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Enzyme-catalyzed Acylation of Plant Polyphenols for Interpretation of Their Functions

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Abstract

The one-step acylation of plant polyphenols, naringin (naringenin 7-neohesperioside) and rutin (quercetin 3-rutinoside), to the corresponding cinnamate esters was achieved by a lipase-catalyzed regioselective transesterification in a dry organic solvent. For acylation to the narigin molecule, the lipase preferentially catalyzed the acylation to the primary alcohol (C6" position of glucose) with less steric hindrance, while, in rutin, the secondary alcohol (C3" position of glucose) was acylated to the cinnamate ester by the lipase.

Keywords: acylation, lipase, plant polyphenol.

Plant polyphenols are very important compounds among natural products. For example, natural plant pigments have been used as food ingredients and cosmetic additives. Furthermore, recently, the immunomodulatory (Liang *et al.*, 1997), antioxidant (Grinberg *et al.*, 1994), and antivirus activities (Formica *et al.*, 1995) of the flavonoids have been reported. Flower colors, a type of naturally occurring pigments such as flavonoid glucosides are often present acylated with a few aromatic acids at specific hydroxy groups in their sugar moieties (Harborne and Williams, 1988). These flavonoid glucosides are reported to be stabilized in plant tissues because of their intra- and intermolecular hydrophobic interactions caused by the acylation with some aromatic acids (Kondo *et al.*, 1992; Yoshida *et al.*, 1992, 1995). As a result, it has been recognized that the physiological functions such as UV-resistibility and radical scavenging ability of higher plants are due to the existence of these acylated flavonoids in the plant tissues (Bros and Saran, 1987; Robak and Gryglewski, 1988; Balakumar *et al.*, 1997). However, commercially available plant polyphenols (i.e., isoquercitrin, rutin, naringin, hesperigin, callistephin, and so on) are present as the non-acylated forms or as mixtures.

To date, the preparations of some acylated polyphenols using subtilisin via the malonate ester have been studied (Danieli *et al.*, 1990, 1993; Riva *et al.*, 1996), however, there is no report with respect to the direct or one-step acylation to polyphenols. Recently, we investigated the lipase-catalyzed regioselective and direct acylation to plant polyphenols for synthesis of stable plant polyphenols having new bioactivities (Nakajima *et al.*, 1997, 1999). Herein, we describe an enzymatic acylation for functional modification of the plant polyphenols, naringin (naringenin 7-neohesperidoside) and rutin (quercetin 3-rutinoside), by a lipase-catalyzed regioselective and one-step transesterification.

A reaction mixture (closed system) containing naringin (2 mmol), vinyl cinnamate (10 mmol), Chirazyme L-2, c.-f., lyo (Roshe Diagnostics, lipase B from *Candida antarctica*) (1.0 g), molecular sieves 4A (500 mg) and dry acetone (40 ml) was incubated with shaking at 37 ℃ for 14 days, and then the reaction was stopped by filtration. The synthesis of rutin cinnamate was carried out under the same conditions except for the addition of DMF (2.0 ml) to the mixture. The resulting crude product

in the reaction mixture was washed with *n*-hexane to remove the excess vinyl cinnamate. Evaporation of the solvent and purification of the product on a column of silica-gel (Silica-gel BW-127ZH, Fuji-sylisia, $CHCl_3$: 2-propanol = 30 : 1 - 3 : 1) gave the corresponding cinnamate ester as a white (naringin cinnamate) or yellow (rutin cinnamate) powder. The reaction scheme of the formation of the naringin cinnamate ester is shown in **Fig. 1**.

The formation of the cinnamate esters of flavonoid glucosides was confirmed by ^1H- and ^{13}C-NMR and TLC analyses. The FAB-MS analyses of

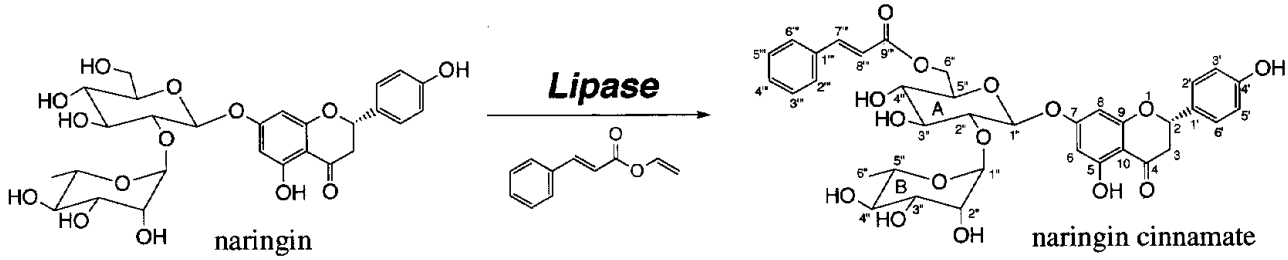

Fig. 1 Reaction scheme for lipase-catalyzed regioselective acylation for synthesis of naringin cinnamate.

