Inhibitory Effect of Perillosides A and C, and Related Monoterpene Glucosides on Aldose Reductase and Their Structure–Activity Relationships

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Monoterpene glucosides, perillosides A and C, obtained from the leaves of *Perilla frutescens*, were found to be inhibitors of aldose reductase (EC 1.1.1.21) which is considered to be a key enzyme in diabetic complications such as cataract. The apparent type of inhibition of rat lens aldose reductase by perillosides A and C was competitive with respect to glyceraldehyde and their K_i values were 1.4×10^{-4} and 2.3×10^{-4} M, respectively. The type of inhibition by their tetraacetates was non-competitive with respect to the same substrate, although their inhibitory effects were increased by about one order of magnitude compared with those of the perillosides and the K_i values were 2.5×10^{-5} and 7.1×10^{-5} M, respectively. We also prepared related monoterpene glucosides and their tetraacetates and determined their inhibitory activities towards aldose reductase in order to elucidate the relationship between structure and inhibitory activity.

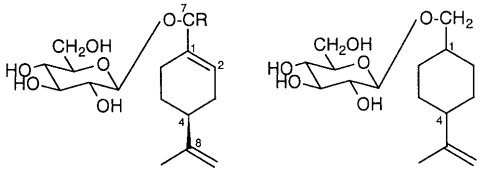
Key words perilloside A; perilloside C; aldose reductase inhibitor; *Perilla frutescens*; monoterpene glucoside; structure–activity relationship

Aldose reductase (AR) is a key enzyme of the polyol pathway, which catalyzes the reduction of hexoses to sugar alcohols.[1] It is expected that AR inhibitors will play an important role in the management of diabetic complications such as cataract, retinopathy, neuropathy, and nephropathy.[2,3] Flavonoids, hydantoin derivatives, carboxylic acids, *etc.* have been examined as AR-inhibitory drugs,[4,5] but only a few terpenoids and their glucosides have been reported.[6] In the previous study,[7] we isolated four new monoterpene glucosides, perillosides A—D, along with five known glucosides from *Perilla frutescens* BRITTON forma *viridis* MAKINO and elucidated their structures. The glucosides were investigated for hypoglycemic or anti-obesity and anti-diabetic activities,[8] and we observed inhibitory effects of perillosides A (**1a**) and C (**3a**) on rat lens AR (RLAR) and human recombinant AR (HRAR). Furthermore, derivatives of these glucosides were synthesized and their activities on ARs were determined. In this paper, we report the inhibitory effects of the perillosides, some synthetic monoterpene glucosides and their tetraacetates, and describe the relationship of structure to the AR-inhibitory activity of monoterpene glucosides.

Results and Discussion

Inhibitory Effects of the Isolated Glucosides from *Perilla frutescens* The inhibitory actions of perillosides A—D (**1a**—**4a**, Fig. 1) and six known glucosides,[7] including newly identified methyl α-D-galactopyranoside, isolated from *Perilla frutescens* BRITTON forma *viridis* MAKINO on RLAR are shown in Table 1. Perillosides A (**1a**) and C (**3a**), and the cyanogenic glucoside prunasin showed a higher inhibitory activity on RLAR than the other glucosides, including perillosides B (**2a**) and D (**4a**). The inhibitory activities of **2a** and **4a** were lower, though the structures are similar to those of **1a** and **3a**. The difference between **3a** and **4a** is the *trans*- and *cis*-form of the *p*-menthane skeleton at the C-1 and C-4 positions, respectively. It was presumed that **1a** and **3a** have a 1-*p*-menthene skeleton with an equatorial side-chain at the C-4 position, while **4a** has an axial side-chain.

Inhibitory Effects of Synthetic Glucosides and Their Tetraacetates Twenty-three glucosides containing **1a**—**4a** and their tetraacetates were prepared from the corresponding alcohols (**1c**—**21c**, shown in Fig. 2) and acetobromoglucose by Koenigs–Knorr methods.[7] Thus, twelve monoterpene glucosides (**5a**—**16a**) and five related glucosides (**17a**—**21a**) were synthesized in order to clarify



1a : R = H, H
2a : R = O

3a : 1,4-*trans*
4a : 1,4-*cis*

Fig. 1. Structures of Perillosides A—D (**1a**—**4a**) from *Perilla frutescens*

Table 1. Inhibitory Effect of Ten Glucosides Isolated from *Perilla frutescens* BRITTON on RLAR

Compound	Inhibition (%)	
	0.1 mM	0.01 mM
Perilloside A (**1a**)	54.5	6.7
Perilloside B (**2a**)	14.4	11.1
Perilloside C (**3a**)	46.4	26.1
Perilloside D (**4a**)	28.2	14.8
Eugenyl β-D-glucoside (**20a**)	35.2	1.1
Benzyl β-D-glucoside (**18a**)	2.3	0
β-Sitosteryl β-D-glucoside	30.9	1.0
Prunasin	68.5	18.5
Sambunigrin	32.9	0
Methyl α-D-galactoside	30.1	0

