The Role of Diglycosides as Tea Aroma Precursors: Synthesis of Tea Diglycosides and Specificity of Glycosidases in Tea Leaves

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Two general synthetic routes were established in order to synthesize two diglycosides, primeverosides (**1**) and vicianosides (**2**), found in tea leaves. Procedure 1 is based on the Koenig−Knorr type of condensation of aglycon alcohols and 1-α-bromohexabenzoylprimeverose (**6**) and is suitable for the condensation of primary alcohols. Procedure 2 is to combine tribenzoyl-β-D-glucoside (**8**) and 1-α-bromotribenzoylxylose (**4**). The primeveroside of a tertiary alcohol was synthesized by this method which is also applicable to the synthesis of vicianosides. The hydrolysis rate of each of the 12 synthesized glycosides by a crude tea enzyme was evaluated, which suggest that the main glycosidase is primeverosidase and the enzyme mixture shows substrate specificity to both the carbohydrate and aglycon moieties.

Keywords: *Tea; flavor precursor; synthesis; primeveroside; vicianoside*

INTRODUCTION

Aroma is one of the important factors to determine the character and quality of various teas. In a recent review of tea research (Teranishi and Hornstein, 1995), more than 600 volatile compounds concerned with tea aroma were described, among which aliphatic alcohols and mono- and sesquiterpene alcohols were the main constituents of the aroma volatiles of black and oolong teas. These teas are called fermented and semifermented teas, respectively, because their manufacture involves the enzymatic activity in fresh tea leaves to different extents. Biochemical aroma formation is thought to proceed by two different processes.

One type follows normal biosynthetic routes such as the formation of (*Z*)-3-hexenol from linolenic acid via peroxidation by lipoxygenase and cleavage of the C−C bond by a lyase (Hatanaka, 1993) or the formation of various terpene alcohols via the mevalonate pathway (Erman, 1985).

The other type involves the hydrolysis of glycosides having aroma compounds as their aglycons. Various glycosides have recently been identified from fruits and other parts of plants (Schreier and Winterhalter, 1993). In fresh tea leaves, benzyl-β-D-glucoside (Yano *et al.*, 1991) and (*Z*)-3-hexenyl-β-D-glucoside (Kobayashi *et al.*, 1994) have been identified as precursors of tea aroma, as well as diglycosides, such as 6-*O*-β-xylopyrasyl-β-D-glucopyranosides (primeverosides, **1**) of benzyl alcohol, 2-phenylethanol and (*S*)-linalool (Guo *et al.*, 1994) and of some linalool oxides (Moon *et al.*, 1994) from fresh leaves of an oolong tea cultivar. Geranylvicianoside (**2c**), the 6-*O*-α-L-arabinopyranosyl-β-D-glycopyranoside, has also recently been identified in fresh tea leaves of a green tea cultivar (Nishikitani *et al.*, 1996). Studies on the chemical structures of these glycosides to investigate the character and specificity of the glycosidase in tea leaves have shown that the main glycosidase was primeverosidase (Guo *et al.*, 1995). However, the primeverosides used in that study as enzyme substrates were natural products separated from tea leaves in such small amounts that limited their use in further investigations to clarify the formation mechanism of tea aroma from the glycosidically bound aroma compounds. It thus became necessary to obtain greater amounts of various glycosides by synthetic methods.

p-Nitrophenyl-β-D-primeveroside has been synthesized for biochemical studies on xyloglucan (Sone and Masaki, 1986), and only two primeverosides, moridone 6-*O*-β-primeveroside (Vermes *et al.*, 1980) and neohancosides (Konda *et al.*, 1996), have been synthesized to elucidate the chemical structure of the natural products.

We tried to find a more general synthetic method for obtaining diglycosides, primeverosides and vicianosides, having typical tea aroma compounds as their aglycons, to clarify the enzyme specificity for these glycosides in tea leaves, as well as to elucidate their chemical structures.

