [b]	CH_3	7	>97	176—179	−4.4	60.91	9.11	61.17	8.83
10 [b]	CH_3	9	>97	189—192	−3.5	62.35	9.42	62.00	9.62
11 [d]	CH_3	11	>97	179—181	−3.3	65.96	9.64	65.65	9.67

a) Measured for solution in methanol. *b*) Contains one and a half mole of water. *c*) Liquid crystal. *d*) Contains one-half mole of water.

TABLE V. ^{1}H-NMR Data for Alkoxyphenyl β-D-Glucopyranosides **1**—**11** in CD_3OD

Compound	Glucopyranosyl moiety	Aglycone
1	3.30—3.90 (7H, m)	0.97 (3H, t, J=7.0 Hz), 3.92 (2H, t, J=6.8 Hz), 6.86 (2H, d, J=6.8 Hz), 6.99 (2H, d, J=6.8 Hz)
2	3.35—3.87 (7H, m)	0.97 (3H, t, J=6.6 Hz), 1.20—1.90 (4H, m), 3.92 (2H, t, J=6.2 Hz), 6.86 (2H, d, J=6.8 Hz), 6.99 (2H, d, J=6.8 Hz)
3	3.35—3.90 (7H, m)	0.92 (3H, t, J=5.5 Hz), 1.05—1.93 (8H, m), 3.91 (2H, t, J=6.2 Hz), 6.86 (2H, d, J=6.8 Hz), 6.99 (2H, d, J=6.8 Hz)
4	3.35—3.85 (7H, m)	0.90 (3H, t, J=5.0 Hz), 1.06—1.90 (12H, m), 3.91 (2H, t, J=6.2 Hz), 6.90 (2H, d, J=6.8 Hz), 6.99 (2H, d, J=6.8 Hz)
5	3.25—3.85 (7H, m)	0.90 (3H, t, J=6.0 Hz), 1.05—1.85 (16H, m), 3.91 (2H, t, J=6.3 Hz), 6.86 (2H, d, J=6.8 Hz), 6.99 (2H, d, J=6.8 Hz)
6	3.25—3.85 (7H, m)	0.90 (3H, t, J=6.0 Hz), 1.05—1.90 (20H, m), 3.91 (2H, t, J=6.2 Hz), 6.86 (2H, d, J=6.8 Hz), 6.99 (2H, d, J=6.8 Hz)
7	2.95—3.25 (1H, m), 3.35—3.71 (5H, m), 4.53 (1H, d, J=7.4 Hz)	0.98 (3H, t, J=7.4 Hz), 1.25—1.85 (4H, m), 2.07 (3H, s), 2.24 (3H, s), 2.30 (3H, s), 3.89 (2H, t, J=6.2 Hz), 6.54 (H, s)
8	2.95—3.23 (1H, m), 3.35—3.75 (5H, m), 4.53 (1H, d, J=7.4 Hz)	0.92 (3H, t, J=5.9 Hz), 1.10—1.90 (8H, m), 2.07 (3H, s), 2.24 (3H, s), 2.30 (3H, s), 3.88 (2H, t, J=5.9 Hz), 6.53 (H, s)
9	2.95—3.23 (1H, m), 3.35—3.75 (5H, m), 4.52 (1H, d, J=7.3 Hz)	0.90 (3H, t, J=5.9 Hz), 1.00—1.89 (12H, m), 2.07 (3H, s), 2.24 (3H, s), 2.30 (3H, s), 3.89 (2H, t, J=6.2 Hz), 6.53 (H, s)
10	2.95—3.23 (1H, m), 3.35—3.72 (5H, m), 4.52 (1H, d, J=7.3 Hz)	0.90 (3H, t, J=5.9 Hz), 1.00—1.90 (16H, m), 2.08 (3H, s), 2.24 (3H, s), 2.30 (3H, s), 3.89 (2H, t, J=6.2 Hz), 6.53 (H, s)
11	2.95—3.23 (1H, m), 3.35—3.72 (5H, m), 4.55 (1H, d, J=7.3 Hz)	0.89 (3H, t, J=5.9 Hz), 1.00—1.85 (20H, m), 2.08 (3H, s), 2.24 (3H, s), 2.30 (3H, s), 3.89 (2H, t, J=6.2 Hz), 6.54 (H, s)

TABLE VI. ^{13}C-NMR Data for Alkoxyphenyl β-D-Glucopyranosides **1**—**11** in Pyridine-d_5