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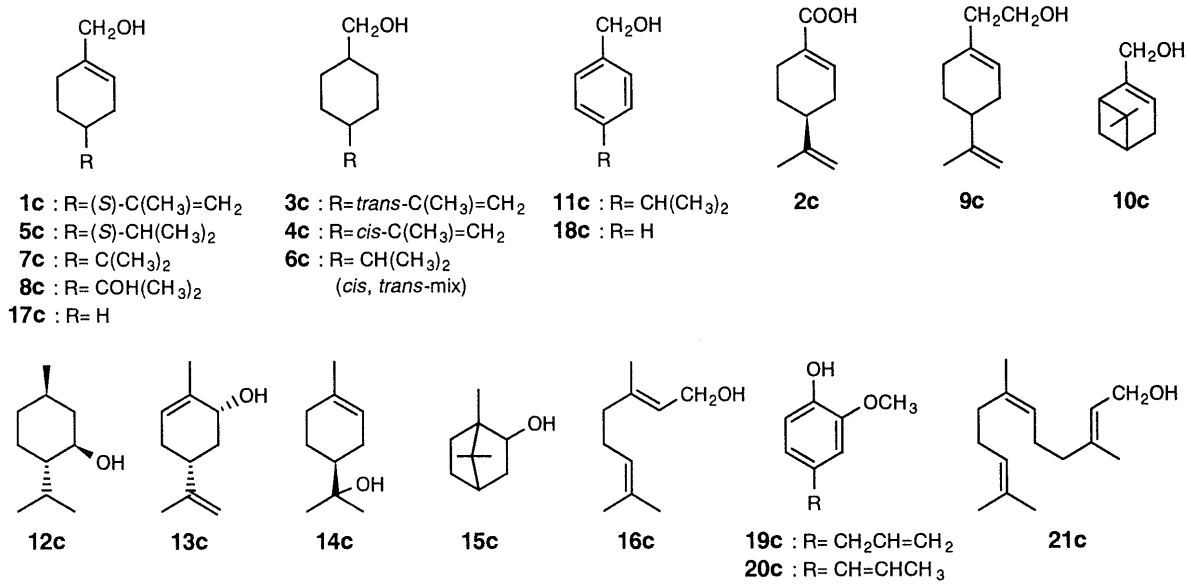

Fig. 2. The Alcohols (**1c**, **3c**—**21c**) and Acid (**2c**) Corresponding to the Synthetic Glucosides (**1a**—**21a**, **1e**, and **5e**) and the Tetraacetylglucosides (**1b**—**21b**, **1d**, and **5d**)

Table 2. Inhibitory Effect of Glucosides and Their Tetraacetates on RLAR

Compound	Inhibition (%)		Compound	Inhibition (%)	
	0.1 mM	0.01 mM		0.1 mM	0.01 mM
1a	54.5	6.7	**1b**	87.1	39.6
2a	14.4	11.1	**2b**	30.7	25.5
3a	46.4	26.1	**3b**	67.0	30.7
4a	28.2	14.8	**4b**	45.2	13.7
5a	43.6	15.7	**5b**	65.4	10.5
6a	26.8	13.9	**6b**	62.3	40.7
7a	41.9	32.9	**7b**	42.3	9.1
8a	14.8	3.8	**8b**	26.3	9.5
9a	35.6	17.4	**9b**	16.2	0
10a	16.3	8.7	**10b**	37.7	10.5
11a	9.5	5.5	**11b**	—[b]	—[b]
12a	38.7	11.6	**12b**	73.9[a]	4.2
13a	9.1	0	**13b**	38.5	16.2
14a	14.2	0	**14b**	20.8	17.5
15a	10.4	8.7	**15b**	33.5	23.8
16a	—[a,b]	0	**16b**	66.8[a]	—[b]
17a	2.0	0	**17b**	62.3	36.6
18a	2.3	0	**18b**	23.5	20.0
19a	30.2	0	**19b**	30.7	12.1
20a	11.4	3.3	**20b**	—[b]	—[b]
21a	47.1[a]	0	**21b**	30.7	9.1
α-Glc			α-Glc(OAc)$_4$		
1e	20.1	9.1	**1d**	37.6	7.1
5e	31.7	16.4	**5d**	32.9	18.7

a) Slight turbidity. *b*) Not measured.

Table 3. Inhibitory Effect of Glucosides and Their Tetraacetates on HRAR

Compound	Inhibition (%)		Compound	Inhibition (%)	
	0.1 mM	0.01 mM		0.1 mM	0.01 mM
1a	44.3	29.5	**1b**	50.2	27.9
3a	40.4	11.3	**3b**	51.7	25.3
5a	40.5	18.0	**5b**	53.4	21.3

the relation between the structure of the aglycone moiety and the inhibitory effect. Six compounds (**5c**—**9c**, **11c**) having a hydroxy group at the C-7 position in the *p*-menthane skeleton were prepared, to examine the effects of structural changes in the aglycone moiety, except at C-7. In addition, the stereoisomers of **1a** and **5a** at the glucosyl linkage, that is, α-D-glucopyranosides (**1e** and **5e**) and their tetraacetates (**1d** and **5d**) were prepared.

The inhibitory effects of these glucosides and their tetraacetates are shown in Table 2. All of the corresponding alcohols for which these effects could be evaluated in this assay system were inactive. Compound **1a** and its homologues such as **3a**, **5a**, and **7a** were quite inhibitory to RLAR. These compounds have a β-glucosyloxy substituent at the C-7 position in the *p*-menthane skeleton. Compound **12a** also depressed the RLAR activity at the same concentration. On the other hand, their tetraacetates demonstrated an approximately one order higher activity than the corresponding glucosides. In this case, compound **1b** and its homologues showed higher activity than the others. α-Type glucosides and tetraacetates had lower inhibitory activity in each case.

The inhibitory effects of the monoterpene glucosides, **1a**, **3a**, and **5a** and their tetraacetates on HRAR were screened by the same method (Table 3). Although the activities were somewhat weaker at the same concentration, the inhibitory tendencies of the glucosides and their tetraacetates were similar to those in the case of RLAR.

