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Glycosylation of sesamol by cultured plant cells

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ABSTRACT

The glycosylation of sesamol was investigated using cultured cells of *Nicotiana tabacum* and *Eucalyptus perriniana*. The cultured suspension cells of *N. tabacum* converted sesamol into its β-glucoside (7%) as well as the disaccharide, sesamyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (β-gentiobioside, 30%). On the other hand, sesamyl 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (β-rutinoside, 56%), together with the β-glucoside (3%), was produced when sesamol was incubated with suspension cells of *E. perriniana*.

1.Introduction

Sesame (*Sesamum indicum*) seed is one of the most important edible oil seeds. Its oil is said to remove wrinkles and prevent aging, and has been used worldwide in various forms such as traditional health foods and cosmetics (Charak, 1981; Fukuda et al., 1994). In addition, it has been used for centuries in ancient Chinese medicine. The compounds in sesame seed oil, which are responsible for such physiological effects, have attracted considerable attention. *Sesamol* (3,4-methylenedioxyphenol), a principal component of sesame seed oil, has emerged as a strong candidate due to its antioxidant activity (Suja et al., 2004). Furthermore, it has been reported to act as a metabolic regulator and to possess anti-aging, antitumor, antimutagenic, antihepatotoxic, chemopreventive, and anticarcinogenic properties (Ito and Hirose, 1989; Hirose et al., 1990; Naish-Byfield et al., 1991; Hasegawa et al., 1992; Ohta et al., 1994; Kaur and Saini, 2000; Kapadia et al., 2002; Jacklin et al., 2003; Chen et al., 2005; Sharma and Kaur, 2006). However, *sesamol* has shortcomings due to its insolubility in aqueous solution and decomposition under light. These disadvantages limit it further pharmacological exploitation.

In higher plants, many types of useful secondary metabolites such as saponins and anthocyanins are produced in the form of glycosides (Voutquenne et al., 2005; Leo et al., 2006; Magid et al., 2006; Mandal et al., 2006a; Mandal et al., 2006b; Temraz et al., 2006; Zhang et al., 2006; Bjorøy et al., 2007; Fossen et al., 2007; Shao et al., 2007). Glycosylation with plant cells has been considered to be an important method for the conversion of water-insoluble and unstable compounds into water-soluble and stable ones. In addition, it has been reported that the glycosides of phenolic compounds such as flavones were better absorbed than the aglycones themselves (Hollman et al., 1995). Recently, we reported that cultured plant cells showed high potential for glycosylation of phenolic compounds to afford the corresponding mono- and disaccharides (Shimoda et al., 2006; Shimoda et al., 2007a; Shimoda et al., 2007b; Shimoda et al., 2007c).

There have been no reports on glycosylation of *sesamol* by cultured plant cells so far, nor have glycosides of *sesamol* been isolated as natural products from plants, from a biochemical and pharmacological point of view, the ability of cultured plant cells to convert *sesamol*, which has a methylenedioxyphenol structure, is of interest. These encouraged us to investigate its glycosylation with cultured plant cells. We report here its biotransformation into β-glucoside, β-gentiobioside, and β-rutinoside with higher water-solubility using cultured cells of *Nicotiana tabacum* and *E. perriniana*.

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2. Results and discussion

2.1. Glycosylation of sesamol (1) by cultured cells of N. tabacum

Biotransformation of sesamol (1) with cultured cells of *N. tabacum* led to isolation of the two products 2 and 3 from the MeOH-extracts of cells. No additional products were detected by careful HPLC analyses. The products were identified as sesamyl β-D-glucopyranoside (2, 7%) and sesamyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (β-gentiobioside, 3, 30%). The β-gentiobioside 3 has not been identified before. No glycoside products were detected in the medium.