Compound	Glucopyranosyl moiety	Aglycone
1	103.1, 78.5, 78.4, 74.8, 71.3, 62.4	154.5, 152.6, 118.3, 115.6, 63.9, 14.9
2	103.2, 78.6, 78.4, 74.9, 71.3, 62.5	154.8, 152.6, 118.4, 115.7, 68.2, 31.5, 19.4, 13.9
3	103.2, 78.6, 78.4, 74.9, 71.3, 62.5	154.9, 152.6, 118.4, 115.7, 68.6, 31.7, 29.5, 25.9, 22.7, 14.1
4	103.1, 78.3, 74.7, 71.2, 62.4	154.8, 152.5, 118.4, 115.7, 68.7, 31.9, 29.5, 26.3, 22.8, 14.1
5	103.2, 78.2, 74.7, 71.3, 62.4	154.9, 152.6, 118.5, 115.7, 68.6, 32.0, 29.7, 29.6, 29.5, 26.3, 22.8, 14.2
6	103.2, 78.6, 78.4, 74.9, 71.4, 62.5	154.9, 152.6, 118.4, 115.7, 68.5, 32.2, 29.7, 26.3, 22.8, 14.3
7	106.2, 78.2, 77.8, 75.6, 71.8, 62.2	153.8, 131.8, 129.5, 123.7, 112.0, 68.3, 31.8, 19.6, 17.9, 14.2, 13.9, 12.1
8	106.4, 78.4, 78.0, 75.7, 71.9, 62.9	153.8, 131.8, 129.5, 123.6, 112.0, 68.6, 31.7, 29.7, 26.1, 22.8, 18.1, 14.2, 12.2
9	106.1, 78.2, 77.6, 75.5, 71.8, 62.9	153.8, 131.8, 129.5, 123.5, 112.1, 68.7, 31.9, 29.8, 29.5, 29.4, 26.5, 22.8, 18.0, 14.2, 12.2
10	106.2, 78,2, 77.7, 75.5, 71.8, 62.8	153.8, 131.8, 129.5, 123.7, 112.1, 68.6, 31.9, 29.8, 29.5, 29.4, 26.5, 22.8, 18.0, 14.2, 12.2
11	106.2, 78.2, 77.7, 75.5, 71.8, 62.8	153.8, 131.8, 129.5, 123.7, 112.0, 68.6, 32.0, 29.8, 29.6, 29.5, 26.5, 22.8, 18.0, 14.2, 12.2

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TABLE VII. Inhibitory Effects of 4-Alkoxyphenyl β-D-Glucopyranosides **1**—**11** on Histamine Release from Rat Peritoneal Mast Cells Induced by Concanavalin A

Compound	Concentration (μM)	Inhibition (%)	Compound	Concentration (μM)	Inhibition (%)
1	3	−1.4	**8**	3	35.0
2	3	2.9	**9**	3	67.3
3	3	3.3	**10**	3	84.2
4	3	9.6	**11**	3	34.3
5	3	32.0	VIE-Glu[a]	100	60.0
6	3	26.6	Azelastin	30	17.3
7	3	20.9			

a) *dl*-α-Tocopheryl β-D-glucopyranoside.

this compound is higher than that of any other compound tested. Thus, it appeared that high hydrophobicity decreased the inhibitory potency of aryl β-D-glucopyranosides on histamine release. The hydrophobicity of compound **6** was nearly the same as that of compound **10**, but its inhibitory effect was approximately a third of that of **10**. In addition, the inhibitory effects of compounds **6** and **7** were almost equal, but the difference between their hydrophobicities was large. These results suggested that the 2,3,6-trimethyl group on the benzene ring increases the inhibitory effect on Con A-induced histamine release. On the basis of previous studies[10] showing that arylglycopyranosides have almost no inhibitory effect on the histamine release induced by compound 48/80 and calcium ionophore A23187, it seems probable that the inhibitory effects of the 4-alkoxyaryl β-D-glucopyranosides tested arise from the inhibition of the binding of Con A to IgE. Moreover, it was reported by Becker *et al.*[16] that the binding site of Con A for saccharides is located in a deep cavity which contains distinct hydrophilic and hydrophobic subunits. Thus, it is considered that a suitable shape and size of the aglycone of aryl β-D-glycosides are necessary for maximal interaction with the hydrophobic subunits. Taking this previous finding together with the fact that the inhibitory effect on Con A-induced histamine release is enhanced by the 2,4,6-trimethyl group on the benzene ring, we consider that the binding site for aryl β-D-glucopyranoside is located in Con A and that the binding of the aryl β-D-glucopyranoside tested to Con A causes the inhibition of Con A-induced histamine release. It was also reported by this laboratory that the histamine release induced by egg albumin is inhibited by some saccharides.[9,11] Though the mechanism by which the histamine release is inhibited is unknown, the facts that egg albumin contains carbohydrate chains[17] and that no saccharide chain exists in the antigenic determinant which is located in the Fab region of the IgE molecule[18] suggest that the antigenic determinant of IgE contains binding sites for saccharides and that the binding of carbohydrate there interferes with the antigen–antibody reaction. Further, since some saccharides inhibit histamine release induced by both Con A and antigen–antibody reaction,[8-10] a saccharide showing an inhibitory effect on Con A-induced histamine release might inhibit the binding of antigen to antibody.

