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Structural aspects of acylated plant pigments: stabilization of flavonoid glucosides and interpretation of their functions

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Dedicated to Professor Dr. Kenji Soda in honor of his 70th birthday

Abstract

The enzymatic synthesis of acylated isoquercitrins was accomplished by the lipase-catalyzed transesterification with carboxylic acid vinyl esters as acyl donors in an organic solvent. The introduction of an acyl group into isoquercitrin improved its thermostability and light-resistivity. In particular, isoquercitrin *p*-coumarate was the most stable of all the acylated isoquercitrins tested.

Keywords: Naturally plant pigment; Acylation; Flavonoid glucoside; Thermostability; Light-resistivity

1. Introduction

Plant polyphenols are very important compounds among natural products. For example, natural plant pigments have been used as food ingredients and cosmetic additives. Furthermore, the immunomodulatory [1], antioxidant [2], and antivirus activities [3] of plant flavonoids have been recently reported. Flower colors, a type of naturally occurring pigment such as flavonoid glucosides are often present acylated with a few aromatic carboxylic acids at specific hydroxy groups in their sugar moieties [4]. 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 carboxylic acids [5–7]. As a result, it has been recognized that the physiological functions such as UV-resistivity and the radical scavenging ability of higher plants are due to the existence of these acylated flavonoids in the plant tissues [8–10]. However, little information is known about the contribution of the acyl moiety in the acylated flavonoid glucosides on their physiological functions. To investigate the structure-stability relationship between the flavonoid aglycon and aromatic carboxylic acid moiety, we previously investigated and reported the synthesis of arbutin cinnamate [11] and isoquercitrin cinnamate [12] via the direct acylation by the lipase-catalyzed transesterification with vinyl cinnamate as an acyl donor. Herein, we describe the enzymatic synthesis of some acylated isoquercitrins and also discuss the contribution of the acyl moiety on the basis of the thermostability and light-resistivity among the acylated isoquercitrins.

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2. Materials and methods

2.1. Instruments

The ^{1}H and ^{13}C NMR spectra were recorded using a JEOL 270EX FT-NMR spectrometer in $CDCl_3$ or DMSO-d_6 solution and the chemical shifts were expressed in δ (ppm) referring to TMS. The FAB-MS spectrum was measured using a JEOL The MStation JMS-700 spectrometer. The molecular weight was estimated from the *m*/*z* value of the quasimolecular ion $[M + H]^+$ peak. The visible absorption spectra of the isoquercitrin solutions were recorded with a Beckman DU-640 spectrophotometer fitted with a thermostated 1 cm path length quartz cuvette.

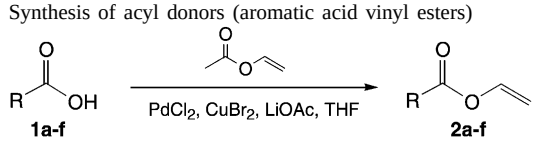
2.2. Materials

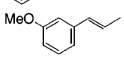
Dihydrocinnamic acid (3-phenylpropionic acid) (**1a**), 3-methoxycinnamic acid (**1b**), 2-methylcinnamic acid (**1c**), 4-phenyl-*n*-butyric acid (**1e**), 2-naphthylacetic acid (**1f**), vinyl cinnamate (**1g**) and vinyl *n*-hexanoate (**2i**) were purchased from Tokyo Kasei Kogyo, Japan. Benzoic acid (**1d**) was obtained from Wako Pure Chemical Industries Ltd., Japan. Vinyl *p*-coumarate (vinyl 4-hydroxycinnamic acid) (**2h**) was prepared according to the literature procedure [13]. Amano-PS (30 kU/g of the lyophilized powder, in protocol) were obtained from Amano Pharmaceutical Co., Japan. Chirazyme L-2 (lipase B from *Candida antarctica*, 5 kU/g of the lyophilized powder, in protocol) was purchased from Roche Diagnostics, Switzerland. Lipoprotein lipase (LPL-311, 20 U/mg of the lyophilized powder, in protocol) was purchased from Toyobo Co. Ltd., Japan. All other chemicals used in this study were of analytical grade and commercially available.

