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Glycosylation of capsaicin and 8-nordihydrocapsaicin by cultured cells of *Catharanthus roseus*

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Abstract

The glycosylation of capsaicin and 8-nordihydrocapsaicin was investigated using cultured cells of *Catharanthus roseus*. In addition to capsaicin 4-*O*-β-D-glucopyranoside (170 μg/g fr. wt of cells), the biotransformation products, capsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (116 μg/g fr. wt of cells) and capsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside (83 μg/g fr. wt of cells), were isolated from the cell suspension after three days of incubation with capsaicin. Two other compounds, 8-nordihydrocapsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (171 μg/g fr. wt of cells) and 8-nordihydrocapsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside (122 μg/g fr. wt of cells), together with the known 8-nordihydrocapsaicin 4-*O*-β-D-glucopyranoside (204 μg/g fr. wt of cells) were also isolated from the cell suspension after incubation with 8-nordihydrocapsaicin.

Keywords: *Catharanthus roseus*; Apocynaceae; Cultured plant cells; Glycosylation; Capsaicin; 8-Nordihydrocapsaicin; β-Glucoside; β-Primeveroside; β-Vicianoside

1. Introduction

Plant cell cultures are ideal systems for propagating rare plants and for studying the biosynthesis of secondary metabolites such as flavors, pigments, and agrochemicals, except for a very limited number of compounds (e.g. pyrethrins, bialaphos, and nicotin). Furthermore, the biotransformation of various organic compounds has been investigated as a target in the biotechnological application of plant cell culture systems. Glycosylation is a characteristic biotransformation reaction in cultured plant cells because glycosyltransferases are widespread in plants. There have been many reports on the glycosylation of exogenously supplied phenolic compounds by cultured plant cells (Moyer and Gustine, 1987; Furuya et al., 1988, 1989; Tabata et al., 1988; Upmeier et al., 1988; Ushiyama et al., 1989; Ushiyama and Furuya, 1989; Kamel et al., 1992; Kometani et al., 1993; Lewinson et al., 1996; Hamada et al., 2003).

Plants of the *Capsicum* species have been important sources of food, spices, and medicines for centuries worldwide. The chemical constituents in the fruits of *Capsicum* plants, such as hot chilli pepper fruits, which are responsible for the sensory effects associated with pungency, are a series of branched or straight-chain alkylvanillylamides, capsaicinoids. Capsaicin (**1**), *N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-(*E*)-6-nonenamide, is the most pungent principle among naturally occurring capsaicinoids (Tylor et al., 1981). Capsaicin (**1**) has also been reported to decrease adipose tissue weight and serum triacylglycerol content in rats by enhancing energy metabolism (Kawada

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et al., 1985). It has also shown a wide range of pharmacological properties, such as analgesic, antigenotoxic, antimutagenic, and anticarcinogenic effects, and has been used to treat various peripheral painful conditions, including rheumatoid arthritis and diabetic neuropathy (Surh and Lee, 1995; Park et al., 1998; Surh et al., 1998; Ward and Lopez-Carrillo, 1999). However, capsaicinoids possess extensive neurological toxicity, and direct irritant effects on skin and mucous membrane (Watanabe et al., 1987). Furthermore, capsaicinoids are scarcely soluble in water and poorly absorbed after oral administration. These disadvantages prevent capsaicinoids from being used as food additives and medicines. It has been postulated that the glycosylation of capsaicinoids may reduce their pungency and enhance their water-solubility, to give glycoconjugates as potential prodrugs and weight-loss formulations. Additionally, capsaicinoid glycosides are of physiological interest because glycosides such as saponins appear to be the active principles of widely used folk medicines. Recently, it has been reported that capsaicin (**1**) was glucosylated by cultured plant cells to its mono-glucoside (Kometani et al., 1993; Hamada et al., 2003). To our knowledge, however, there have been no reports on the biotransformation of capsaicinoids to give disaccharides by cultured plant cells. We report here the biotransformations of capsaicin (**1**) and 8-nordihydrocapsaicin, both of which are naturally occurring capsaicinoids, into the corresponding monoglucosides and disaccharides, β-primeverosides and β-vicianosides, which are more soluble in water, by cultured cells of *Catharanthus roseus*.