It seemed clear that the aglycone moiety of the glucosides and their tetraacetates was recognized by the RLAR and HRAR. The results may be summarized as follows: (1) the *p*-menthane skeleton having a glucosyloxy moiety at the C-7 position is essential for the appearance of inhibitory action; (2) double bonds in the *p*-menthane skeleton, *e.g.*, 1-cyclohexenyl ring or 1,8-*p*-menthadiene moiety, increased the activity; (3) equatorial substituents of **1a**, **3a**, **5a** and **12a** when the compounds have a stable chair form were better than axial ones, as in **4a** and **6a**; (4) acetylation of the glucosides resulted in a one order

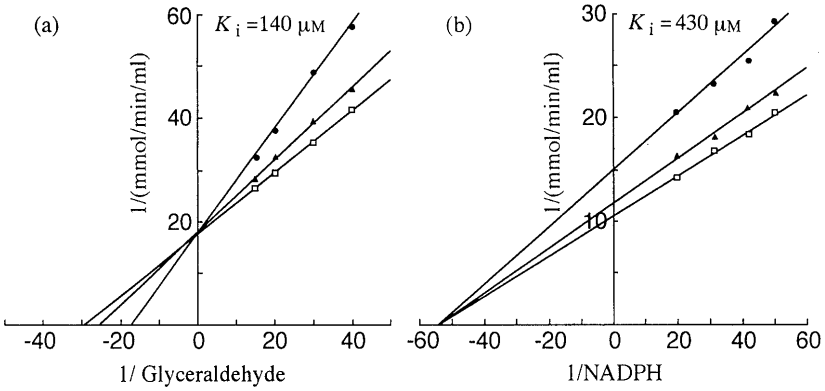

Fig. 3. Lineweaver–Burk Plots of Rat Lens AR Activity for **1a** against Glyceraldehyde and NADPH

Double-reciprocal plots for glyceraldehyde (a) and for NADPH (b). The substrate and NADPH concentrations were as indicated in the presence and absence of inhibitor. Key: (□), control; (▲), 50 μM **1a**; (●), 100 μM **1a**.

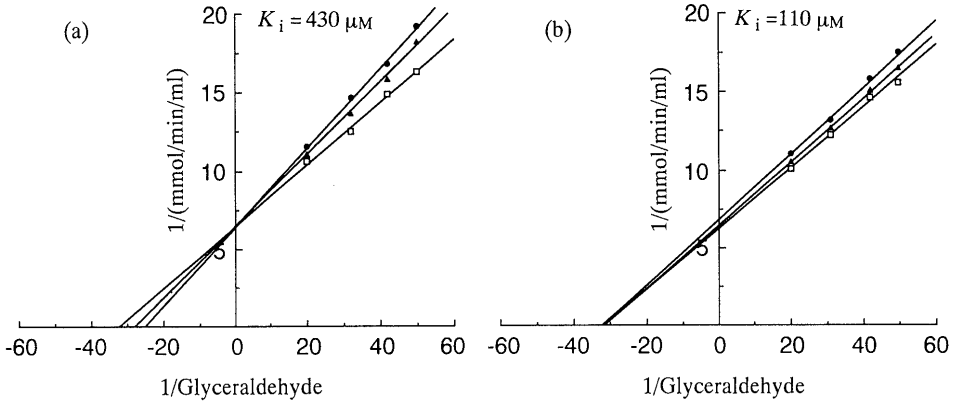

Fig. 4. Lineweaver–Burk Plots of Human Recombinant AR Activity for **1a** and **1b** against Glyceraldehyde

Enzyme activity was measured at the indicated substrate concentration in the presence and absence of inhibitors. (a) Double-reciprocal plots of **1a**. Key: (□), control; (▲), 100 μM **1a**; (●), 150 μM **1a**. (b) Double-reciprocal plots of **1b**. Key: (□), control; (▲), 100 μM **1b**; (●), 150 μM **1b**.

higher activity; (5) the glucosidic β-linkage with aglycone was preferred to the α-linkage the former favors a planar structure; (6) an ether-type glucoside was better than an ester-type. In summary, a planar monoterpene glucoside consisting of a *p*-menthane skeleton with an equatorial side-chain and a β-D-glucosyloxy moiety at the C-7 position is expected to have a potent inhibitory effect on ARs.

Kinetic studies were conducted on **1a** and its tetraacetate **1b** in order to determine the type of inhibition and inhibition constant (K_i). The Lineweaver–Burk plots of **1a** against RLAR are shown in Fig. 3a, 3b. The K_i values of **1a** for the substrates, glyceraldehyde and reduced NADP (NADPH), were calculated from the Lineweaver–Burk plots. The apparent type of enzyme inhibition by **1a** was competitive with respect to glyceraldehyde, but non-competitive to NADPH. On the other hand, the type of inhibition of **1b** was non-competitive with respect to both substrate and NADPH. The other compounds, **3a** and **5a** and their tetraacetates, were similarly examined, and the type of inhibition and the K_i values are shown in Table 4. Kinetic studies of **1a** and **1b** with HRAR were also conducted, and the Lineweaver–Burk plots are shown in Fig. 4a, 4b, respectively. The type of inhibition was similar to that with RLAR, but less potent.

It is hoped that AR inhibitors will play an important role in the management of diabetic complications such as cataract. Historically, many flavonoids, carboxylic acids, and tannins have been examined as AR-inhibitory drugs.[4,5] The inhibitor binding should result from a combination of hydrophobic binding and a reversible charge-transfer reaction.[3,9] Assuming that a compound with higher affinity for the receptor is more potent, the inhibitory potency of an AR inhibitor would be expected to be increased by the incorporation of suitable lipophilic substituents or by the addition of groups which could increase the charge-transfer capability. This is the first report of monoterpene glucosides, such as perillosides, as AR inhibitors. In the present experiments, the introduction of acetyl groups into monoterpene glucosides, as lipophilic substituents, increased the magnitude of inhibition of AR, as shown in Fig. 3. Furthermore, the β-form of mono-

Table 4. Kinetic Properties of Glucosides and Their Tetraacetates on RLAR

	K_i (10^{-4} M)			K_i (10^{-5} M)	
	Glyceraldehyde	NADPH		Glyceraldehyde	NADPH
1a	1.4 (C)	4.3 (NC)	**1b**	2.5 (NC)	4.0 (NC)
3a	2.3 (C)	2.0 (NC)	**3b**	7.1 (NC)	8.9 (NC)
5a	5.4 (C)	3.0 (NC)	**5b**	4.7 (NC)	4.8 (NC)

C, competitive inhibition; NC, non-competitive inhibition.

terpene glucosides showed higher inhibition than the α-form. This result indicates that ARs have stereochemical requirements for binding, as well as lipophilic binding regions and a charge-transfer pocket. More specific studies are required to elucidate the stereochemical requirements for binding to ARs. However, the present results should contribute to the design of more effective AR inhibitors.