2.3. Synthesis of acyl donors (vinyl esters)

The aromatic carboxylic acids, **1a**–**1f**, were readily converted to the corresponding vinyl esters (**2a**–**2f**) by $PdCl_2$-catalyzed transesterification with vinyl acetate, lithium acetate, and copper dibromide [14,15]. As a typical procedure, $PdCl_2$ (0.2 mmol), LiOAc (19.5 mmol), and $CuBr_2$ (0.5 mmol) were added to a solution of vinyl acetate (0.4 mol) and THF (20 ml) at room temperature. The reaction mixture was heated to 70 °C and then the aromatic carboxylic acid (**1a**–**1f**) (40 mmol, dissolved in 20 ml of THF) was slowly added. After stirring for 24 h at 70 °C, the mixture was filtered and washed with ethyl acetate, and then concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (*n*-hexane:EtOAc = 10:1). These results are summarized in Table 1. The formation of the ester linkage was confirmed by ^{1}H and ^{13}C NMR analyses.

Table 1
Synthesis of acyl donors (aromatic acid vinyl esters)



Products	R	Yield (%)[a]
2a		89
2b		64
2c		34
2d		64
2e		45
2f		47

[a] Isolate yield.

Vinyl dihydrocinnamate (vinyl 3-phenylpropionate) (**2a**): ^{1}H NMR ($CDCl_3$) δ = 2.69 (t, 2H, J = 15.9 Hz), 2.96 (t, 2H, J = 15.4 Hz), 4.55 (1H, dd, J = 14.0, 1.6 Hz), 4.86 (1H, dd, J = 6.3, 1.6 Hz), 7.19–7.29 (5H, m); ^{13}C NMR ($CDCl_3$) δ = 30.5, 35.4, 97.6, 126.3, 128.2, 128.5, 140.0, 141.0, 169.8.

Vinyl 2-methoxycinnamate (**2b**): ^{1}H NMR ($CDCl_3$) δ = 3.80 (3H, s), 4.62 (1H, dd, J = 6.2, 1.6 Hz), 4.97 (1H, dd, J = 14.0, 1.6 Hz), 6.42 (1H, d, J = 15.9 Hz), 6.93 (1H, dd, J = 8.1, 1.6 Hz), 7.03 (1H, t, J = 1.6 Hz), 7.10 (1H, d, J = 7.8 Hz), 7.28 (1H, t, J = 7.8 Hz), 7.42 (1H, dd, J = 14.3, 6.5 Hz), 7.73 (1H, d, J = 15.7 Hz); ^{13}C NMR ($CDCl_3$) δ = 54.8, 97.3, 112.9, 116.2, 116.6, 120.6, 129.6, 135.1, 141.0, 146.2, 159.6, 163.4.

Vinyl 2-methylcinnamate (**2c**): ^{1}H NMR ($CDCl_3$) δ = 3.80 (3H, s), 4.64 (1H, dd, J = 6.2, 1.6 Hz), 4.99 (1H, dd, J = 14.0, 1.6 Hz), 6.39 (1H, d, J = 16.2 Hz), 7.22 (1H, d, J = 10.8 Hz), 7.23 (1H, d, J = 5.4 Hz), 7.30 (1H, d, J = 16.2 Hz), 7.45 (1H, dd J = 13.5, 6.2 Hz), 7.58 (1H, d, J = 8.1 Hz), 8.08

(1H, d, $J = 16.2$ Hz); ^{13}C NMR ($CDCl_3$) $\delta = 19.5$, 97.5, 117.3, 126.2, 126.2, 130.2, 130.8, 132.9, 137.9, 141.3, 144.1, 163.9.

Vinyl benzoate (**2d**): ^{1}H NMR ($CDCl_3$) $\delta = 4.67$ (1H, dd, $J = 6.2$, 1.6 Hz), 5.05 (1H, dd, $J = 13.8$, 1.6 Hz), 7.39–7.55 (4H, m), 8.08 (2H, d, $J = 5.4$ Hz); ^{13}C NMR ($CDCl_3$) $\delta = 98.5$, 128.8, 129.2, 130.3, 133.9, 141.7, 163.9.