Our synthetic strategy toward primeverosides is based on the Koenig−Knorr reaction. The first route involves combining the primeverose moiety with aglycon alcohol, and the second one combines the xylose moiety with the already prepared glucoside. The former route proved to be convenient for primary alcohols (procedure 1) and the latter for the less reactive tertiary alcohols such as linalool as aglycon (procedure 2). As a variation of latter procedure, the arabinose moiety substituted for xylose was condensed with glucoside to give several vicianosides in reasonable yield.

MATERIALS AND METHODS

Analytical Instruments. *GC and GC−MS Analyses.* Quantitative analysis of the volatiles was conducted with Shimadzu GC-7A gas chromatograph equipped with FID. The GC conditions were as follows: column, 50 m × 0.25 mm (i.d.) fused silica capillary type, coated with PEG-20M (CP-Wax, film thickness, 0.25 μm); N_2 carrier gas flow rate, 1.1 mL/min; split ratio, 35:1; column temperature, held at 60 °C for 4 min and

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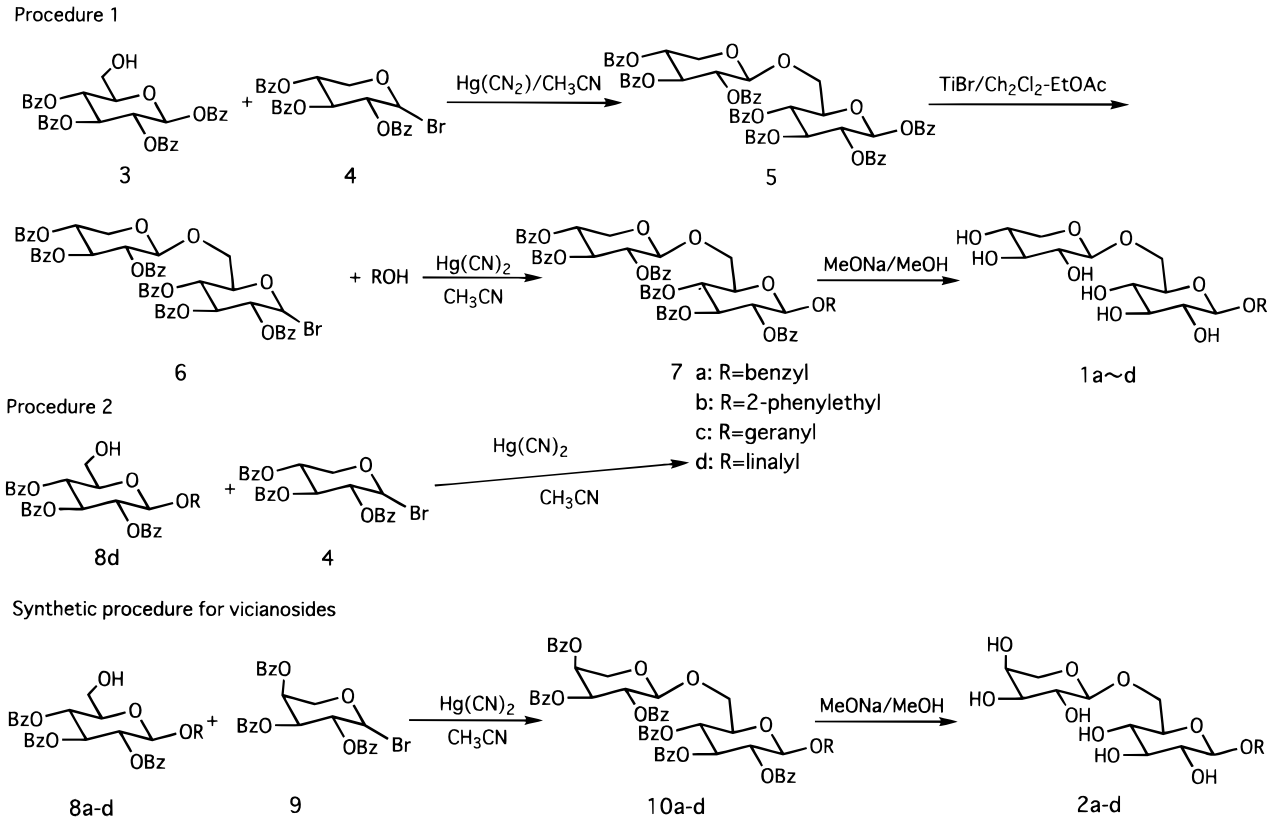