2. Results and discussion

2.1. Glycosylation of capsaicin (*1*)

Glycoside products **3** (170 μg/g fr. wt of cells), **4** (116 μg/g fr. wt of cells), and **5** (83 μg/g fr. wt of cells) were isolated from cultured cells of *C. roseus* that had been previously treated with capsaicin (**1**) by extraction with MeOH. The substrate, capsaicin (**1**), was detected in both the media and cell extracts. The glycoside products were purified and isolated by preparative HPLC for structure identification by spectroscopic methods. The product **3** was identified as capsaicin 4-*O*-β-D-glucopyranoside by comparison of its ^{1}H and ^{13}C NMR spectroscopic data (Table 1) with previously reported data (Kometani et al., 1993; Hamada et al., 2003; Higashiguchi et al., 2006).

The HRFABMS spectrum of **4** showed a peak at *m*/*z* 622.2850 $[M+Na]^+$ suggesting a molecular formula of $C_{29}H_{45}NO_{12}$ (calcd. 622.2839 for $C_{29}H_{45}NO_{12}Na$). The ^{1}H and ^{13}C NMR spectroscopic data of the sugar moiety of **4** agreed with those of β-primeverose (Li et al., 2006). Each signal in the NMR spectra of **4** was assigned by H–H COSY, C–H COSY, and HMBC analyses. The HMBC spectrum of **4** showed correlations between the proton signal at δ 4.91 (H-1′) and the carbon resonance at δ 147.1 (C-4), and between the proton signal at δ 4.32 (H-1″) and the carbon resonance at δ 69.4 (C-6′), which confirmed that the inner β-D-glucopyranosyl residue was attached to the 4-hydroxyl group of capsaicin (**1**) and that the second β-D-xylopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Therefore, the structure of **4** was determined to be capsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside. Compound **4** has not been identified previously.

Table 1
^{13}C chemical shifts of the biotransformation products **3**–**5** and **6**–**8** in CD_3OD

Product		**3**	**4**	**5**	**6**	**7**	**8**
Aglycone	1	134.9	135.0	134.9	135.1	135.2	135.1
	2	113.0	113.0	113.0	113.2	113.2	113.1
	3	150.7	150.7	150.8	151.0	151.0	150.8
	4	147.0	147.1	147.0	146.8	146.9	146.7
	5	117.9	118.0	117.9	118.4	118.4	118.3
	6	121.2	121.2	121.2	121.4	121.5	121.4
	7	43.7	43.7	43.7	43.8	43.8	43.8
	8	175.9	175.9	175.9	176.1	176.2	176.0
	9	36.9	36.8	36.9	37.2	37.2	37.1
	10	26.5	26.5	26.5	27.1	27.1	27.1
	11	30.3	30.3	30.3	30.3	30.5	30.3
	12	33.2	33.2	33.2	30.3	30.5	30.3
	13	127.8	127.9	127.9	30.3	30.5	30.3
	14	139.0	139.0	139.0	33.0	33.0	32.9
	15	32.2	32.3	32.2	23.7	23.7	23.7
	16	23.1	23.1	23.1	14.4	14.5	14.4
	17	23.1	23.1	23.1			
	OCH_3	56.6	56.6	56.6	56.7	56.7	56.7
Glc	1′	102.7	102.5	102.6	102.8	102.6	102.6
	2′	74.8	75.2	75.2	74.9	75.1	75.3
	3′	78.0	77.9	78.0	78.0	78.0	78.0
	4′	71.2	71.6	71.8	71.4	71.6	71.7
	5′	77.7	77.9	78.0	77.8	77.9	78.0
	6′	62.4	69.4	69.3	62.5	69.7	69.5
	1″		105.0	104.6		105.1	104.6
	2″		74.7	72.2		74.7	72.2
	3″		77.8	74.0		77.9	74.1
	4″		71.4	70.0		71.4	70.0
	5″		67.1	67.0		67.2	67.1

Product **5** was assigned a *Mr* of 622.2841 $[M+Na]^+$ in the HRFABMS spectrum, which suggested a molecular formula of $C_{29}H_{45}NO_{12}$ (calcd. 622.2839 for $C_{29}H_{45}NO_{12}Na$). In the ^{1}H and ^{13}C NMR spectra of **5**, the coupling pattern of the sugar proton signals and the chemical shifts of the sugar carbon signals indicated that the sugar component in **5** was β-vicianose (Kawahara et al., 2006). Correlations were observed in the HMBC spectrum between the proton signal at δ 4.91 (H-1′) and the carbon resonance at δ 147.0 (C-4), and between the proton signal at δ 4.30 (H-1″) and the carbon resonance at δ 69.3 (C-6′). These results confirmed that the inner β-D-glucopyranosyl residue was attached to the phenolic hydroxyl group of capsaicin (**1**) and that the second α-L-arabinopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Thus, compound **5** was identified as capsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside, which is a new compound.