Vinyl 4-phenyl-*n*-butyrate (**2e**): ^{1}H NMR ($CDCl_3$) $\delta = 1.87$ (2H, m), 2.28 (2H, t, $J = 7.3$ Hz), 2.55 (2H, t, $J = 7.3$ Hz), 4.44 (1H, dd, $J = 6.2$, 1.4 Hz), 4.76 (1H, dd, $J = 14.0$, 1.4 Hz), 7.05–7.23 (6H, m); ^{13}C NMR ($CDCl_3$) $\delta = 26.0$, 33.0, 34.9, 97.4, 126.0, 128.3, 128.4, 141.0, 141.1, 170.3.

Vinyl 2-naphthylacetate (**2f**): ^{1}H NMR ($CDCl_3$) $\delta = 3.85$ (2H, s), 4.59 (1H, dd, $J = 6.2$, 1.6 Hz), 4.91 (1H, dd, $J = 13.8$, 1.6 Hz), 7.23–7.48 (4H, m), 7.73–7.83 (3H, m); ^{13}C NMR ($CDCl_3$) $\delta = 40.9$, 98.0, 125.9, 126.1, 127.6, 128.1, 128.3, 130.5, 132.5, 133.3, 141.2, 168.5.

2.4. Enzymatic synthesis of acylated isoquercitrins

A reaction mixture (closed system) containing isoquercitrin (**3**) (2 mmol), a prepared or commercially available acyl donor (carboxylic acid vinyl ester, **2a**–**2i**) (10 mmol), lipase (1.0 g), molecular sieves 4A (500 mg) and dry acetone or acetonitrile (40 ml) was incubated with gentle shaking at 37 °C for 10 days, and then the reaction was stopped by filtration. The resulting crude product in the reaction mixture was washed with *n*-hexane to remove the excess acyl donor. 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 acylated isoqercitrin (**4a**–**4i**). These results were summarized in Table 2. The formation of the acylated isoquercitrins was confirmed by ^{13}C NMR and FAB-MS analyses.

Isoquercitrin 6″-*O*-3‴-phenylpropionate (**4a**): ^{13}C NMR (DMSO-d_6) $\delta = 30.3$ (C3‴), 35.1 (C2‴), 63.2 (C6″), 70.1 (C4″), 74.1 (C2″), 74.3 (C3″), 76.4 (C5″), 93.7 (C8), 98.9 (C6), 100.9 (C1″), 104.0 (C4a), 115.3 (C2′), 116.3 (C5′), 121.2 (C6′), 121.7 (C1′), 126.1 (C4‴), 128.2 (C3‴′, C5‴′), 128.4 (C2‴′, C6‴′), 133.2 (C3), 140.4 (C1‴′), 145.0 (C3′), 148.7 (C4′), 156.4 (C8a), 156.5 (C2), 161.4 (C5), 164.3 (C7), 171.8 (C1‴), 177.6 (C4); FAB-MS *m*/*z* 597 $[M + H]^+$.

Isoquercitrin 6″-*O*-3‴′-methoxycinnamate (**4b**): ^{13}C NMR (DMSO-d_6) $\delta = 54.0$ (C7‴′), 63.4 (C6″), 69.9 (C4″), 74.1 (C2″), 74.4 (C3″), 76.3 (C5″), 93.5 (C8), 98.8 (C6), 100.8 (C1″), 103.8 (C4a), 112.6 (C4‴′), 115.2 (C2′), 116.1 (C5′), 116.8 (C2‴′), 117.8 (C2‴), 120.9 (C6‴), 121.1 (C6′), 121.5 (C1′), 129.9 (C5‴′), 133.1 (C1‴′), 135.3 (C3), 144.4 (C3‴′), 144.8 (C3′), 148.6 (C4′), 156.3 (C2), 156.4 (C8a), 159.6 (C3‴′), 161.1 (C5), 164.1 (C7), 165.9 (C1‴), 177.5 (C4); FAB-MS *m*/*z* 625 $[M + H]^+$.