Enzymatic Hydrolysis of 4-Alkoxy-2,3,6-trimethylphenyl β-D-Glucopyranoside Lack of susceptibility to glycosidases is an essential requirement for glycosides as candidates for novel anti-allergic drugs. Thus, we measured the susceptibility of compounds **3** and **8** to hydrolysis by β-glucosidase. The results are shown in Fig. 2. Compound **3** was completely hydrolyzed within 15 min. In contrast, the hydrolysis of compound **8** was very slow. These results agree well with the previous finding that the hydrolysis of *dl*-δ-tocopheryl β-D-glucopyranoside was faster than that of *dl*-α-tocopheryl β-D-glucopyranoside.[11] These results suggest that the 2,6-dimethyl group protects the glycosidic linkage from hydrolytic cleavage by glycosidases.

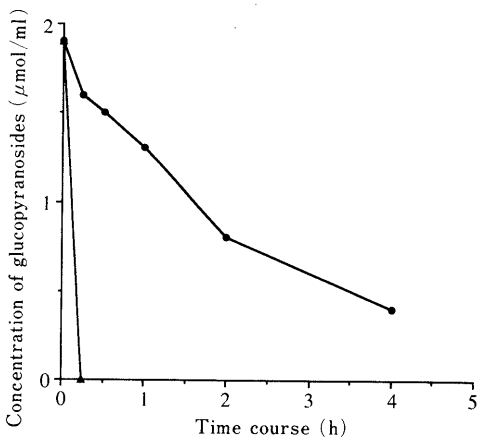

Fig. 2. Hydrolysis of 4-Hexyloxyphenyl-2,3,6-trimethyl-β-D-Glucopyranoside (●) and 4-Hexyloxylphenyl β-D-Glucopyranoside (▲) by β-Glucosidase (110 units/ml)

The inhibitory effects of the present 4-alkoxyaryl β-D-glucopyranosides on Con A-induced histamine release, and their resistance to glucosidase, suggest that they may be potential lead compounds for the development of new anti-allergic drugs.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus, and are uncorrected. Nuclear magnetic resonance spectra were obtained on a JEOL FX-90Q spectrometer. Chemical shifts were reported in parts per million relative to tetramethylsilane (δ units) as an internal standard. Optical rotations were measured with a DIP 140 (JASCO). Elemental analyses were recorded with a Hitachi Perkin–Elmer 240C apparatus. Hydrophobicity was examined with a Cosmosil $_5C_{18}$ column (4.6 × 50 mm). The amounts of 4-alkoxyphenyl β-D-glucopyranosides were determined by using a Cosmosil $_{10}C_{18}$ column (4.6 × 150 mm). Concanavalin A was purchased from Wako Pure Chemical Co., Osaka. L-α-Phosphatidyl-L-serine (from bovine brain) and β-glucosidase (from almonds) were purchased from Sigma Chemical Co. Hanks' solution was purchased from Nissui Pharmaceutical Co., Sugamo.

Chemistry 4-Alkoxyphenols (**Q1**—**Q11**): Phosphomolybdic acid (0.7 g) was added to a solution of a hydroquinone (21.0 mmol) in an alcohol (30 ml). The reaction solution was refluxed for 6 h, then allowed to cool to room temperature, diluted with ethyl acetate and washed with water. The solvents were removed under reduced pressure, then the residue was chromatographed on silica gel with hexane–ethyl acetate, and then recrystallized from hexane to give the corresponding 4-alkoxyphenol (**Q1**—**Q11**). The physical properties and ^{1}H-NMR data are given in Table I.