The product 3 had a molecular formula of $C_{19}H_{26}O_{13}$ established from its HRFABMS ($[M+Na]^+$, *m*/*z* 485.1297). Two anomeric proton signals at δ 4.84 (1H, *d*, *J* = 7.6 Hz) and 4.88 (1H, *d*, *J* = 7.6 Hz) were observed in its ^{1}H NMR spectrum of 3, indicating the presence of two β-anomers in 3. The ^{13}C NMR spectrum of 3 showed 19 carbon signals including two anomeric carbon resonances at δ 97.8 and 99.9. The sugar moiety of 3 was determined to be β-gentiobiose from the chemical shifts of the carbon signals due to the sugar moiety of 3 (Shimoda et al., 2006). The resonance corresponding to C-6' in the ^{13}C NMR spectrum was comparatively shifted downfield to δ 67.5. In addition, HMBC correlations were observed between the anomeric proton signal at δ 4.88 (H-1') and at δ 150.6 (C-3), and between the anomeric proton resonance at δ 4.84 (H-1'') and that at δ 67.5 (C-6'). This confirmed that the inner glucopyranosyl residue was attached to the phenolic hydroxyl group of sesamol (1), and that the pair of β-D-glucopyranosyl residues were 1,6-linked to each other. Thus, product 3 was identified as sesamyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside.

Next, the biotransformation of sesamol (1) in cultured *N. tabacum* cells was investigated (Fig. 1). Sesamol (1) was converted into its β-glucoside 2 as the major product and β-gentiobioside 3 as the minor product at an early stage of incubation (10 h). However, β-gentiobioside 3 was predominantly produced after 20 h and the amount of β-glucoside 2 then decreased. These indicated that the β-glucoside 2 was first produced and that the β-gentiobioside 3 was produced from 2 as shown in Scheme 1.

2.2. Glycosylation of sesamol (1) by cultured cells of E. perriniana

Sesamol (1) was subjected to the biotransformation system with cultured cells of *E. perriniana*. After a five-day incubation, products 2 and 4 were isolated from the MeOH-extracts of the cells. Their structures were determined to be sesamyl β-D-glucoside (2, 3%) and sesamyl 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (β-rutinoside, 4, 56%). The biotransformation product 4 had not been identified before.

The HRFABMS spectrum of the product 4, which included $[M+Na]^+$ peak at *m*/*z* 469.1333), suggested the molecular formula of $C_{19}H_{26}O_{12}$. The chemical shifts of the carbon signals of the sugar moiety were similar to those of β-rutinose (Ishimaru et al., 2003). The ^{1}H NMR spectrum of 4 showed two anomeric proton resonances at δ 4.67 (1H, *d*, *J* = 8.0 Hz) and 4.71 (1H, *d*, *J* = 1.6 Hz), indicating the presence of each of the α- and β-anomers in the sugar moiety. Correlations were observed in the HMBC spectrum between the anomeric proton signal at δ 4.67 (H-1') and the carbon resonance at δ 154.1 (C-3), and between the anomeric proton signal at δ 4.71 (H-1'') and the carbon resonance at δ 67.8 (C-6'), indicating that the inner β-D-glucopyranosyl residue was attached to the phenolic hydroxyl group of sesamol (1), and that the second α-L-rhamnopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Thus, compound 4 was identified as sesamyl 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside.

A time-course experiment for the biotransformation of sesamol (1) (Fig. 2) with cultured cells of *E. perriniana* established that sequential glycosylation of 1 gave β-glucoside 2 and β-rutinoside 4 as shown in Scheme 2. The disaccharide was formed more effectively in comparison with the case of biotransformation with *N. tabacum*.

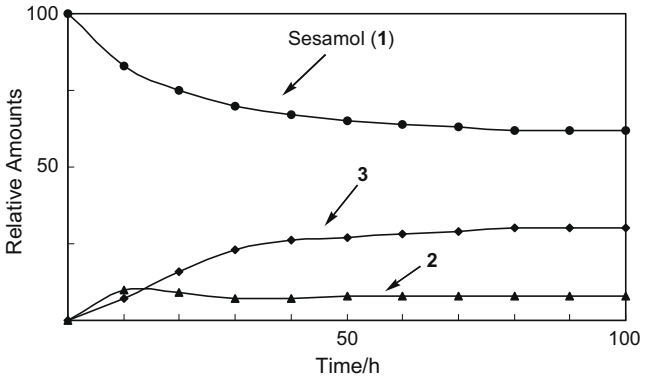


Fig. 1. Time-course of the biotransformation of sesamol (1) by cultured cells of *N. tabacum*. [a]Amounts are expressed as relative percentage.