Figure 1. General synthetic procedures for primeverosides and vicianosides.

raised to 180 °C at 2 °C/min; injection temperature, 170 °C. Identification and structural investigation were carried out by GC−MS using a Hewlett-Packard 5972 mass spectrometer combined with a Hewlett-Packard 5890 GC. The GC conditions were the same as those for the corresponding GC analyses, except that He was used as the carrier gas. HR-FABMS data were obtained with a JEOL JMS-AX505WA instrument under the following conditions: acceleration voltage, 3.0 kV; matrix, glycerol; gas, Xe; standard, polyethylene glycol.

NMR Analysis. PMR and CMR spectra were collected with a JEOL-JNM-GX-270 (270 MHz) spectrometer, each sample being dissolved in $CDCl_3$ containing TMS as the internal standard.

TLC Analysis. TLC was performed with precoated plates of silica gel and a solvent system of 12:3:3:2 v/v (EtOAc/AcOH/MeOH/H_2O). To detect the glycosidic fraction, 0.2% (v/v) naphthoresorcinol/EtOH:H_2SO_4 (19:1 v/v) was used before heating to 110 °C.

General Synthetic Method for Primeverosides. All reagents are purchased from Sigma-Aldrich Japan and are used without any purification.

Procedure 1. 1,2,3,4-Tetra-*O*-benzoyl-*β*-D-glucose (**3**, mp 179.5 °C; Peter and Per, 1986) was prepared by following the known method for preparing 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucose (Reynolds and Evans, 1953) using benzoyl chloride.

To a mixture of 8.0 g of **3** (13.4 mmol) and 10 g (19.0 mmol) of freshly prepared 2,3,4-tri-*O*-benzoyl-α-D-xylosyl bromide (**4**, mp 137 °C; Fletcher *et al.*, 1947) dissolved in acetonitrile was added 7.5 g of mercuric cyanide (30 mmol) in one portion while stirring, which was continued for 3 h at room temperature. The reaction mixture was then diluted with dichloromethane, successively washed with a saturated $NaHCO_3$ solution and water, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel and eluted with toluene−ethyl acetate (20:1 v/v) to give 1,2,3,4-tetra-*O*-benzoyl-6-(2,3,4-tri-*O*-benzoyl-D-xylopyranosyl)-*β*-D-glucose (**5**), which was then recrystallized from ether; mp 181 °C.

To 1.5 g of **5** (1.4 mmol) dissolved in dichloromethane−ethylacetate (9:1 v/v, 20 mL), titanium tetrabromide (1.1 g, 3.0 mmol) was added under N_2 gas flow. After stirring for 1 h at room temperature, the reaction mixture was poured onto ice−water and extracted with dichloromethane. After successively washing with a sat. $NaHCO_3$ solution and water, and then drying with $MgSO_4$, the solvent was distilled off under reduced pressure.

The residue was treated with ether to give 2,3,4,2′,3′,4′-hexa-*O*-benzoyl-α-D-primeverosyl bromide (**6**, 480 mg, 33.3% yield) as a solid material (mp 178 °C dec) which was used for the next step without purification.

To a stirred mixture of an aglycon alcohol (0.9 mmol) and **6** (350 mg, 0.3 mmol) dissolved in acetonitrile (3 mL) was added mercuric cyanide (250 mg, 0.9 mmol) at room temperature while stirring, which was continued for 3 h. The reaction mixture was diluted with dichloromethane and filtered. The filtrate was successively washed with a saturated $NaHCO_3$ solution and water, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel and eluted with toluene−ethyl acetate (20:1 v/v) to give **7a**−**c**. The solid material was recrystallized from benzene−cyclohexane (1:1 v/v).