Experimental

General Procedures Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Specific rotations were measured on a Horiba SEPA 300 polarimeter in MeOH. IR spectra were recorded on Hitachi 260-10 and Perkin Elmer 1760X Fourier transform (FT)-IR spectrometers. UV spectra were measured with a Hitachi U-3210 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM-GX 270, using tetramethylsilane as an internal standard. Secondary ion (SI) mass spectra were measured on a Hitachi M-80 instrument [SI-MS (negative mode): matrix; glycerol]. HPLC was carried out with a Hitachi L-6200 chromatography system using Chemcosorb 5-ODS-H (4.6 i.d. × 150 mm) and Inertsil octadecyl silica (ODS)-2 (4.6 i.d. × 150 mm) columns.

Materials Crude RLAR was prepared as follows: rat lenses were removed from Wistar strain rats weighing 200—250 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to Hayman and Kinoshita[10] and then partially purified according to Inagaki *et al.*,[11] except that polyethylene glycol 6000 fractionation was used instead of ammonium sulfate fractionation. The resultant crude AR[8] was purified approximately 20-fold. It was used for determining the inhibition by glucosides. HRAR was purchased from Wako Chemicals Co. (Japan). All other chemicals were of the highest grade commercially available.

Assay of Aldose Reductase Activity Aldose reductase assays were conducted according to Hayman and Kinoshita.[10] Assay using RLAR was done at 37 °C in a 50 mM potassium phosphate buffer (pH 7.0) containing 100 μM methyl glyoxal, 200 μM NADPH and the enzyme (0.01 to 0.015 units) in a total volume of 1.0 ml. HRAR assay was done at 37 °C in a 100 mM potassium phosphate buffer (pH 6.2) containing 10 mM glyceraldehyde, 150 μM NADPH and the enzyme (0.01 to 0.015 units) in a total volume of 1.0 ml. The effects of monoterpene glucosides on AR were determined by adding 10 μl of aqueous solution (10 or 100 μM at desired reaction mixture concentrations), while the tetraacetates were dissolved in ethanol. The reaction was initiated by the addition of substrate and the rate of NADPH oxidation was followed by recording the decrease of absorbance at 340 nm on a Hitachi UV-3210 spectrophotometer. The inhibitory activities of the synthetic glucosides and their tetraacetates on RLAR and HRAR are shown in Tables 2 and 3, respectively. The kinetic study of each sample was done with glyceraldehyde as a substrate. The apparent type of inhibition and the inhibition constant (K_i) are summarized in Table 4.

Preparation of Perillosides A—D Perillosides A—D (**1a—4a**), along with five known glucosides, were prepared from the methanolic extract of fresh leaves of *Perilla frutescens* Britton forma *viridis* Makino. The isolation procedure and structural elucidation of these compounds were described in detail in our previous papers.[7]

Synthesis of Monoterpene Glucosides Compounds **1a**, **3a—21a** were prepared from the corresponding alcohols (**1c**, **3c—21c**) and acetobromoglucose by the modified Koenigs–Knorr method, followed by deacetylation as described previously.[7] The ^{1}H- and ^{13}C-NMR spectral data for some synthetic monoterpene glucosides and their tetraacetates are summarized in Tables 5—8.

(−)-Perillyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (1b) Compound **1b** was prepared from (−)-perillyl alcohol (**1c**, α_D^{20} −102.1 (neat), d_4^{20} 0.959, n_D^{20} 1.5010), which was rectified by fine distillation, according to the above methods.[7] Mercuric cyanide (11.0 g) was added to a solution of acetobromoglucose (17.0 g) and **1c** (6.5 g) in benzene, and the mixture was stirred for 2 h at 50 °C. The reaction mixture was worked up as usual and the residue was subjected to silica gel column chromatography followed by crystallization to give **1b** (7.2 g) as colorless needles.

Perilloside A (=(−)-Perillyl β-D-Glucopyranoside (1a)) A solution of **1b** (7.2 g) in methanol (50 ml) was treated with 10% methanolic potassium hydroxide (50 ml), and the mixture was stirred for 5 h at room temperature, then worked up as usual. The residue was chromatographed on silica gel, followed by crystallization to give **1a** (3.5 g) as colorless needles. The spectral data of **1a** were identical with those of perilloside A.

(−)-Perillyl 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranoside (1d) The mother liquor, which was obtained upon the purification of crude **1b** as a by-product, was rechromatographed on silica gel (BW-350, Fuji Silysia Co.) with hexane–EtOAc (10 : 1) to give a small amount of the α-isomer (**1d**) as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 6 and 8.

(−)-Perillyl α-D-Glucopyranoside (1e) Compound **1d** was deacetylated to give **1e** as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 5 and 7.

(−)-Phellandryl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (5b) Compound **5b** was prepared from acetobromoglucose and (−)-phellandrol (**5c**, α_D^{20} −77.8 (neat), d_4^{20} 0.936, n_D^{20} 1.4831), which was itself prepared from **1c** by selective hydrogenation followed by repeated rectification, according to the same method as above to give **5b** as colorless needles. mp 57—58 °C, $[\alpha]_D^{20}$ −58.3° (c=0.45, MeOH). ^{1}H- and ^{13}C-NMR, see Tables 6 and 8.

(−)-Phellandryl β-D-Glucopyranoside (5a) Compound **5b** was deacetylated to give **5a** as colorless needles, mp 107—109 °C, $[\alpha]_D^{20}$ −83.3° (c=0.37, MeOH), ^{1}H- and ^{13}C-NMR, see Tables 5 and 7.

(−)-Phellandryl 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranoside (5d) The mother liquor, which was obtained upon the purification of crude **5b** as a by-product, was rechromatographed on silica gel (BW-350, Fuji Silysia Co.) with hexane–EtOAc (10 : 1) to give a small amount of the α-isomer (**5d**) as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 6 and 8.