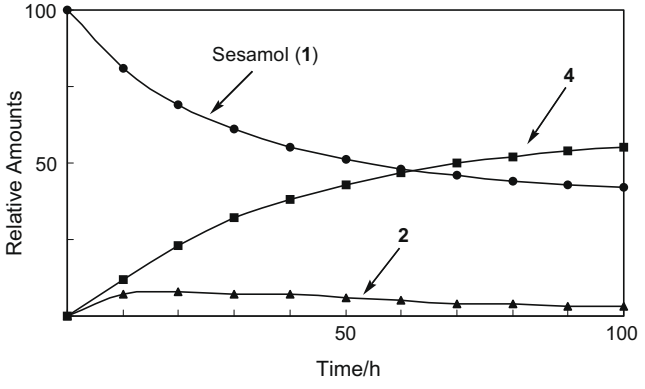
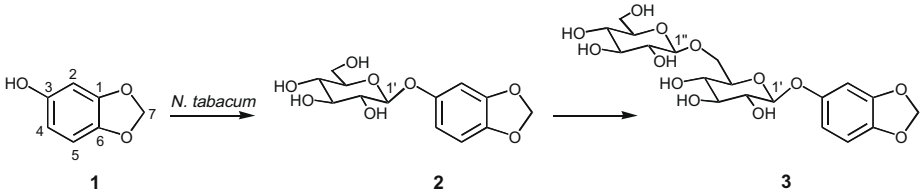
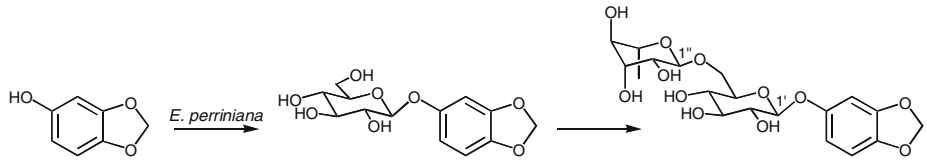


Fig. 2. Time-course of the biotransformation of sesamol (1) by cultured cells of *E. perriniana*. [a]Amounts are expressed as relative percentage.



Scheme 1. Glycosylation of sesamol (1) by cultured cells of *N. tabacum*.



Scheme 2. Glycosylation of sesamol (1) by cultured cells of *E. perriniana*.

3. Conclusion

It was demonstrated that cultured cells of *N. tabacum* are able to glycosylate sesamol to the corresponding β-glucoside and β-gentiobioside, and that *E. perriniana* can convert sesamol into its β-glucoside and β-rutinoside. It was found that the rate of formation of sesamyl disaccharide in *E. perriniana* cells was faster than that in *N. tabacum* cells as judged by results of the time course experiments. The total yield of glycosides produced by *E. perriniana* was 1.5 fold higher than that by *N. tabacum*, indicating that the glycosylation activity of *E. perriniana* was higher than that of *N. tabacum*. Recently, we reported that cultured *E. perriniana* cells glycosylated thymol and carvacrol to their disaccharides, i.e., β-gentiobiosides (Shimoda et al., 2006). On the other hand, cultured *E. perriniana* cells converted tocopherols into two disaccharides, that is, β-gentiobiosides and β-rutinosides (Shimoda et al., 2007c). In this work, sesamyl β-gentiobioside was not produced from *sesamol* by *E. perriniana* cells. It should be emphasized that cultured cells of *E. perriniana* glycosylate these phenolic compounds to the corresponding disaccharides with high substrate specificity. This is probably due to the substrate specificity of each glycosyltransferases responsible for β-gentiobioside and β-rutinoside formation in *E. perriniana* cells.

There have been no reports on biocatalytic glycosylation of *sesamol* so far. This is the first description of production of sesamyl glycosides, such as the β-glucoside, β-gentiobioside, and β-rutinoside, by cultured plant cells. This study demonstrated that each sesamyl disaccharides, i.e., β-gentiobioside and β-rutinoside, can be individually prepared by selective use of two cell cultures of *N. tabacum* and *E. perriniana*. This method would be useful for practical preparation of sesamyl glycosides as drugs and food additives. Further studies on the physiological activities of the glycosides are now in progress.