The synthesized hexa-*O*-benzoyl-*β*-D-primeverosides (**7a**−**c**, 0.2 mmol) were dissolved in methanol (10 mL). A 0.1 M sodium methoxide solution (1.2 mmol) was added to methanolyze the ester linkage. After stirring for 15 h at room temperature, the mixture was neutralized with H^+ type Dowex 50W-X8 ion exchange resin (Dow Chemical Co., Midland, MI). The resulting neutral mixture was filtered and the methanol was evaporated to give a syrup (**1a**−**c**), which was purified by HPLC with a solvent system of acetonitrile−water (2:3 v/v).

Procedure 2. (*R*,*S*)-Linalyl-2,3,4-tri-*O*-benzoyl-*β*-D-glucopyranoside (**8d**) was prepared from glucose and (*R*,*S*)-linalool by the same method described in the literature (Sone and Misaki, 1986). The Koenig−Knorr reaction was performed under the same conditions as those used in procedure 1, 0.5 g of **8d** (0.8 mmol) and 1.3 g of **4** (2.5 mmol) being dissolved in 10 mL of acetonitrile, 0.65 g of mercuric cyanide (2.6 mmol) being added, and stirring being continued for 3 h. After being diluted with dichloromethane and after the organic layer was washed and dried, the solvent was distilled off by following procedure 1. The eluate from the silica gel column with toluene−ethyl acetate (20:1 v/v) gave 416 mg of (*R*,*S*)-linalyl-2,3,4-tri-*O*-

Table 1. HRMS Data for the Synthesized Diglycoside

	observed	Δmmu	molecular formula
primeveroside type			
benzyl alcohol (**1a**)	403.1605	+0.1	$C_{18}H_{27}O_{10}$ [M + H]$^+$
2-pehnylethanol (**1b**)	417.1798	+3.7	$C_{19}H_{29}O_{10}$ [M + H]$^+$
geraniol (**1c**)	471.2227	+2.1	$C_{21}H_{36}O_{10}Na$ [M + Na]$^+$
linalool (**1d**)	471.2227	+2.1	$C_{21}H_{36}O_{10}Na$ [M + Na]$^+$
vicianoside type			
benzyl alcohol (**2a**)	425.1463	+4.0	$C_{18}H_{27}O_{10}Na$ [M + Na]$^+$
2-phenylethanol (**2b**)	417.1768	+0.7	$C_{19}H_{29}O_{10}$ [M + H]$^+$
geraniol (**2c**)	471.2227	+0.6	$C_{21}H_{36}O_{10}Na$ [M + Na]$^+$
linalool (**2d**)	471.2227	+0.6	$C_{21}H_{36}O_{10}Na$ [M + Na]$^+$

benzoyl-6-β-(2,3,4-tri-*O*-benzoylxylopyranosyl)glucopyranoside (**7d**). To a methanolic solution (10 mL) of 215 mg of **7d** (0.2 mmol) was added 1.2 mL of 0.1 M sodium methoxide while stirring. After 15 h of stirring at room temperature, the solution was neutralized with Dowex-50W, filtered, and concentrated *in vacuo* to a syrupy material. Under the same HPLC conditions as those already described, 77 mg of (*R*,*S*)-linalyl-1-β-D-primeveroside (**1d**) was obtained.

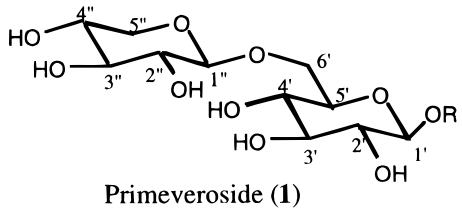
General Synthetic Method for Vicianosides. Benzyl-, phenylethyl-, geranyl-, and (*R*,*S*)-linalyl-2,3,4-tetra-*O*-benzoyl-β-D-glucopyranosides (**8a**−**d**) and 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl bromide (**9**) were prepared under the same conditions as those used for procedure 2.