A time-course experiment was carried out to investigate the ability of cultured cells of *C. roseus* to biotransform capsaicin (**1**). The glycoside yields of **3**–**5** at 24 h of incubation were 266, 44, and 27 μg/g fr. wt of cells, respectively, while those of **3**–**5** at 48 h of incubation were 224, 107, and 80 μg/g fr. wt of cells, respectively. β-Glucoside **3** was produced at an early stage of incubation and products **4** and **5** were accumulated after 18 h of incubation (Fig. 1), which indicated that **1** was first converted to **3** and further glycosylation of **3** gave **4** and **5**, as shown in Scheme 1.

2.2. Glycosylation of 8-nordihydrocapsaicin (2)

8-Nordihydrocapsaicin (**2**) was biotransformed by the same transformation system as with capsaicin (**1**) to give glycosides **6** (204 μg/g fr. wt of cells), **7** (171 μg/g fr. wt of cells), and **8** (122 μg/g fr. wt of cells). Substrate **2** was detected in both the media and cell extracts. The structure of product **6** was determined to be 8-nordihydrocapsaicin 4-*O*-β-D-glucopyranoside by NMR spectroscopic methods (Hamada et al., 2003).

The HRFABMS spectrum of product **7** ($[M+Na]^+$ peak at *m/z* 610.2833) suggested a molecular formula of $C_{28}H_{45}NO_{12}$ (calcd. 610.2839 for $C_{28}H_{45}NO_{12}Na$). The coupling pattern of the proton signals and the chemical shifts of the carbon signals due to the sugar moiety indicated that the sugar component in **7** was β-primeverose (Li et al., 2006). HMBC correlations were observed between the proton signal at δ 4.91 (H-1′) and the carbon resonance at δ 146.9 (C-4), and between the proton signal at δ 4.33 (H-1″) and the carbon resonance at δ 69.7 (C-6′). These findings established that the inner β-D-glucopyranosyl residue was attached to the phenolic hydroxyl group of 8-nordihydrocapsaicin (**2**) and that the second β-D-xylopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Based on these spectroscopic data, the structure of **7** was determined to be 8-nordihydrocapsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside, which has not been identified previously.

Product **8** showed a pseudo-molecular ion peak at *m/z* 610.2838 $[M+Na]^+$ (HRFABMS) consistent with a molecular formula of $C_{28}H_{45}NO_{12}$ (calcd. 610.2839 for $C_{28}H_{45}NO_{12}Na$). The sugar component in **8** was shown to be β-vicianose based on the coupling pattern of the sugar proton signals and the chemical shifts of the sugar carbon resonance (Kawahara et al., 2006). HMBC correlations between the proton signal at δ 4.92 (H-1′) and the carbon resonance at δ 146.7 (C-4) and between the proton signal at δ 4.31 (H-1″) and the carbon resonance at δ 69.5 (C-6′)

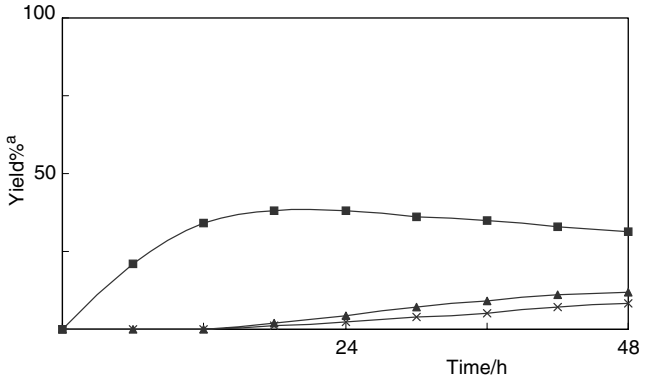


Fig. 1. Time-course of the biotransformation of capsaicin (**1**) by cultured cells of *C. roseus*. [a]Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of **3** (■), **4** (▲), and **5** (×) are plotted.