(−)-Phellandryl α-D-Glucopyranoside (5e) Compound **5d** was

Table 5. ^{1}H-NMR Spectral Data for **1e**, **5a**, **5e**, **7a**, and **8a** (δ ppm in CD_3OD)

H	1e	5a	5e	7a	8a
2	5.78 (1H, br s)	5.73 (1H, br s)	5.74 (1H, br s)	5.72 (1H, br s)	5.76 (1H, br s)
3	—	—	—	2.79 (2H, br s)	—
4	—	—	—		—
5	1.48 (1H, m), 1.84 (1H, m)	—	1.48 (1H, m), 1.84 (1H, m)	2.13 (2H, m)[c]	—
6	—	—	—	2.34 (2H, m)[c]	—
7a	3.92 (1H, d, J=11.6)	4.01 (1H, d, J=11.6)	3.90 (1H, d, J=11.6)	4.05 (1H, d, J=11.6)	4.02 (1H, d, J=11.6)
7b	4.08 (1H, d, J=11.6)	4.21 (1H, d, J=11.6)	4.06 (1H, d, J=11.6)	4.21 (1H, d, J=11.6)	4.22 (1H, d, J=11.6)
8		—	1.47 (1H, dquin, J=6.4, 6.7)		
9	4.71 (2H, s)	0.90 (3H, d, J=6.7)[a]	0.90 (3H, d, J=6.7)[b]	1.65 (3H, s)[d]	1.16 (3H, s)
10	1.74 (3H, s)	0.91 (3H, d, J=6.7)[a]	0.91 (3H, d, J=6.7)[b]	1.69 (3H, s)[d]	1.16 (3H, s)
1′	4.77 (1H, d, J=3.7)	4.25 (1H, d, J=7.9)	4.77 (1H, d, J=3.7)	4.25 (1H, d, J=7.6)	4.26 (1H, d, J=7.9)
2′	3.38 (1H, dd, J=3.7, 9.8)	—[e]	3.37 (1H, dd, J=3.7, 9.8)	—[e]	—[e]
3′	3.65 (1H, dd, J=9.2, 9.2)	—[e]	3.65 (1H, dd, J=8.9, 9.8)	—[e]	—[e]
4′	3.27 (1H, m)	—[e]	3.28 (1H, m)	—[e]	—[e]
5′	3.58 (1H, ddd, J=2.1, 5.5, 9.7)	—[e]	3.57 (1H, ddd, J=2.4, 5.5, 9.8)	—[e]	—[e]
6′a	3.66 (1H, dd, J=5.5, 11.6)	3.66 (1H, dd, J=5.5, 11.9)	3.66 (1H, dd, J=5.2, 11.9)	3.66 (1H, dd, J=5.2, 11.9)	3.66 (1H, dd, J=5.5, 11.9)
6′b	3.80 (1H, dd, J=2.1, 11.6)	3.85 (1H, dd, J=2.4, 11.9)	3.80 (1H, dd, J=2.1, 11.9)	3.86 (1H, dd, J=2.1, 11.9)	3.86 (1H, dd, J=2.1, 11.9)

Figures in parentheses are coupling constants (Hz). *a—d*) Assignments may be interchanged within the same column. *e*) Signals overlapped with each other within δ 3.15—3.40 ppm. (—), not assigned.

Table 6. ^{1}H-NMR Spectral Data for **1d**, **5b**, **5d**, **7b**, and **8b** (δ ppm in $CDCl_3$)

H	1d	5b	5d	7b	8b
2	5.73 (1H, br s)	5.68 (1H, br s)	5.70 (1H, br s)	5.67 (1H, br s)	5.69 (1H, br d, J=4.9)
3	—	—	—	2.80 (2H, br s)	—
4	—	—	—		—
5, 6	—	—	—	—	—
7a	3.88 (1H, d, J=11.9)	3.97 (1H, d, J=11.9)	3.87 (1H, d, J=11.9)	4.00 (1H, d, J=11.6)	3.97 (1H, d, J=11.6)
7b	4.05 (1H, d, J=11.9)	4.18 (1H, d, J=11.9)	4.04 (1H, d, J=11.9)	4.18 (1H, d, J=11.6)	4.20 (1H, d, J=11.6)
8		—	—		
9	4.71 (1H, s) 4.73 (1H, t like, J=1.5)	0.89 (3H, d, J=6.7)[a]	0.89 (3H, d, J=6.7)[b]	1.66 (3H, s)[c]	1.19 (3H, s)[d]
10	1.74 (3H, s)	0.90 (3H, d, J=6.7)[a]	0.90 (3H, d, J=6.7)[b]	1.70 (3H, s)[c]	1.21 (3H, s)[d]
1′	5.06 (1H, d, J=3.7)	4.52 (1H, d, J=7.9)	5.06 (1H, d, J=3.7)	4.51 (1H, d, J=7.9)	4.52 (1H, d, J=7.9)
2′	4.88 (1H, dd, J=3.7, 10.4)	5.02 (1H, dd, J=7.9, 9.5)	4.87 (1H, dd, J=3.7, 10.1)	5.01 (1H, dd, J=7.9, 9.5)	5.01 (1H, dd, J=7.9, 9.5)
3′	5.51 (1H, dd, J=9.5, 10.1)	5.22 (1H, dd, J=9.5, 9.5)	5.51 (1H, dd, J=9.5, 10.1)	5.21 (1H, dd, J=9.1, 9.5)	5.22 (1H, dd, J=9.2, 9.5)
4′	5.07 (1H, dd, J=9.5, 10.1)	5.09 (1H, dd, J=9.5, 9.8)	5.07 (1H, dd, J=9.5, 10.1)	5.09 (1H, dd, J=9.5, 9.8)	5.09 (1H, dd, J=9.2, 9.8)
5′	4.05 (1H, m)	3.68 (1H, ddd, J=2.1, 4.6, 9.8)	4.05 (1H, m)	3.68 (1H, ddd, J=2.4, 4.9, 9.8)	3.68 (1H, ddd, J=2.7, 4.6, 9.8)
6′a	4.09 (1H, dd, J=2.4, 12.5)	4.14 (1H, dd, J=2.1, 12.2)	4.08 (1H, dd, J=2.5, 12.2)	4.14 (1H, dd, J=2.1, 12.2)	4.14 (1H, dd, J=2.7, 12.2)
6′b	4.28 (1H, dd, J=4.6, 12.5)	4.27 (1H, dd, J=4.6, 12.2)	4.28 (1H, dd, J=4.5, 12.2)	4.26 (1H, dd, J=4.6, 12.2)	4.26 (1H, dd, J=4.6, 12.2)
OAc	2.02 (3H, s), 2.03 (3H, s), 2.06 (3H, s), 2.10 (3H, s)	2.01 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.09 (3H, s)	2.01 (3H, s), 2.03 (3H, s), 2.06 (3H, s), 2.10 (3H, s)	2.01 (3H, s), 2.02 (3H, s), 2.04 (3H, s), 2.09 (3H, s)	2.01 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.09 (3H, s)