To a stirred chloroform solution (50 mL) of 1,2,3,4-tetra-*O*-benzoyl-β-L-arabinopyranose (6.23 g, 10 mmol) was added 22.3 mL of a 33% HBr/AcOH solution dropwise during 15 min at 0 °C, stirring and cooling being continued for 1.5 h. The mixture was poured onto 50 mL of ice−water, the organic layer separated was washed and dried, and the solvent was distilled off. The residue crystallized from methanol had mp 146 °C, the yield of **9** being 3.01 g (57.3%). A mixed solution of 2,3,4-tetra-*O*-benzoyl-β-D-glucosides (**8a**−**d**, 1.6 mmol) and **9** (0.3 mmol) in acetonitrile was condensed by the Koenig−Knorr reaction in the presence of 0.35 g of mercuric cyanide (1.3 mmol) as described for procedure 2. Debenzoylation of vicianoside hexabenzoates (**10a**−**d**) was conducted with sodium methoxide/methanol as described for procedure 2 to yield vicianosides (**2a**−**d**) in over 90% yield respectively.

Preparation of the Crude Enzyme Solution. Acetone powder was prepared from fresh tea leaves of *Camellia sinensis* var. *sinensis* Okumusashi, as previously described (Yano *et al.*, 1990). In 60 mL of a 50 mM citrate buffer solution, 3.0 g of the acetone powder, 0.3 g of ascorbic acid, and 0.5 g of Polyclar AT (General Anilin & Film Corp. New York) were homogenized twice for 30 s while cooling at 0 °C. After centrifuging at 10000*g* for 20 min at −5 °C, the resulting supernatant was used as the crude enzyme solution.

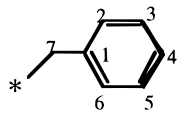
Hydrolysis of Glycosides with the Crude Enzyme Solution. Each synthetic glycoside (12 μM) was dissolved in 4.9 mL of a 50 mM sodium citrate buffer solution (pH = 5.0). After adding 100 μL of an acetone solution of nonanol (7 ppm) as internal standard, the solution was incubated with 100 μL of the crude enzyme solution for 15 min at 37 °C. The freed aglycons were extracted with ether and analyzed by GC and

Table 2. CMR Data for the Synthesized Disglycosides

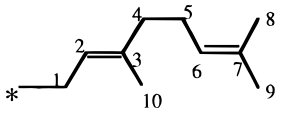


Primeveroside (**1**)

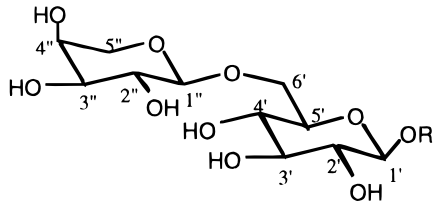
R=



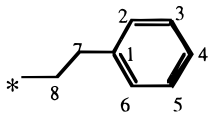
Benzyl (a)



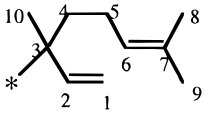
Geranyl (c)



Vicianoside (**2**)



2-Phenylethyl (b)



Linalyl (d)

	1a	1b	1c	1d	2a	2b	2c	2d
aglycon								
1	139.7	139.5	66.5	115.9,115.2	137.5	139.7	66.5	116.0,116.2
2	130.0	129.7	121.6	144.4	132.0	129.9	120.0	144.3
3	130.0	129.4	142.0	81.6,81.5	129.6	129.5	142.0	81.5
4	129.4	129.3	40.7	42.7,42.5	129.0	127.4	40.0	42.7,42.5
5	130.0	129.4	27.4	23.7,23.6	132.0	129.5	26.8	23.6
6	130.0	129.7	125.1	125.8,125.7	132.0	129.9	125.1	125.7,125.6
7	72.8	35.9	132.6	132.1	73.9	36.1	131.6	132.0
8		73.4	25.9	25.9		73.2	26.0	25.6
9			17.8	17.8			18.0	17.7
10			16.6	23.3			16.6	23.2
glucose								
1′	104.0	105.0	102.8	99.6,99.3	102.2	103.1	101.7	99.8,100.0
2′	75.6	75.7	74.9	75.2,75.0	74.0	73.9	73.9	75.1,75.0
3′	78.7	78.4	77.9	78.1	76.6	76.3	76.8	78.2
4′	71.9	72.1	71.4	71.4	71.6	71.5	70.1	72.4
5′	76.5	76.7	76.9	76.5	75.9	75.9	75.8	75.8
6′	70.6	70.8	69.7	69.8,69.6	70.3	70.2	69.2	68.7
xyl or ara								
1″	106.3	106.2	105.5	105.4	104.6	104.6	104.5	104.1
2″	75.8	75.7	74.8	74.9	72.4	71.8	71.6	71.8
3″	77.8	77.3	77.7	77.6	73.1	73.2	73.2	74.2
4″	72.2	71.6	71.1	71.2	69.2	69.2	69.0	69.4
5″	67.7	67.6	66.9	66.8	67.1	67.1	67.1	66.1