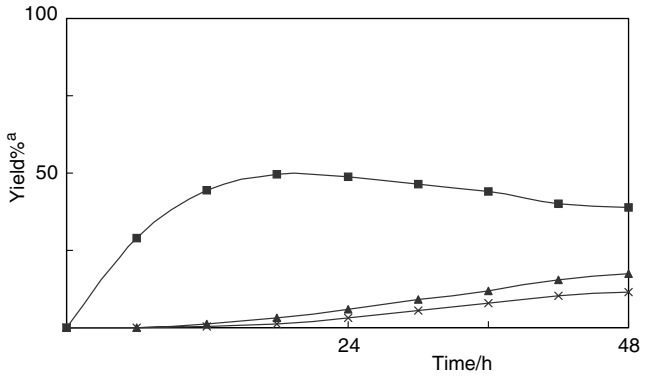
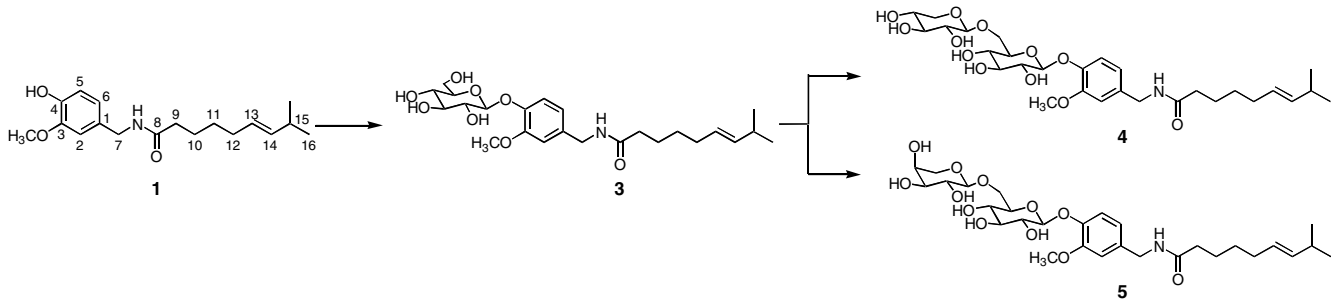
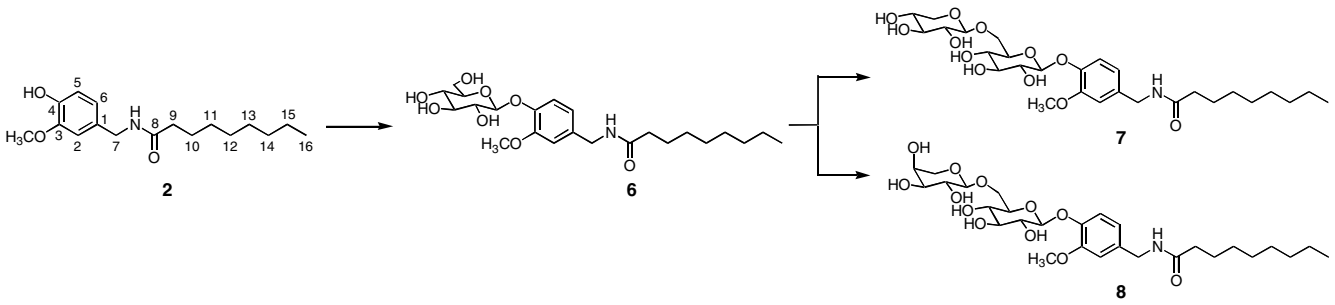


Fig. 2. Time-course of the biotransformation of 8-nordihydrocapsaicin (**2**) by cultured cells of *C. roseus*. [a]Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of **6** (■), **7** (▲), and **8** (×) are plotted.



Scheme 1. Glycosylation of capsaicin (**1**) by cultured cells of *C. roseus*.



Scheme 2. Glycosylation of 8-nordihydrocapsaicin (**2**) by cultured cells of *C. roseus*.

established that the inner β-D-glucopyranosyl residue was attached to the 4-hydroxyl group of 8-nordihydrocapsaicin (**2**) and that the second α-L-arabinopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Consequently, compound **8** was determined to be 8-nordihydrocapsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside, which is a new compound.