Figures in parentheses are coupling constants (Hz). *a—d*) Assignments may be interchanged within the same column. (—), not assigned.

Table 7. ^{13}C-NMR Data for Synthetic Monoterpene Glucosides (δ ppm in CD_3OD)

Carbon	1a	1e	3a	4a	5a	5e	7a	8a	9a	11a
1	135.4	135.4	39.3	35.2	135.6	135.5	135.9	135.6	135.5	136.4
2	125.9	126.0	31.06[a]	27.85[b]	126.4	126.5	126.2	126.3	123.2	129.5
3	31.6	31.7	32.5	28.01[b]	29.9	29.8	30.3	28.1[d]	31.9[e]	127.3
4	42.3	42.5	46.9	45.5	41.5	41.6	128.5	46.3	42.4	149.7
5	28.7	28.8	32.5	27.97[b]	27.2	27.2	27.3[c]	24.9	30.1[e]	127.3
6	27.4	27.3	31.03[a]	27.93[b]	27.7	27.6	28.2[c]	27.8[d]	29.1[e]	129.5
7	74.3	72.0	76.4	72.8	74.5	72.1	74.0	74.4	38.6	71.6
8	150.9	151.0	151.9	151.1	33.4	33.5	122.8	73.1	69.6	35.1
9	109.2	109.2	108.7	109.2	20.3	20.3	20.3	27.1	151.1	24.4
10	21.0	20.9	21.1	21.5	20.1	20.1	19.9	26.3	109.0	24.4
11									21.0	
1′	102.8	98.4	104.6	104.6	102.9	98.4	102.8	103.0	104.3[f]	103.1
2′	74.9	73.6	75.2	75.2	75.1	73.6	75.1	75.1	75.0	75.1
3′	78.0	75.1	78.1	78.1	78.1	75.1	78.1	78.1	78.1	78.1
4′	71.5	71.9	71.7	71.7	71.7	71.9	71.7	71.7	71.6	71.7
5′	77.7	73.7	77.9	77.9	77.8	73.7	77.9	77.9	77.9	78.0
6′	62.7	62.7	62.8	62.8	62.8	62.7	62.8	62.8	62.7	62.8

a—e) Assignments may be interchanged within the same column. *f*) The signal was split due to (±)-form.

deacetylated to give **5e** as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 5 and 7.

***p*-Menthan-7-yl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranoside (6b)** Compound **6b** was prepared from acetobromoglucose and *p*-menthan-7-ol (**6c**), which was derived from **1c** as a mixture of *cis* and *trans* isomers (*ca.* 2:3) at the C-1 and C-4 positions by hydrogenation, to give **6b** as a colorless powder. The ratio of *cis* and *trans* isomers of **6b** was about 3:7. ^{1}H-NMR ($CDCl_3$) δ: 0.847 and 0.853 (total 6H, each *trans* and *cis* isomer, d, J=7.0 Hz, $-CH_3\times2$), 2.01, 2.02, 2.04 (and 2.03), 2.09 (3H each, s, $COCH_3$), 3.24 (J=7.0, 9.5 Hz), 3.71 (J=6.1, 9.5 Hz) (*trans* isomer, H-7a and 7b), 3.41 (J=7.9, 9.5 Hz), 3.81 (J=6.7, 9.5 Hz) (*cis* isomer, H-7a and 7b), 3.66 (1H, m, H-5′), 4.13 (1H, dd, J=2.1, 12.2 Hz, H-6′a), 4.27 (1H, dd, J=4.6, 12.2 Hz, H-6′b), 4.47 and 4.49 (total 1H, each *trans* and *cis* isomer, d, J=7.9 Hz, H-1′α), 5.00 (1H, dd, J=7.9, 9.5 Hz, H-2′), 5.08 (1H, dd, J=9.5, 9.8 Hz, H-4′), 5.21 (1H, dd, J=9.5, 9.5 Hz, H-3′).

***p*-Menthan-7-yl *β*-D-Glucopyranoside (6a)** Compound **6b** was deacetylated to give **6a** as a colorless syrup. The ratio of *cis* and *trans* isomers of **6a** was about 1:3. ^{1}H-NMR (CD_3OD) δ: 0.87 (6H, *trans* and *cis* isomers, d, J=7.0 Hz, $-CH_3\times2$), 3.48 (*cis* isomer, H-7a), 3.67 (1H, dd, J=4.6, 11.6 Hz, H-6′a), 3.74 (*trans* isomer, H-7b), 3.86 (1H, br d, J=11.6 Hz, H-6′b), 4.23 and 4.25 (total 1H, each *trans* and *cis* isomer, d, J=7.6 Hz, H-1′α).

1,4(8)-*p*-Menthadien-7-yl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranoside (7b) Compound **7b** was prepared from acetobromoglucose and 1,4(8)-*p*-menthadien-7-ol (**7c**, d_4^{20} 0.983, n_D^{20} 1.5162), which was derived from **8c** by pyrolysis, to give **7b** as a colorless syrup. ^{1}H- and ^{13}C-NMR, see Tables 6 and 8.