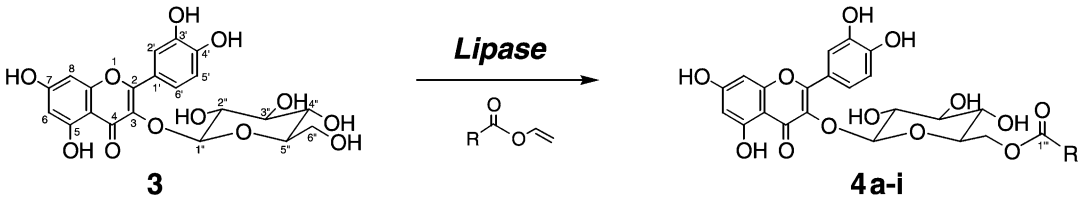
Isoquercitrin 6″-*O*-2‴′-methylcinnamate (**4c**): ^{13}C NMR (DMSO-d_6) $\delta = 25.9$ (C10‴), 63.1 (C6″), 69.9 (C4″), 73.9 (C2″), 74.3 (C5″), 77.2 (C3″), 93.5 (C8), 98.7 (C6), 100.8 (C1″), 103.9 (C4a), 115.2 (C2′), 116.2 (C5′), 118.4 (C2‴), 121.2 (C6′), 121.7 (C1′), 126.5 (C5‴′), 126.6 (C4‴′), 130.3 (C6‴′), 130.8 (C3‴′), 132.6 (C2‴′), 133.2 (C3), 137.4 (C1‴′), 141.8 (C3‴), 144.8 (C3′), 148.5 (C4′), 156.4 (C8a), 156.7 (C2), 161.0 (C5), 165.9 (C7), 167.1 (C1‴), 177.4 (C4); FAB-MS *m*/*z* 609 $[M + H]^+$.

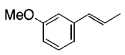
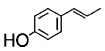
Isoquercitrin 6″-*O*-benzoate (**4d**): ^{13}C NMR (DMSO-d_6) $\delta = 62.6$ (C6″), 70.1 (C4″), 74.1 (C2″), 74.3 (C3″), 76.4 (C5″), 93.8 (C8), 99.0 (C6), 100.7 (C1″), 103.7 (C4a), 115.3 (C2′), 116.1 (C5′), 121.1 (C6′), 121.5 (C1′), 128.6 (C3‴′, C5‴′), 128.9 (C1‴′), 129.6 (C2‴′, C6‴′), 132.9 (C4‴′), 133.2 (C3), 145.0 (C3′), 148.7 (C4′), 156.3 (C8a), 156.4 (C2), 161.3 (C5), 164.9 (C7), 165.5 (C1‴), 177.4 (C4); FAB-MS *m*/*z* 409 $[M + H]^+$.

Isoquercitrin 6″-*O*-4‴-phenyl-*n*-butyrate (**4e**): ^{13}C NMR (DMSO-d_6) $\delta = 26.2$ (C3‴), 32.7 (C4‴), 34.3 (C2‴), 63.0 (C6″), 70.1 (C4″), 74.0 (C2″), 74.1 (C3″), 76.3 (C5″), 93.6 (C8), 98.9 (C6), 100.9 (C1″), 103.8 (C4a), 115.2 (C2′), 116.2 (C5′), 121.1 (C6′), 121.6 (C1′), 125.9 (C4‴′), 128.3 (C3‴′, C5‴′), 128.4 (C2‴′, C6‴′), 133.1 (C3), 141.4 (C1‴′), 144.9 (C3′), 148.6 (C4′), 156.4 (C8a), 156.5 (C2), 161.3 (C5, 165.5 (C7), 172.3 (C1‴), 177.4 (C4); FAB-MS *m*/*z* 611 $[M+H]^+$.

Isoquercitrin 6″-*O*-2‴-naphthylacetate (**4f**): ^{13}C NMR (DMSO-d_6) $\delta = 40.5$ (C2‴), 62.8 (C6″), 69.9 (C4″), 74.0 (C2″), 74.2 (C3″), 76.4 (C5″), 93.7 (C8), 98.9 (C6), 101.0 (C1″), 104.1 (C4a), 115.3 (C2′), 116.4 (C5′), 121.2 (C6′), 121.7 (C1′), 125.9 (C6‴′), 126.2 (C7‴′), 127.5 (C1‴′, C4‴′), 127.7 (C5‴′, C8‴′), 127.8 (C3‴′), 131.9 (C4a‴′), 132.0 (C8a‴′), 133.0 (C2‴′), 133.3 (C3), 145.0 (C3′), 148.7 (C4′), 156.5 (C8a), 156.6 (C2), 161.5 (C5), 164.3 (C7), 170.8 (C1‴), 177.5 (C4); FAB-MS *m*/*z* 631 $[M + H]^+$.