GC−MS. The amounts of the aglycons were determined from the peak area ratio to that of nonanol and transformed into a mol ratio. In order to compare the enzyme activity toward each glycoside, the percentage of each mol yield to the highest one was used as the hydrolysis ratio (%).

The aqueous layer after the ether extraction was concentrated *in vacuo*, and the resulting sugars were determined qualitatively by TLC. Solutions without enzyme or without substrate were also incubated at the same time as blanks.

RESULTS AND DISCUSSION

Synthetic Procedures (Figure 1). To prepare primeverosides with various aglycons by a simple synthetic procedure, the Koenig−Knorr reaction between the halogenated primeverose moiety and an aglycon alcohol was thought to be the most suitable for a simple and effective preparation of diglycosides, as well as for the formation of a β-glycoside linkage. The traditional method used acetyl protective groups for the glycoside moiety but orthoester formation occurred during the glycosidation reaction. We found that the benzoate derivatives did not exhibit such a side reaction and, moreover, were more favorable for 1-α-halogenation.

The bromination of heptabenzoylprimeverose (**5**) with the usual brominating reagent of HBr/CH_3COOH failed, because of cleavage of the internal glycosidic linkage, while bromination with titanium tetrabromide gave stable bromide **6**. The most suitable Koenig−Knorr reaction conditions for the synthesis of these diglycosides were provided by using mercuric cyanide as the condensing agent in acetonitrile. The hexabenzoylprimeverosides (**7a**−**c**) were also stable and their stereostructures were confirmed by NMR. In particular, the anomeric protons in the xylose and glucose moieties appeared at 4.94 ppm (J = 6.1 Hz) and 4.83 ppm (J = 7.9 Hz), respectively, showing that both the glycosidic linkages had the β-configuration.

As linalool is a tertiary alcohol, which is less reactive toward glycosylation, others (Konda *et al*., 1996) have recommended primeverosyl fluoride as Koenig−Knorr reagent; however, we prefer linalyl tribenzoylglucoside (**8d**) as the condensing alcohol in the Koenig−Knorr reaction with xylosyl bromide (**4**), because procedure 2 is expected to be applicable for the general synthesis of vicianosides and the starting material **8d** is readily obtainable by a known synthetic method (Sone and Misaki, 1986). With procedure 2, the newly formed glycosidic linkage was proved to have the β-configuration by NMR as described later.

All the hexabenzoylprimeverosides were hydrolyzed with sodium methoxide in methanol with good yields. After the synthetic products were purified by HPLC, their molecular structures were confirmed by FABMS and NMR spectrometry. The FABMS data are summarized in Table 1, showing the observed millimass values to be in excellent agreement with the calculated.

The CMR data, summarized in Table 2, are consistent with those of natural products in the literature (Otsuka *et al*., 1990; Inagaki *et al*., 1995; Guo *et al*., 1993, 1994; Pabst *et al*., 1991; Watanabe *et al*., 1994; Nishikitani *et al*., 1996). Thus the chemical structures of these natural products are confirmed by synthesis.