1,4(8)-*p*-Menthadien-7-yl *β*-D-Glucopyranoside (7a) Compound **7b** was deacetylated to give **7a** as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 5 and 7.

8-Hydroxy-1-*p*-menthen-7-yl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranoside (8b) Compound **8b** was prepared from acetobromoglucose and 1-*p*-menthene-7,8-diol (**8c**), which was derived from *β*-pinene oxide by hydration to give **8b** as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 6 and 8.

8-Hydroxy-1-*p*-menthen-7-yl *β*-D-Glucopyranoside (8a) Compound **8b** was deacetylated to give **8a** as a colorless powder. ^{1}H- and ^{13}C-NMR, see Tables 5 and 7.

Homoperillyl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranoside (9b) Compound **9b** was prepared from acetobromoglucose and homoperillyl alcohol (**9c**, α_D^{20} −2.4 (neat), d_4^{20} 0.952, n_D^{20} 1.4994), which was derived from nopol by pyrolysis, to give **9b** as a colorless powder. ^{1}H-NMR ($CDCl_3$) δ: 1.73 (3H, s, H-11), 2.01, 2.03, 2.04, 2.09 (3H each, s, $COCH_3$), 2.23 (2H, br t, J=7.0 Hz, H-7), 3.57 (1H, ddt, J=1.2, 7.0, 9.5 Hz, H-8a), 3.69 (1H, ddd, J=2.4, 4.6, 9.8 Hz, H-5′), 3.93 (1H, ddt, J=2.7, 7.0, 9.5 Hz, H-8b), 4.14 (1H, dd, J=2.4, 12.2 Hz, H-6′a), 4.27 (1H, dd, J=4.6, 12.2 Hz, H-6′b), 4.52 (1H, d, J=7.9 Hz, H-1′α), 4.70 (1H, s, H-10a), 4.71

Table 8. ^{13}C-NMR Data for Synthetic Tetraacetates of Monoterpene Glucosides (δ ppm in $CDCl_3$)

Carbon	1b	1d	3b	4b	5b	5d	7b	8b	9b	11b
1	133.0	132.8	37.7	33.5	133.1	132.9	133.6	133.3	133.9[e]	133.9
2	125.8	126.4	29.7[a]	26.25[b]	126.4	127.0	126.1	125.8	122.3	127.9
3	30.4	30.3	31.0	26.76[b]	28.6	28.7	29.3	26.6	30.7[d]	126.5
4	40.8	40.8	45.3	43.7	39.8	39.9	126.6	44.8	40.9	148.8
5	27.2	27.2	31.0	26.59[b]	25.8	25.8	26.1[c]	23.4	29.1[d]	126.5
6	26.1	26.0	29.5[a]	26.51[b]	26.3	26.2	26.8[c]	26.6	27.8[d]	127.9
7	73.5	71.5	75.7	72.1	73.6	71.6	73.2	73.5	37.2[e]	70.6
8	149.5	149.4	150.6	149.7	32.1	32.1	122.6	72.5	68.7[e]	33.8
9	108.8	108.8	108.1	108.7	19.8	19.9	20.2	27.3	149.9	23.9
10	20.72	20.72	20.60	21.4	19.5	19.6	19.7	26.3	108.5	23.9
11									20.72	
1′	98.9	93.7	101.1	101.1	98.7	93.5	98.8	99.0	100.6[e]	99.1
2′	71.2	70.7	71.4	71.3	71.2	70.7	71.3	71.3	71.3	71.3
3′	72.9	70.2	72.8	72.8	72.8	70.2	72.9	72.9	72.9	72.8
4′	68.4	68.5	68.5	68.4	68.4	68.6	68.5	68.4	68.4	68.4
5′	71.6	67.2	71.8	71.7	71.6	67.1	71.6	71.6	71.7	71.8
6′	61.9	61.9	62.0	62.0	61.9	61.9	61.9	61.9	61.9	61.9
CO	170.7	170.6	170.7	170.7	170.6	170.6	170.7	170.7	170.6	170.6
	170.3	170.1	170.3	170.3	170.3	170.1	170.3	170.3	170.3	170.2
	169.4	170.0	169.4	169.4	169.3	170.0	169.4	169.4	169.4	169.3
	169.2	169.6	169.2	169.3	169.2	169.6	169.2	169.2	169.2	169.2
CH_3	20.72	20.69	20.91	20.75	20.66	20.69	20.7	20.7	20.7	20.7
	20.65	20.66	20.72	20.62	20.61	20.66	(2×C)	20.6	20.6	20.5
	20.60	20.60	20.61	(2×C)	20.54	20.60	20.5	20.5	(3×C)	(3×C)
	20.57	(2×C)	(2×C)	20.60	20.52	(2×C)	(2×C)	(2×C)		

a—d) Assignments may be interchanged within the same column. *e*) The signals were split due to (±)-form.

(1H, d-like, J=1.5 Hz, H-10b), 4.99 (1H, dd, J=7.9, 9.5 Hz, H-2′), 5.09 (1H, dd, J=9.5, 9.8 Hz, H-4′), 5.21 (1H, dd, J=9.2, 9.5 Hz, H-3′), 5.45 (1H, br s, H-2). ^{13}C-NMR, see Table 8.

Homoperillyl β-D-Glucopyranoside (9a) Compound **9b** was deacetylated to give **9a** as a colorless paste. ^{1}H-NMR (CD_3OD) δ: 1.72 (3H, d-like, J=0.9 Hz, H-11), 2.29 (2H, br t, J=7.0 Hz, H-7), 3.61 (1H, m, H-8a), 3.67 (1H, dd, J=4.5, 11.9 Hz, H-6′a), 3.87 (1H, br d, J=11.9 Hz, H-6′b), 3.97 (1H, m, H-8b), 4.26 (1H, d, J=7.6 Hz, H-1′α), 4.69 (2H, s, H-10), 5.50 (1H, br s, H-2). ^{13}C-NMR, see Table 7.