Table 2
Lipase-catalyzed direct and regioselective acylation of isoquercitrin



Acyl donor (R)		Lipases	Solvent	**4**, Yield (%)
2a		Chirazyme L-2	Acetone	68
2b		Chirazyme L-2	Acetone	30
2c		Chirazyme L-2	Acetone	22
2d		Amano-PS	Acetone	42
2e		Chirazyme L-2	Acetonitrile	49
2f		Chirazyme L-2	Acetone	43
2g		Chirazyme L-2	Acetone	59
2h		Chirazyme L-2	Acetone	11
2i		Lipoprotein lipase	Acetone	49

Isoquercitrin 6″-*O*-cinnamate (**4g**): ^{13}C NMR (DMSO-d_6) δ = 63.6 (C6″), 70.2 (C4″), 74.3 (C2″), 76.8 (C5″), 77.8 (C3″), 93.8 (C8), 98.9 (C6), 101.1 (C1″), 104.2 (C4a), 115.4 (C2′), 116.5 (C5′), 119.4 (C2″), 121.4 (C1′), 121.9 (C6′), 128.4 (C3‴, C5‴), 129.1 (C2‴, C6‴), 130.4 (C4‴), 133.6 (C3), 134.4 (C1‴), 144.1 (C3‴), 145.1 (C3′), 148.7 (C4′), 156.3 (C8a), 156.5 (C2), 161.5 (C5), 164.2 (C7), 165.8 (C1‴), 177.7 (C4); FAB-MS *m*/*z* 595 $[M + H]^+$.

Isoquercitrin 6″-*O*-4‴-hydroxycinnamate (**4h**): ^{13}C NMR (DMSO-d_6) δ = 63.5 (C6″), 70.1 (C4″), 74.3 (C2″), 76.7 (C5″), 77.8 (C3″), 93.7 (C8), 98.8 (C6), 101.0 (C1″), 103.9 (C4a), 104.2 (C2‴), 115.4 (C2′), 115.9 (C3‴, C5‴), 116.4 (C5′), 121.4 (C1′), 121.8 (C6′), 130.0 (C2‴, C6‴), 133.5 (C3), 133.9 (C1‴), 144.1 (C3‴), 145.0 (C3′), 148.7 (C4′), 156.3 (C8a), 156.5 (C2), 159.3 (C4‴), 161.4 (C5), 164.3 (C7), 170.7 (C1‴), 177.6 (C4); FAB-MS *m*/*z* 611 $[M+H]^+$.

Isoquercitrin 6″-*O*-*n*-hexanoate (**4i**): ^{13}C NMR (DMSO-d_6) δ = 14.0 (C6‴), 21.8 (C3‴), 24.1 (C5‴), 30.7 (C4‴), 33.4 (C2‴), 63.0 (C6″), 70.3 (C4″), 74.1 (C2″), 74.3 (C3″), 76.4 (C5″), 93.7 (C8), 99.0 (C6), 100.8 (C1″), 103.9 (C4a), 115.3 (C2′), 116.2 (C5′), 121.2 (C6′), 121.7 (C1′), 133.1 (C3), 145.0 (C3′), 148.7 (C4′), 156.4 (C8a), 156.6 (C2), 161.4 (C5), 164.8 (C7), 172.7 (C1‴), 177.5 (C4); FAB-MS *m*/*z* 563 $[M + H]^+$.

2.5. Measurement of thermostability

Isoquercitrin or the prepared acylated isoquercitrin was dissolved in McIlvain buffer (pH 7.0) [16], and then each solution was diluted to 4.0×10^{-5} M with the same buffer. A 1 ml aliquot pigment solution in a cuvette (closed system) was incubated at 50 °C in the dark. The pigment solution was placed in the thermostated cell (at 50 °C), and its absorption at 350 nm was recorded. The remaining absorbance was calculated by setting the absorbance without incubation to 100.