4. Experimental

4.1. General

The substrate sesamol (1) was purchased from Aldrich Chemical Co. The ^{1}H and ^{13}C NMR, H–H COSY, C–H COSY, NOE, and HMBC spectra were recorded using a Varian XL-400 spectrometer in CD_3OD solution. The HRFABMS spectra were measured using a JEOL MStation JMS-700 spectrometer.

4.2. Cell line and culture conditions

Each cell suspension culture of *N. tabacum* and *E. perriniana* was initiated from static cultured cells in 300 ml conical flasks containing Murashige and Skoog's medium (100 ml, pH 5.7). Prior to use for this work, part of the callus tissues (fr. wt 40 g) was individually transplanted to freshly prepared Murashige and Skoog's medium (100 ml in a 300 ml conical flask) containing 3% sucrose and grown with continuous shaking for 2 weeks on a rotary shaker (120 rpm) at 25 °C in the dark.

4.3. Biotransformation and purification of products

0.15 mmol of each substrate was individually administered to each of 10 flasks (1.5 mmol/l) containing cultured suspension cells, and the cultures were incubated at 25 °C for five days on a rotary shaker (120 rpm) in the dark. After the incubation period, the cells and medium were separated by filtration with suction. The extraction and purification of products and unreacted substrate were performed according to previously reported procedures (Shimoda et al., 2006).

4.4. Product identification

The structures of the products were determined on the basis of analysis, their HRFABMS, ^{1}H and ^{13}C NMR, H–H COSY, C–H COSY, NOE, and HMBC spectra.

Spectral data for selected products are as follows.

Sesamyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (3): HRFABMS: *m/z* 485.1297 $[M+Na]^+$ calcd. 485.1271 for $C_{19}H_{26}O_{13}Na$; ^{1}H NMR (400 MHz, CD_3OD): δ3.30-3.95 (12H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5', 5'', 6', 6''), 4.84 (1H, *d*, *J* = 7.6 Hz, H-1''), 4.88 (1H, *d*, *J* = 7.6 Hz, H-1'), 5.89 (2H, *m*, H-7), 6.65 (1H, *d*, *J* = 8.0 Hz, H-5), 6.72 (1H, *dd*, *J* = 8.0, 2.4 Hz, H-4), 7.02 (1H, *d*, *J* = 2.0 Hz, H-2); for ^{13}C NMR (100 MHz, CD_3OD) spectroscopic assignment, see Table 1.

Sesamyl 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (4): HRFABMS: *m/z* 469.1333 $[M+Na]^+$; calcd. 469.1322 for $C_{19}H_{26}O_{12}Na$ ^{1}H NMR (400 MHz, CD_3OD): δ1.19 (3H, *d*, *J* = 6.0 Hz, H-6''), 3.32-3.90 (10H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5', 5'', 6'), 4.67 (1H, *d*, *J* = 8.0 Hz, H-1'), 4.71 (1H, *d*, *J* = 1.6 Hz, H-1''), 5.89 (2H, *m*, H-7), 6.61 (1H, *d*, *J* = 8.0 Hz, H-5), 6.70 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-

Table 1
Spectroscopic assignments of biotransformation products 2–4 in CD_3OD.

Product		2	3	4
Aglycone	1	149.3	148.7	149.3
	2	101.1	105.9	101.1
	3	154.3	150.6	154.1
	4	110.3	116.2	110.1
	5	108.7	108.2	108.7
	6	144.3	144.9	144.3
	7	102.5	102.4	102.5
Glc	1'	103.6	97.8	103.5
	2'	74.9	73.4	74.8
	3'	78.1	76.1	77.9
	4'	71.4	71.6	71.5
	5'	77.9	75.2	76.8
	6'	62.5	67.5	67.8
	1''		99.9	102.1
	2''		73.7	72.1
	3''		78.4	72.3
	4''		71.4	73.9
	5''		75.2	69.7
	6''		62.4	18.0

4), 7.00 (1H, *d*, *J* = 1.8 Hz, H-2); for ^{13}C NMR (100 MHz, CD_3OD) spectroscopic assignment ,see Table 1.

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