Cuminyl (=p-Cymen-7-yl) 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (11b) Compound **11b** was prepared from acetobromoglucose and cuminyl alcohol (**11c**) to give **11b** as a colorless powder. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (6H, d, J=6.7 Hz, –CH_3), 2.00, 2.04, 2.11 (6H, 3H, 3H each, s, $COCH_3$), 2.91 (1H, dq, J=6.7 Hz, H-8), 3.67 (1H, ddd, J=2.4, 4.6, 9.8 Hz, H-5′), 4.17 (1H, dd, J=2.4, 12.2 Hz, H-6′a), 4.28 (1H, dd, J=4.6, 12.2 Hz, H-6′b), 4.54 (1H, d, J=7.6 Hz, H-1′α), 4.59 (1H, d, J=12.2 Hz, H-7a), 4.86 (1H, d, J=12.2 Hz, H-7b), 5.05 (1H, dd, J=7.6, 9.8 Hz, H-2′), 5.10 (1H, dd, J=8.9, 9.0 Hz, H-4′), 5.17 (1H, dd, J=9.0, 9.2 Hz, H-3′), 7.21 (4H, s, aromatic protons). ^{13}C-NMR, see Table 8.

Cuminyl (=p-Cymen-7-yl) β-D-Glucopyranoside (11a) Compound **11b** was deacetylated to give **11a** as a colorless powder. ^{1}H-NMR (CD_3OD) δ: 1.23 (6H, d, J=7.0 Hz, –CH_3), 2.88 (1H, dq, J=6.7, 7.0 Hz, H-8), 3.69 (1H, dd, J=5.5, 11.9 Hz, H-6′a), 3.89 (1H, dd, J=2.1, 11.9 Hz, H-6′b), 4.34 (1H, d, J=7.6 Hz, H-1′α), 4.63 (1H, d, J=11.6 Hz, H-7a), 4.88 (1H, d, J=11.6 Hz, H-7b), 7.19 (2H, d, J=7.9 Hz, H-3, 5 or H-2, 6), 7.33 (2H, d, J=7.9 Hz, H-2, 6 or H-3, 5). ^{13}C-NMR, see Table 7.

1-Cyclohexenylmethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (17b) Compound **17b** was prepared from acetobromoglucose and 1-cyclohexenylmethanol (**17c**) to give **17b** as a colorless powder. ^{1}H-NMR ($CDCl_3$) δ: 1.5—1.7 (4H, m), 1.9—2.3 (4H, m), 2.00, 2.02, 2.04, 2.09 (3H each, s, $COCH_3$), 3.67 (1H, ddd, J=2.4, 4.6, 9.8 Hz, H-5′), 3.96 (1H, d, J=11.6 Hz, H-7a), 4.14 (1H, dd, J=2.4, 12.2 Hz, H-6′a), 4.15 (1H, d, J=11.6 Hz, H-7b), 4.26 (1H, dd, J=4.6, 12.2 Hz, H-6′b), 4.51 (1H, d, J=7.9 Hz, H-1′α), 5.01 (1H, dd, J=7.9, 9.5 Hz, H-2′), 5.08 (1H, dd, J=9.5, 9.8 Hz, H-4′), 5.21 (1H, dd, J=9.2, 9.5 Hz, H-3′), 5.68 (1H, br s, H-2). ^{13}C-NMR ($CDCl_3$) δ: 20.5 (q), 20.6 (q), 20.6 (q), 20.7 (q), 22.2 (t), 22.4 (t), 25.0 (t), 25.5 (t), 62.0 (t), 68.5 (d), 71.3 (d), 71.7 (d), 73.0 (d), 73.8 (t), 98.8 (d), 126.5 (d), 133.4 (s), 169.2 (s), 169.4 (s), 170.3 (s), 170.6 (s).

1-Cyclohexenylmethyl β-D-Glucopyranoside (17a) Compound **17b** was deacetylated to give **17a** as a colorless powder. ^{1}H-NMR (CD_3OD) δ: 1.63 (4H, m), 2.04 (4H, m), 3.66 (1H, dd, J=5.5, 11.9 Hz, H-6′a), 3.86 (1H, dd, J=2.1, 11.9 Hz, H-6′b), 4.01 (1H, d, J=11.6 Hz, H-7a), 4.18 (1H, d, J=11.6 Hz, H-7b), 4.24 (1H, d, J=7.9 Hz, H-1′α), 5.74 (1H, br s, H-2). ^{13}C-NMR (CD_3OD) δ: 23.5 (t), 23.6 (t), 26.0 (t), 26.8 (t), 62.8 (t), 71.7 (d), 74.6 (t), 75.1 (d), 77.9 (d), 78.1 (d), 102.8 (d), 126.6 (d), 135.8 (s).

Myrtenyl β-D-glucopyranoside (**10a**),[12] (–)-menthyl β-D-glucopyranoside (**12a**),[13] (–)-*cis*-carvyl β-D-glucopyranoside (**13a**),[14] α-terpinyl β-D-glucopyranoside (**14a**),[15] bornyl β-D-glucopyranoside (**15a**),[16] geranyl β-D-glucopyranoside (**16a**)[17], benzyl β-D-glucopyranoside (**18a**),[18] isoeugenyl β-D-glucopyranoside (**20a**),[19] and farnesyl β-D-glucopyranoside (**21a**),[17] and their tetraacetates were prepared in a similar manner to that described for the preparation of **1a**. The starting materials were commercial products unless otherwise indicated. (–)-*cis*-Carveol (**13c**, α_D^{20} –19.1 (neat), d_4^{20} 0.959, n_D^{20} 1.4976) used in this experiment was prepared from (–)-carvone by stereoselective reduction. The structures of these glucosides were confirmed by comparison with the spectral data (^{1}H- and ^{13}C-NMR) in the literature.

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