Enzymatic Hydrolysis. The hydrolysis rates (%) of twelve glycosides by the crude enzyme solution are summarized in Table 3. Geranyl primeveroside has the highest rate (=100), as well as the relatively high rates for the other primeverosides, supports the assertion that primeverosidase is the main glycosidase in tea leaves (Guo *et al*., 1995). The data in Table 3 also show that, among a series of diglycosides with the same aglycon, the hydrolysis rates of primeveroside are high without exception. This specificity of the tea glycosidases does explain our previous experimental results in which the composition of volatiles freed from the glycoside fraction of tea leaves by crude tea glycosidase differed greatly from that obtained with commercial β-glucosidase or a nonspecific glycosidase (Morita *et al*., 1994).

Table 3. Hydrolysis Rates (%) of Twelve Glycosides by the Crude Enzyme Solution

	glycosides		
	glucoside	vicianoside	primeveroside
aglycons			
benzyl-	4.1	1.2	11.9
2-phenylethyl-	5.4	1.2	9.5
geranyl-	44.5	3.0	100.0
linalyl-	22.2	9.3	36.7

By comparing the hydrolysis rate of each glycoside with the same glycoside moiety but different aglycons, those of the geranyl- and linalyl-diglycosides were much higher than those of the benzyl and 2-phenylethyldiglycosides. It may be concluded that the primeverosidase in tea leaves was specific not only to the glycoside but also to the aglycon, and the double specificity of the enzyme may explain the formation of a large amount of terpene alcohols with a low content of aromatic alcohols during black tea fermentation, in spite of the high content of benzyl-β-D-glucoside in fresh tea leaves (Morita *et al*., 1994).

The species of mono- or disaccharoses after the enzymatic hydrolysis were determined by TLC. A main spot (R_f = 0.23) was identical to synthetic primeverose and there were two minor spots of glucose and xylose; therefore, the main glycosidase in tea leaves really is β-primeverosidase, as claimed by Guo *et al*., 1995).

Regarding the stereostructure of the aglycons, racemic linalool yielded a diastereomeric mixture of a glycoside. The NMR spectra of **1d** and **2d** show anomeric protons in the glucose moiety at 4.31 ppm (J = 7.8 Hz) and at 4.33 ppm (J = 7.7 Hz) with the same intensity. The stereospecificity of the primeverosidase will be discussed elsewhere as well as the synthesis of (*R*)- and (*S*)-linalylprimeverosides and the naturally occurring primeverosides with optically active linalool in fresh tea leaves.

LITERATURE CITED

Erman, W. F. *Chemistry of the Monoterpenes*; Gassman, P. G., Ed.; Studies in Organic Chemistry 11; Dekker: New York, 1985; pp 34−126.

Fletcher, H. G.; Hudson, C. S. 1,5-Anhydroxylitol. *J. Am. Chem. Soc.* **1947**, *69*, 921−924.

Guo, W.; Sakata, K.; Watanabe, N.; Nakajima, R.; Yagi, A.; Ina, K.; Luo, S. Geranyl 6-*O*-β-D-Xylopyranosyl-β-D-glucopyranoside Isolated as an Aroma Precursor from Tea Leaves for Oolong Tea.

Guo, W.; Hosoi, R.; Sakata, K.; Watanabe, N.; Yagi, A.; Ina, K.; Luo, S. (*S*)-Linalyl, 2-Phenylethyl and Benzyl Disaccharide, Glycosides Isolated as Aroma Precursors from Oolong Tea Leaves. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1532−1534.

Guo, W.; Yamauchi, K.; Watanabe, N.; Usui, T.; Luo, S.; Sakata, K. A. Primeverosidase as a Main Glycosidase Concerned with the Alcoholic Aroma Formation in Tea Leaves. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 962−964.

Hatanaka, A. The Biogeneration of Green Odour by Green Leaves. *Phytochemistry* **1993**, *34*, 1201−1218.