2.6. Measurement of light-resistibility

Isoquercitrin or the prepared acylated isoquercitrin was dissolved in McIlvain buffer (pH 7.0) [16], and then each solution was diluted to 4.0×10^{-5} M with

the same buffer. A 1 ml aliquot pigment solution in a cuvette (closed system) was incubated at 30 °C with illumination by a white fluorescent light (40,000 lx, one side). The pigment solution was placed in the thermostated cell (at 30 °C), and its absorption at 350 nm was recorded. The remaining absorbance was calculated by setting the absorbance without incubation to 100.

3. Results and discussion

3.1. Preparation of acyl donors (vinyl esters)

As shown in Table 1, the isolated yields of vinyl 2-methylcinnamate (**2c**) and vinyl 2-naphthylacetate (**2f**) were low (34 and 47%, respectively). The starting material (**1c** or **1f**) was recovered (30 and 28%, respectively). These results appear to be attributable to the bulkiness of the substrate. During the preparation of vinyl 4-phenyl-*n*-butyrate (**2e**), the substrate (**1e**) was not recovered, however, the yield of product was not above 50% due to the formation of by-products. Other acyl donors were synthesized in good yields (from 64 to 89%).

3.2. Enzymatic synthesis of acylated isoquercitrins

When using vinyl cinnamate (**2g**), other cinnamate derivatives (**2a**–**2c**, **2h**), 2-naphthyl acetate (**2f**), and 4-phenyl-*n*-butyrate (**2e**) were used as the acyl donors, Chirazyme L-2 was a good catalyst for the acylation toward isoquercitrin. During the preparation of isoquercitrin benzoate (**4d**) and isoquercitrin *n*-hexanoate (**2i**), Amano-PS and lipoprotein lipase were suited, respectively. In almost all cases, the lipase-catalyzed transesterification proceeded in dry acetone to give the corresponding acylated isoquercitrin. However, the reaction proceeded when using acetonitrile as the solvent in the preparation of **4e**. During the enzymatic acylation with the cinnamate derivative having the substituent group such as **2b**, **2c**, and **2h**, the conversion ratio was low (below 50%). In particular, the conversion ratio for the acylation with **2h** was not over 30%. These low ratios are presumably caused by the steric hindrance of the substrate.

The regioselectivity of the lipase-catalyzed acylation was specific at the C6″ position (primary alcohol) in the glucose moiety. In any case, the acylation toward the phenolic hydroxyl groups in the flavonoid skeleton was not observed at all. For example, in ^{13}C NMR spectra of isoquercitrin *p*-coumarate (**4h**), the signals of the carbonyl carbon of the ester moiety (C1‴ position) shifted by −2.1 ppm with the production of the ester from 172.8 ppm for *p*-coumaric acid to 170.7 ppm for isoquericitrin *p*-coumarate. Furthermore, the signals of the C6″ position for the D-glucose moiety shifted by 2.3 ppm (from 61.2 to 63.5 ppm). However, the shifts of C5, C7, C3′, C4′, C2″ and C3″ with the product formation were not observed. These results show the formation of an ester linkage between the primary aliphatic alcohol (C6″) of the glucose moiety in isoquercitrin and the carboxyl residue (C1‴) of vinyl *p*-coumarate, and deny the esterification of the phenolic and secondary alcohols of isoquercitrin.

Thus, the preparation of nine acylated isoquercitrins was accomplished by the lipase-catalyzed transesterification with carboxylic acid vinyl esters in an organic solvent.