Based on the time course in the biotransformation of 8-nordihydrocapsaicin (**2**) (Fig. 2), the pathway shown in Scheme 2 was proposed for the metabolism of **2** by cultured cells of *C. roseus*. The glycoside yields of **6**–**8** at 24 h of incubation were 335, 52, and 26 μg/g fr. wt of cells, respectively, while those at 48 h of incubation were 266, 157, and 105 μg/g fr. wt of cells, respectively. The products were obtained in higher yields than in the biotransformation of capsaicin (**1**).

3. Conclusions

The results of this study established that cultured cells of *C. roseus* can convert capsaicin (**1**) and 8-nordihydrocapsaicin (**2**), which are important ingredients in most spicy foods, into the water-soluble capsaicinoids such as the β-glucosides, β-primeverosides, and β-vicianosides. Cultured *C. roseus* cells preferentially converted glycosated 8-nordihydrocapsaicin (**2**) rather than capsaicin (**1**), probably due to the difference in the structure of the side-chain between these substrates. There have been several reports on the biosynthesis of capsaicin (**1**) in *Capsicum* cultivar (Kopp and Jurenitsch, 1980, 1981; Fujiwake et al., 1982a,b; Rao and Ravishankar, 2000; Kang et al., 2005). A recent metabolism study demonstrated that capsaicin (**1**) was hydroxylated at the 5-position to give ω-hydroxycapsaicin in rats (Surh et al., 1995). However, there have been only a few reports on the metabolism of capsaicinoids by plant cell cultures. Capsaicin (**1**) has been reported to be glucosylated to its mono-glucoside by cultured cells of *Coffea arabica* (Kometani et al., 1993) and *Phytolacca americana* (Hamada et al., 2003). Recently, we also reported the synthesis of capsaicinoid oligosaccharides by a chemoenzymatic method using cyclodextrin glucanotransferase (Hamada et al., 2001). On the other hand, plant cell cultures may be useful for the production of disaccharides such as β-primeverosides, which are difficult to synthesize chemically (Furuya et al., 1989; Ushiyama and Furuya, 1989). This is thus the first description of the formation of capsaicinoid disaccharides, β-primeverosides and β-vicianosides, by cultured plant cells.

Recently, Higashiguchi et al. discovered and isolated the naturally occurring capsaicinoid glucosides capsaicin 4-*O*-β-D-glucopyranoside and dihydrocapsaicin 4-*O*-β-D-glucopyranoside from fruits of *Capsicum* (Higashiguchi et al., 2006). The pungency of the isolated capsaicin 4-*O*-β-D-glucopyranoside was 1/100 that of capsaicin. On the other hand, a chemically synthesized capsaicinoid glucoside, vanillylnonanamide β-D-glucopyranoside (Mihara et al., 1992), has been reported to have a remarkable in vivo effect on lipid metabolism in rats (Tani et al., 2003). The levels of liver lipids and serum lipids decreased when vanillylnonanamide β-D-glucopyranoside was administered orally. Furthermore, the total cholesterol level and the arteriosclerosis index also decreased after supplementation with this glucoside. In this study, the glycosylation of capsaicin (**1**) and 8-nordihydrocapsaicin (**2**) by cultured cells of *C. roseus* was attempted to reduce their pungency and enhance their solubility in water. Since capsaicinoid glycosides are significantly less pungent than capsaicinoids, the capsaicinoid glycosides obtained here may be more suitable for ingestion than previously reported glycosides (Higashiguchi et al., 2006) and may improve lipid metabolism in humans. It is well known that sugar conjugation of drugs reduces their toxicity and enhances their oral absorption. Therefore, water-soluble capsaicinoid glycosides should not only facilitate research on the pharmacological activities of capsaicinoids but may also be useful as potential prodrugs of capsaicinoids. The high capacity of *C. roseus* cells for the glycosylation of capsaicinoids should be useful for the preparation of even more highly water-soluble capsaicinoid glycosides. This procedure overcomes the time-consuming protection and deprotection steps necessary in chemical synthesis. Studies on the physiological activities of glycosides, e.g., how much glycosides are needed to produce the desired positive effects in humans, are now in progress.

4. Experimental

4.1. Substrates

The substrates capsaicin (**1**) and 8-nordihydrocapsaicin (**2**) were purchased from Tokyo Kasei Kogyo Co. Ltd.

and purified by silica gel column chromatography before use.