Inagaki, J.; Watanabe, N.; Moon, J. H.; Yagi, A.; Sakata, K.; Ina, K.; Luo, S. Glycosidic Aroma Precursors of 2-Phenyl-

ethyl and Benzyl Alcohols from *Jasminum sambac* Flowers. *Phytochemistry* **1995**, *59*, 738−739.

Kobayashi, A.; Kubota, K.; Joki, Y.; Wada, E.; Wakabayashi, M. (*Z*)-3-Hexenyl-*β*-D-glucopyranoside in Fresh Tea Leaves as a Precursor of Green Tea. *Biosci., Biotechnol. Biochem.* **1994**, *58*, 592−593.

Konda, Y.; Toida, T.; Kaji, E.; Takeda, K.; Harigaya, Y. First Total Synthesis of New Diglycosides, Neohancosides A and B from *Cynanchum hancokkianum*. *Tetrahedron Lett.* **1996**, *37*, 4015−4018.

Moon, J.; Watanabe, N.; Sakata, K.; Yagi, A.; Ina, K.; Luo, S. *trans*- and *cis*-Linalool 3,6-Oxide 6-*O*-*β*-D-Xyropyranosyl-*β*-D-glucopyranosides Isolated as Aroma Precursors from Leaves for Oolong Tea. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1742−1744.

Morita, K.; Wakabayashi, M.; Kubota, K.; Kobayashi, A.; Herath, N. L. Aglycone Constituents in Flesh Tea Leaves Cultivated for Green and Black Tea. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 687−690.

Nishikitani, M.; Kubota, K.; Kobayashi, A.; Sugawara, F. Geranyl 6-O-α-Arabinopyranosyl-*β*-D-glucopyranoside Isolated as an Aroma Precursor from Leaves of a Green Tea Cultivar. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 929−931.

Otsuka, H.; Takeda, Y.; Yamasaki, K. Xyloglucosides of Benzyl and Phenethyl Alcohols and *Z*-Hexen-1-ol from Leaves of *Alandium platanifolium* Var. Trilobum. *Phytochemistry* **1990**, *29*, 3681−3683.

Pabst, A.; Barron, D.; Semon, E.; Schreier, P. Isolation of a Novel Linalool Disaccharide Glycoside from Raspberry Fruit. *Tetrahedron Lett.* **1991**, *32*, 4885−4888.

Peter, F.; Per, G. A Novel Promoter for the Efficient Construction of 1,2-Trans Linkages in Glycoside Synthesis Using Thioglycosides as Glycosyl Donors. *J. Carbohydr. Res.* **1986**, *149*, C9−C12.

Reynolds, D. D.; Evans, L. W. *β*-D-Glucose-1,2,3,4-tetraacetate. In *Organic Syntheses*; Wiley: New York, 1953; Collect. Vol. 3, pp 432−434.

Schreier, P., Winterhalter, P., Eds. *Progress in Flavour Precursor Studies*; Allured Pub.: Carol Stream, IL, 1993; pp 219−298.

Sone, Y.; Misaki, A. Synthesis of *p*-Nitrophenyl 6-*O*-α- and 6-*O*-*β*-Xylopyranosyl-*β*-D-glucopyranoside. *J. Carbohydr. Chem.* **1986**, *5*, 671−682.

Teranishi, R.; Hornstein, I. Eds. *Food Review Int.* **1995**, *11* (Special Issue on Tea), 480−525.

Vermes, B.; Farkas, L.; Wagner, H. Synthesis and Structural Proof of Moridone 6-*O*-Primeveroside and 6-*O*-Rutinoside. *Phytochemistry* **1980**, *19*, 119−121.

Watanabe, N.; Nakajima, R.; Watanabe, S.; Moon, J. H.; Inagaki, J.; Sakata, K.; Yagi, A.; Ina, K. Linalyl and Bornyl Disaccharides from *Gardenia jasminoides* Flowers. *Phytochemistry* **1994**, *37*, 457−459.

Yano, M.; Joki, Y.; Mutoh, H.; Kubota, K.; Kobayashi, A. Benzyl Glycoside from Tea Leaves. *Agric. Biol. Chem.* **1991**, *55*, 1205−1206.

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