3.3. Thermostability

To investigate the effect of the substituent in the aromatic ring on the thermostability of the acylated isoquercitrins, the depigmentation of isoquercitrin (**3**), isoquercitrin 3-methoxycinnamate (**4b**), isoquercitrin 2-methylcinnamate (**4c**), isoquercitrin cinnamate (**4g**), and isoquercitrin *p*-coumarate (**4h**) at 50 °C were measured as shown in Fig. 1. The absorbance at 350 nm of **4b**, **4c**, **4g**, and **4h** more slowly decreased than that of **3**. In particular, **4h** had a high thermostability and the slowest rate of depigmentation of the five compounds tested. Fig. 2 shows a comparison of the thermostability among **3**, isoquericitrin dihydrocinnamate (**4a**), isoquercitrin benzoate (**4d**), and isoquercitrin 4-phenyl-*n*-butyrate (**4e**). The thermostability of **4a** and **4e** were slightly higher than **3**, however, **4d** had the lowest stability. The depigmentation rate of isoqurecitrin *n*-hexanoate (**4i**), having no aromatic ring in the acyl moiety, was same as that of **3** (shown in Fig. 3). The thermostability of isoquercitrin 2-naphthylacetate (**4f**) was slightly higher than **4a** and **4g**. Briefly, the acylation with the aliphatic carboxylic acid vinyl ester did not increase the thermostability. These results suggest that thermostability

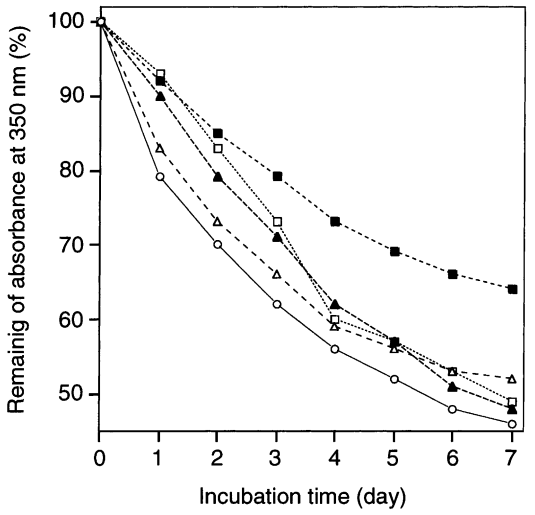


Fig. 1. Thermostability (effects of substitution group in aromatic ring) of acylated isoquercitrins in McIlvain buffer (pH 7.0) (4.0×10^{-5} M) at 50 °C in the dark: (○) isoquercitrin (**3**); (□) isoquercitrin 3-methoxycinnamate (**4b**); (▲) isoquercitrin 2-methylcinnamate (**4c**); (△) isoquercitrin cinnamate (**4g**); (■) isoquercitrin *p*-coumarate (**4h**). The remaining absorbance was calculated by setting the absorbance without incubation to 100.

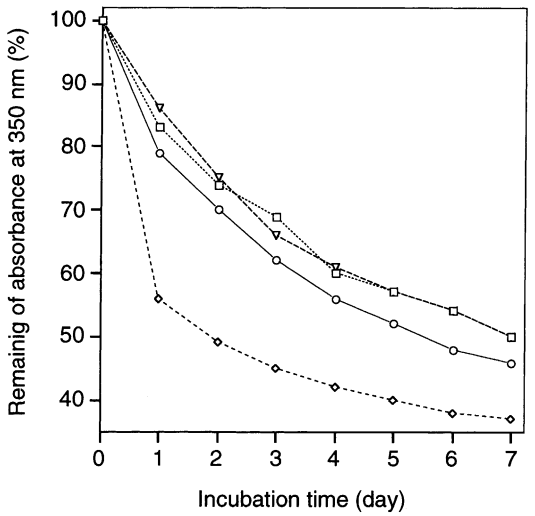


Fig. 2. Thermostability (effects of distance between isoquercitrin and aromatic ring) of acylated isoquercitrins in McIlvain buffer (pH 7.0) (4.0×10^{-5} M) at 50 °C in the dark: (○) isoquercitrin (**3**); (□) isoquercitrin dihydrocinnamate (**4a**); (◇) isoquercitrin benzoate (**4d**); (▽) isoquercitrin 4-phenyl-*n*-butyrate (**4e**). The remaining absorbance was calculated by setting the absorbance without incubation to 100.