4-Hexyloxy-2,3,5-trimethylphenol (**Q12**): 2,3,5-Trimethylhydroquinone (3.5 g, 23.0 ml) was dissolved in CH_2Cl_2, and then pyridine (6 ml) was added. The mixture was cooled to −15 °C, and pivaloyl chloride (2.8 g) was added dropwise at this temperature over 20 min. The mixture was warmed to room temperature, and stirred for 8 h. Acetic acid

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(4.25 ml) and water (20 ml) were added.The organic layer was concentrated to dryness, and the residue was chromatographed on silica gel with benzene–ethyl acetate to afford crude 4-pivaloyloxy-2,3,6-trimethylphenol **Q12a** (2.4 g, 44%), mp 69—75 °C. ^{1}H-NMR ($CDCl_3$): 1.37 (9H, s), 2.01 (3H, s), 2.14 (3H, s), 2.17 (3H, s), 4.63 (H, s), 5.58 (H, s). A solution of compound **Q12a** (2.4 g) in 70 ml of methylethylketone (MEK) was treated with 1-iodohexane (21.2, 100 mmol) and K_2CO_3 (6.9 g, 20.0 mmol). The reaction solution was refluxed for 8 h, then diluted with ethyl acetate and washed with water. The solvents were removed under reduced pressure. The residue was chromatographed on silica gel with hexane–ethyl acetate to afford 1-hexyloxy-2,3,6-trimethyl-4-pivaroyloxybenzene **Q12b** (1.5 g, 45%), mp 83—87 °C. ^{1}H-NMR ($CDCl_3$): 0.91 (3H, t, J=5.9 Hz),1.05—1.90 (17H, m), 2.00 (3H, s), 2.19 (3H, s), 2.22 (3H, s), 3,69 (2H, t, J=6.2 Hz), 6.63 (H, s). A mixture of compound **Q12b**, KOH (0.45 g) and methanol (5 ml) was stirred at room temperature for 6 h. The solution was diluted with ethyl acetate and washed with water. The solvents were removed under reduced pressure. The residue was chromatographed on silica gel with hexane–ethyl acetate, and then recrystallized from hexane to afford 4-hexyloxy-2,3,5-trimethylphenol **Q12** (0.7 g, 44%), mp 32—34 °C. ^{1}H-NMR ($CDCl_3$): 0.91 (3H, t, J=5.9 Hz), 1.23—1.79 (8H, m), 2.05 (3H, s), 2.12 (3H, s), 2.18 (3H, s), 3,66 (2H, t, J=6.4 Hz), 4.53 (1H, s), 6.45 (1H, s).

4-Alkoxyphenyl β-D-Glucopyranosides (**1**—**11**): A mixture of β-D-glucopyranose pentaacetate (2.8 g, 7.3 mmol), a 4-alkoxyphenol (14.5 mmol) and $BF_3\cdot(C_2H_5)_2O$ (1.0 ml, 7.3 mmol) in 50 ml of dry benzene was stirred for 8 h at room temperature. The benzene solution was washed with water and 0.5 M NaOH solution, dried with $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel with hexane–ethyl acetate, and then recrystallized from ethanol to give the corresponding 4-alkoxyphenyl β-D-glucopyranoside tetraacetates **1**Ac—**11**Ac. The yields and physical data are given in Table II. ^{1}H-NMR spectal data are given in Table III. Compounds **1**Ac—**11**Ac were each deacetylated with a catalytic amount of 0.1 N CH_3ONa in dry methanol at room temperature. After the reaction was completed, the reaction mixture was neutralized with Amberlite IR 120 (H^+) and decolorized with activated charcoal. The resin and charcoal were filtered off and the solution was evaporated to a syrup under reduced pressure. The residue was crystallized from benzene–ethanol, to afford the corresponding 4-alkoxyphenyl β-D-glucopyranosides **1**—**11**. The physical data, and ^{1}H-NMR and ^{13}C-NMR spectral data are given in Tables IV, V and VI.

Hydrolysis of 4-Hexyloxyphenyl β-D-Glucopyranoside and 4-Hexyloxy-2,3,6-trimethylphenyl β-D-Glucopyranoside by β-Glucosidase: The test sample (20 μmol) and Triton-100 (250 mg) were dissolved in 2 ml of ethanol. The solvent was removed, and 0.1 M acetate buffer solution (pH 5.0) was added to make 5 ml of sample solution. β-Glucosidase (250 mg) was dissolved in 5 ml of acetate buffer solution (0.1 M, pH 5.0) as enzyme solution. A mixture of sample solution (0.1 ml) and enzyme solution (0.4 ml) was adjusted to 1.0 ml with the buffer solution and incubated at 37 °C. At a suitable time, 3 ml of ethanol–acetone (1 : 1) was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 15 min. The residual glucosides in the supernatant were determined by HPLC.