4.2. Cell suspension culture and culture conditions

Cultured cells of *C. roseus* were prepared as described previously (Hamada et al., 1994). A cell suspension culture was initiated from static cultured callus (fr. wt 40 g (0.40 g fr. wt of cells per ml of medium)) in 300-ml conical flasks, each containing 100 ml of Schenk and Hidebrandt (SH) medium (pH 5.7). The cells were cultivated with continuous shaking for 2 weeks on an illuminated (4000 lx) rotary shaker (120 rpm) at 25 °C.

4.3. Biotransformation conditions

Biotransformation experiments were typically performed by adding 0.08 mmol of substrate to a 300-ml conical flask (100 ml medium) containing a cultured cell suspension of *C. roseus* which was grown for 2 weeks pre-incubation. The cultures were returned to the illuminated (4000 lx) rotary shaker (120 rpm) for an additional three days at 25 °C. After incubation, the products were extracted and purified according to the previously reported method (Shimoda et al., 2006).

Time-course experiments were performed by a procedure similar to normal transformation experiments except that 0.1 mmol of substrate was administered to each of eight flasks containing 50 g of cells. At a regular time interval (6 h), one of the flasks was tested to evaluate the conversion yields of the products by HPLC as described previously (Shimoda et al., 2006).

4.4. Analysis

Chiral-GLC analyses were carried out using a Shimadzu GC-17A (Shimadzu Co.) with FID and a capillary column (0.25 mm × 25 m) coated with 0.25 μm CP cyclodextrin β 236M-19 (Chrompack) (column temp: 100 °C, injector temp: 200 °C, detector temp: 200 °C, split ratio: 50, carrier gas: N_2, 100 kPa). HPLC was carried out with a YMC-Pack R&D ODS column (150 × 30 mm) using MeOH–H_2O (9:11, v/v) as the eluent [detection: UV (280 nm); flow rate: 1.0 ml/min]. ^{1}H and ^{13}C NMR, H–H COSY, C–H COSY, NOE, and HMBC spectra were measured in CD_3OD on a Varian XL-400 spectrometer. HRFABMS spectra were taken on a JEOL MStation JMS-700 spectrometer. The structures of the products were determined by HRFABMS, ^{1}H and ^{13}C NMR, H–H COSY, C–H COSY, NOE, and HMBC spectra.

The absolute configuration of the sugar moieties in disaccharide products was determined as follows. Each of the disaccharides, **4**, **5**, **7**, and **8**, was added to a vial containing 100 μl of 4.0 M HCl, heated to 80 °C for 2 h, and then cooled to room temperature. The solvent was removed in a stream of N_2 and each hydrolysate was converted into the corresponding pentafluoropropionate with pentafluoropropionic anhydride (400 μl) in 400 μl CH_2Cl_2 in a sealed tube at 120 °C for 2 h. Excess reagents were removed under a stream of N_2 and the derivatives were analyzed by chiral GLC on CP cyclodextrin β 236M-19. The peaks of the derivatives from primeverosides **4** and **7** were assigned to those of D-xylose and D-glucose, and the peaks of derivatives from vicianosides **5** and **8** were assigned to those of L-arabinose and D-glucose.

Capsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (**4**): HRFABMS: *m/z* 622.2850 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): δ 0.95 (6H, *d*, $J = 6.8$ Hz, H-16, 17), 1.37 (2H, *q*, $J = 7.6$ Hz, H-11), 1.60 (2H, *q*, $J = 7.6$ Hz, H-10), 1.98 (2H, *m*, H-12), 2.20 (3H, *m*, H-9, 15), 3.15 (1H, *dd*, $J = 11.6$, 9.6 Hz, H-5a″), 3.28-3.60 (7H, *m*, H-2′, 2″, 3′, 3″, 4′, 4″, 5′), 3.75-3.81 (2H, *m*, H-5b″, 6a′), 3.83 (3H, *s*, OCH_3), 4.05 (1H, *dd*, $J = 11.6$, 1.6 Hz, H-6b′), 4.28 (2H, *s*, H-7), 4.32 (1H, *d*, $J = 7.6$ Hz, H-1″), 4.91 (1H, *d*, $J = 7.2$ Hz, H-1′), 5.30–5.40 (2H, *m*, H-13, 14), 6.80 (1H, *dd*, $J = 8.4$, 1.6 Hz, H-6), 6.92 (1H, *d*, $J = 1.6$ Hz, H-2), 7.10 (1H, *d*, $J = 8.4$ Hz, H-5); ^{13}C NMR (100 MHz, CD_3OD), spectra, see Table 1.

Capsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside (**5**): HRFABMS: *m/z* 622.2841 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): δ 0.95 (6H, *d*, $J = 6.8$ Hz, H-16, 17), 1.36 (2H, *q*, $J = 7.6$ Hz, H-11), 1.60 (2H, *q*, $J = 7.6$ Hz, H-10), 1.98 (2H, *m*, H-12), 2.20 (3H, *m*, H-9, 15), 3.28 (1H, *dd*, $J = 12.2$, 1.8 Hz, H-5a″), 3.30–3.60 (7H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′), 3.73–3.81 (2H, *m*, H-5b″, 6a′), 3.83 (3H, *s*, OCH_3), 4.05 (1H, *dd*, $J = 11.6$, 1.6 Hz, H-6b′), 4.28 (2H, *s*, H-7), 4.30 (1H, *d*, $J = 6.4$ Hz, H-1″), 4.91 (1H, *d*, $J = 7.2$ Hz, H-1′), 5.30–5.39 (2H, *m*, H-13, 14), 6.80 (1H, *dd*, $J = 8.4$, 1.6 Hz, H-6), 6.92 (1H, *d*, $J = 1.6$ Hz, H-2), 7.10 (1H, *d*, $J = 8.4$ Hz, H-5); for ^{13}C NMR (100 MHz CD_3OD), spectra, see Table 1.

8-Nordihydrocapsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (**7**): HRFABMS: *m/z* 610.2833 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): δ 0.90 (3H, *t*, $J = 6.8$ Hz, H-16), 1.19–1.26 (10H, *m*, H-11, 12, 13, 14, 15), 1.62 (2H, *q*, $J = 7.6$ Hz, H-10), 2.22 (2H, *t*, $J = 7.6$ Hz, H-9), 3.16 (1H, *dd*, $J = 11.6$, 9.6 Hz, H-5a″), 3.32–3.60 (7H, *m*, H-2′, 2″, 3′, 3″, 4′, 4″, 5′), 3.75–3.81 (2H, *m*, H-5b″, 6a′), 3.85 (3H, *s*, OCH_3), 4.01 (1H, *dd*, $J = 11.6$, 1.6 Hz, H-6b′), 4.30 (2H, *m*, H-7), 4.33 (1H, *d*, $J = 7.6$ Hz, H-1″), 4.91 (1H, *d*, $J = 7.2$ Hz, H-1′), 6.85 (1H, *dd*, $J = 8.4$, 1.6 Hz, H-6), 6.94 (1H, *d*, $J = 1.6$ Hz, H-2), 7.15 (1H, *d*, $J = 8.4$ Hz, H-5); for ^{13}C NMR (100 MHz, CD_3OD) spectra, see Table 1.

8-Nordihydrocapsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside (**8**): HRFABMS: *m/z* 610.2838 $[M+Na]^+$; ^{1}H NMR (CD_3OD): δ 0.89 (3H, *t*, $J = 6.8$ Hz, H-16), 1.18–1.32 (10H, *m*, H-11, 12, 13, 14, 15), 1.63 (2H, *q*, $J = 7.6$ Hz, H-10), 2.24 (2H, t, $J = 7.6$ Hz, H-9), 3.29 (1H, *dd*, $J = 12.0$, 2.0 Hz, H-5a″), 3.32-3.61 (7H, *m*, H-2′, 2″, 3′, 3″, 4′, 4″, 5′), 3.74–3.82 (2H, *m*, H-5b″, 6a′), 3.85 (3H, *s*, OCH_3), 4.05 (1H, *dd*, $J = 11.6$, 1.6 Hz, H-6b′), 4.30 (2H, *m*, H-7), 4.31 (1H, *d*, $J = 6.4$ Hz, H-1″), 4.92 (1H, *d*, $J = 7.6$ Hz, H-1′), 6.85 (1H, *dd*, $J = 8.4$, 1.6 Hz,

H-6), 6.94 (1H, 9id, $J = 1.6$ Hz, H-2), 7.15 (1H, *d*, $J = 8.4$ Hz, H-5); for ^{13}C NMR (100 MHz, CD_3OD) spectra, see Table 1.

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