Table 1 ^{13}C-NMR data (DMSO-d_6) of naringin and naringin cinnamate

Position[1)]	Naringin	Cinnamic acid	Naringin cinnamate
B-C6"	18.0	-	18.1
C3	42.0	-	42.1
A-C6"	60.2	-	63.4
B-C5"	68.2	-	68.4
A-C4"	69.5	-	69.8
B-C2"	70.3	-	70.4
B-C3"	71.6	-	71.5
B-C4"	71.7	-	71.8
A-C5"	75.9	-	73.6
A-C3"	76.1	-	76.1
A-C2"	76.8	-	76.8
C2	78.6	-	78.5
C8	95.0	-	95.2
C6	96.2	-	96.1
A-C1"	97.1	-	97.1
B-C1"	100.3	-	100.5
C10	103.2	-	103.3
C3',C5'	115.1	-	115.2
C8"'	-	119.4	117.8
C3"',C5"'	-	128.4	128.3
C2',C6'	128.4	-	128.4
C1'	128.6	-	128.6
C2"',C6"'	-	129.1	128.9
C4"'	-	130.4	130.6
C1"'	-	134.4	134.0
C7"'	-	144.1	144.7
C4'	157.8	-	157.8
C9	162.6	-	162.6
C5	162.8	-	162.8
C7	164.6	-	164.5
C9"'	-	167.8	165.5
C4	197.1	-	197.2

[1)]The numbers in the position column corresponded to those shown in **Fig. 1**.

the products also indicated the formation of the naringin cinnamate and the rutin cinnamate esters (naringin cinnamate: *m/z* 711 $[M+H]^+$; rutin cinnamate: *m/z* 741 $[M+H]^+$). The isolated yields of naringin cinnamate and rutin cinnamate were 36% (conversion ratio was 64%) and 8% (conversion ratio was 28%), respectively. The results of the ^{13}C-NMR analysis of naringin cinnamate are summarized in **Table 1**. In the spectra, the carbonyl carbon signals of the ester moiety (C9''' position) shifted by -2.3 ppm due to the production of the ester from 167.8 ppm for cinnamic acid to 165.5 ppm for naringin cinnamate. Furthermore, the signals of the A-C6" position for the D-glucose moiety shifted by 3.2 ppm (from 60.2 to 63.4 ppm). However, the shifts of C5, C4' and C3" due to the product formation were not observed. These results show the formation of an ester linkage between the primary alcohol moiety (A-C6") of naringin and the carboxyl residue (C9''') of cinnamic acid, and deny the esterification of the phenolic and secondary alcohols of naringin.

Similarly, in the ^{13}C-NMR analysis of rutin cinnamate, the signal of the carbonyl carbon in the cinnamic acid moiety shifted by the formation of the ester. Furthermore, the signals of the C3" position (Glu, C3") for the D-glucose moiety shifted by 1.5 ppm (from 76.1 to 77.6 ppm) as shown in **Table 2**. These results suggest the formation of an ester linkage between the secondary alcohol (C3" position) of the D-glucose moiety in rutin and the carboxyl residue of cinnamic acid (**Fig. 2**). In both cases (naringin cinnamate and rutin cinnamate), the acylation toward phenolic hydroxyl groups in the flavonoid skeleton was not observed at all.

The regioselectivity of the lipase-catalyzed acylation to naringin was specific at the C6" position (primary alcohol of glucose) in the sugar moiety, however, the selectivity toward rutin, which has no free primary alcohol, was specific at the C3" position of the sugar moiety. According to these results, the lipase preferentially acylates the primary alcohol (C6" position) with less steric hindrance, and the secondary alcohol (C3" position) is next acylated when there is no primary alcohol available in the sugar moiety.

The application of this enzymatic method to stabilize a wide variety of plant polyphenols, such as naturally occurring pigments, is currently under investigation for a large scale production. The contribution of the acyl moiety of the acylated flavonoid glucosides against thermostability and light-resistibility is also now in progress and will be reported in our forthcoming paper.

Table 2 ^{13}C-NMR data (DMSO-d_6) of the disaccharide moiety of rutin and rutin cinnamate

Position[1)]	Rutin	Rutin cinnnamate
Glu,C2"	73.6	72.6
Glu,C3"	76.1	77.6
Glu,C4"	70.3	69.2
Rha,C2'''	70.1	70.3
Rha,C3'''	69.8	70.1
Rha,C4'''	71.7	71.5

[1)]The numbers in position column corresponded to those shown in **Fig. 2.**

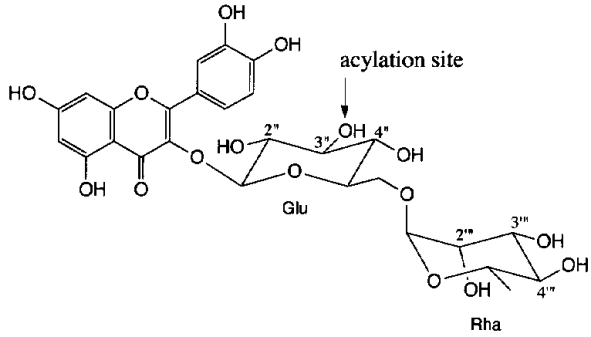

Fig. 2 The regioselectivity of the enzymatic acylation to rutin.

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