Bioassay Preparation of Rat Peritoneal Mast Cells: Male Sprague Dawley rats weighing 250—300 g were exsanguinated and injected intraperitoneally with 10 ml of Hanks' solution containing 0.1% egg albumin. The abdominal region was gently massaged for 2 min and then the peritoneal exudate was collected. The cells were purified by a modified version of the method of Németh and Röhlich.[19] After purification, mast cells of 90% or greater purity were obtained. The cell suspension was adjusted to a concentration of 5×10^5 mast cells/ml.

Histamine Assay: Three-tenths ml of rat peritoneal mast cells was mixed with 1.0 ml of various concentrations of a test compound and 0.2 ml of L-α-phosphatidyl-L-serine (300 μg/ml), and then 0.3 ml of Hanks' solution was added. The mixture was preincubated for 10 min at 37 °C. Test substance was replaced by physiological saline solution in the control. The preincubated mast cell suspensions were mixed with 0.2 ml of Con A solution (400 μg/ml) and incubated for 10 min at 37 °C. Control solution without Con A was treated in the same manner. Each solution was cooled to 4 °C and centrifuged at $2500\times g$ for 10 min at 4 °C. The histamine in the supernatant and residue were each measured according to the method of Shore *et al.*[20] The percentage inhibition was calculated as follows:

$$\text{histamine release} = \frac{P_s}{P_s+P_r}\times 100 = A$$

P_s: histamine in supernatant
P_r: residual histamine in cells

$$\text{inhibition (\%)} = 100 - \frac{S-B}{C-B}\times 100$$

S: A of sample
C: A of control
B: A of blank

References

1) J. B. Sumner, S. F. Howell, *J. Bact.*, **32**, 227 (1936).
2) K. Ishizaka, T. Ishizaka, *J. Immunol.*, **100**, 554 (1968).
3) N. Sharon, H. Lis, *Science*, **177**, 949 (1972).
4) P. A. Siraganian, R. P. Siraganian, *J. Immunol.*, **112**, 2117 (1974).
5) R. P. Siraganian, P. A. Siraganian, *J. Immunol.*, **114**, 886 (1975).
6) T. J. Sullivan, W. C. Greene, C. W. Parker, *J. Immunol.*, **115**, 278 (1975).
7) T. J. Sullivan, C. W. Parker, *Am. J. Pathol.*, **85**, 437 (1976).
8) R. Keller, *Clin. Exp. Immunol.*, **13**, 139 (1973).
9) T. C. Wang, H. Kakegawa, H. Matsumoto, T. Satoh, *Biol. Pharm. Bull.*, **17**, 93 (1994).
10) T. C. Wang, H. Kakegawa, H. Matsumoto, T. Satoh, *Biol. Pharm. Bull.*, **17**, 87 (1994).
11) T. Satoh, H. Miyataka, Y. Masamoto, T. Asai, K. Hasegawa, H. Kakegawa, Eur. Patent Appl. EP 169716 (1986) [*Chem. Abstr.*, **104**, 213266*u* (1986)].
12) K. Taniguchi, Japan. Kokai Tokkyo Koho JP 60215643 (1985) [*Chem. Abstr.*, **104**, 109228*x* (1986)].
13) T. Yoshioka, T. Fujita, T. Kanai, Y. Aizawa, T. Kurumada, K. Hasegawa, H. Horikoshi, *J. Med. Chem.*, **32**, 421 (1989).
14) B. Helferich, H. Scheiber, *Hoppe-Seyl. Z.*, **226**, 276 (1934).
15) K. Onodera, S. Hirano, H. Fukumi, *Agric. Biol. Chem.*, **28**, 173 (1964).
16) J. W. Becker, G. N. Reeke, Jr., B. A. Cunningham, G. M. Edelman, *Nature* (London), **259**, 406 (1976).
17) P. Johansen, R. D. Marshall, A. Neuberger, *Nature* (London), **181**, 1345 (1976).
18) K. Onoue, "The Iwanami Immunology Series," Vol. I, ed. by Y. Yamamura, T. Kishimoto, Iwanami, Tokyo, 1986, p. 47.
19) A. Németh, P. Röhlich, *Eur. J. Cell. Biol.*, **20**, 272 (1980).
20) P. A. Shore, A. Burkhalter, V. H. Cohn, *J. Pharmac. Exp. Ther.*, **127**, 182 (1959).

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