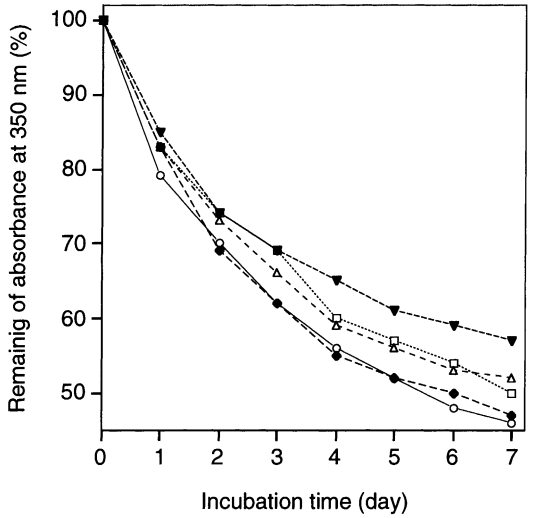


Fig. 3. Thermostability (effects of the number of aromatic ring or carbon–carbon double bond) of acylated isoquercitrins in McIlvain buffer (pH 7.0) (4.0×10^{-5} M) at 50 °C in the dark: (○) isoquercitrin (**3**); (□) isoquercitrin dihydrocinnamate (**4a**); (▼) isoquercitrin 2-naphthylacetate (**4f**); (△) isoquercitrin cinnamate (**4g**); (◆) isoquercitrin *n*-hexanoate (**4i**). The remaining absorbance was calculated by setting the absorbance without incubation to 100.

of isoquercitrin molecule was dependent on the aromatic ring in the acyl moiety.

3.4. Light-resistivity

As shown in Fig. 4, the depigmentations of isoquercitrin (**3**), isoquercitrin dihydrocinnamate (**4a**), isoquercitrin cinnamate (**4g**), and isoquercitrin *p*-coumarate (**4h**) at 30 °C with illumination by white fluorescent light (40,000 lx, one side) were measured to study the effect of the acyl moiety on the light-resistivity. The depigmentaiton rates of **4a**, **4g**, and **4h** proceeded more slowly than that of **3**. In particular, **4h** had the highest light-resistivity of the tested four compounds. These results suggest that the acylation also increased the light-resistivity of isoquercitrin.

In conclusion, the acylation with aromatic carboxylic acids improved both the thermostability and light-resistivity of isoquercitrin. In particular, isoquercitrin *p*-coumarate was the most stable of the nine enzymatically synthesized acylated isoquercitrins. Among the natural plant pigments, the acylation with

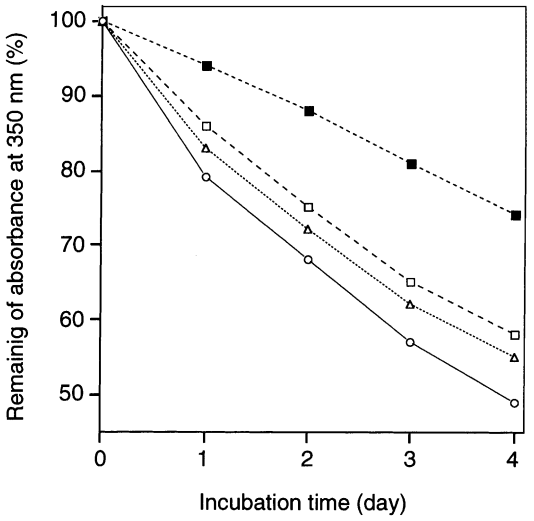


Fig. 4. The light-resistibility of acylated isoquercitrins in McIlvain buffer (pH 7.0) (4.0×10^{-5} M) at 30 °C with illumination by white fluorescent light (40,000 lx, one side): (○) isoquercitrin (**3**); (□) isoquercitrin dihydrocinnamate (**4a**); (△) isoquercitrin cinnamate (**4g**); (■) isoquercitrin *p*-coumarate (**4h**). The remaining absorbance was calculated by setting the absorbance without incubation to 100.

p-coumarate was observed in many pigments. This fact suggests that the acylation with *p*-coumarate is significantly related to the stabilization of their plant pigments. The support data for the introduction of the acyl group to provide stability was obtained by our present study. Although, the mechanism for the improvement of the thermostability and light-resistivity by the acylation is not clear, it seems that the intra- and intermolecular hydrophobic interactions or the π–π stacking between the flavonoid skeleton and the aromatic ring in the acyl moiety would prevent the decomposition of the flavonoid glucoside molecule. Further, detailed studies including the large scale production of the stable flavonoid glucosides by the lipase-catalyzed transesterification are now in progress in our laboratories.

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