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Chemo-enzymatic transformation of naturally abundant naringin to luteolin, a flavonoid with various biological effects

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ABSTRACT

Luteolin [3′,4′,5,7-tetrahydroxyflavone], having multiple biological effects such as anti-inflammation, anti-allergy and anti-cancer, was prepared by chemo-enzymatic synthesis from naringin, a naturally abundant flavonoid glycoside. On the occasion of *Candida antarctica* lipase B (Novozym 435)-catalyzed transesterification on peracetylated form of naringin, an acetate on C-4′ was exclusively deprotected to give the key intermediate. The oxidation with 2-iodoxybenzoic acid (IBX) followed by the reductive workup provided regioselectively C-3′and C-4′ catechol functionality. After protection of the above-mentioned diol with methoxymethyl (MOM) groups and subsequent hydrolysis of all acetyl groups, a dehydrogenative introduction of double bond between C-2 and C-3 was done by the treatment with I_2. Acid-catalyzed simultaneous removal of MOM groups and glycoside provided luteolin in total 8 steps and 36% overall yield from the starting material. Throughout the synthesis, diglycoside side chain effectively worked as the protective group on C-7 hydroxy group.

1. Introduction

Luteolin, 3′,4′,5,7-tetrahydroxyflavone (**1a**, Fig. 1), is a common flavone, which is one of the categories of flavonoids and exists in various plants including fruits, vegetables, and medicinal herbs. It has been shown to possess potent antioxidant [1] and anti-inflammatory/anti-allergic activities [2], and the recent review gives an account of the pharmacological data and many references for luteolin concerning its biological effect, comparing it to other flavonoids [3]. Moreover, it displays the anti-cancer action, and research papers have been reporting cancer preventive effect and anti-cancer effects [4]. Inhibitory activity for osteoclast differentiation has also been reported [5].

However, the amount of luteolin is generally low compared to some of the major flavonols like quercetin. While it is only a minor flavonoid component in food and traditional medicine, high amounts can be isolated from peanut hulls and *Reseda luteola* L. that has been used as a dyeing plant due to its high luteolin content since ancient times. The yield is still *ca.* 0.4% [6]. Luteolin has been synthesized by the traditional Claisen condensation [7] between 2,4,6-trihydroxyacetophenone and bis-*t*-butyldimethylsilylated form of methyl 3,4-dihydroxybenzoate, but the latter component is quite expensive. Recently, palladium-catalyzed carbonylative coupling between an aryl iodide and an arylalkyne was demonstrated for the synthesis of luteolin as its permethylated form [8].

We turned our attention to naringin (**2a**, Fig. 1), which is daily produced in large quantity as the by-product in industries of citrus beverages and foods and envisaged a supply of **1a** as sole aglycone flavonoid, starting from **2a**. By the combination of regioselective introduction of one hydroxy group on C-3′, dehydrogenation to flavanone to flavone, and the subsequent hydrolytic cleavage of glycosidic bond, the desired molecule would be produced in an expeditious manner. Herein we describe the successful chemo-enzymatic approach, which was inspired by the *Candida antarctica* lipase B-catalyzed regioselective transesterification and hydrolysis on aromatic hydroxy groups and acetates [9,10].

2. Experimental

IR spectra were measured as ATR on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR spectra were measured at 400 MHz on an Agilent 400-MR or at 500 MHz on an Agilent INOVA-500 or at 600 MHz on a JEOL FT-NMR ECP-600 spectrometers. ^{13}C NMR spectra were measured at 100 MHz on an Agilent 400-MR or at 125 MHz on an Agilent INOVA-500 spectrometer. Optical rotation values were recorded on a Jasco P-1010 polarimeter. High resolution mass spectra were recorded on a Jeol JMS-700 MStation spectrometer at 70 eV. Silica gel 60 N (spherical and neutral, 100–210 μm, 37560-79) of Kanto Chemical Co. was used for column chromatography.

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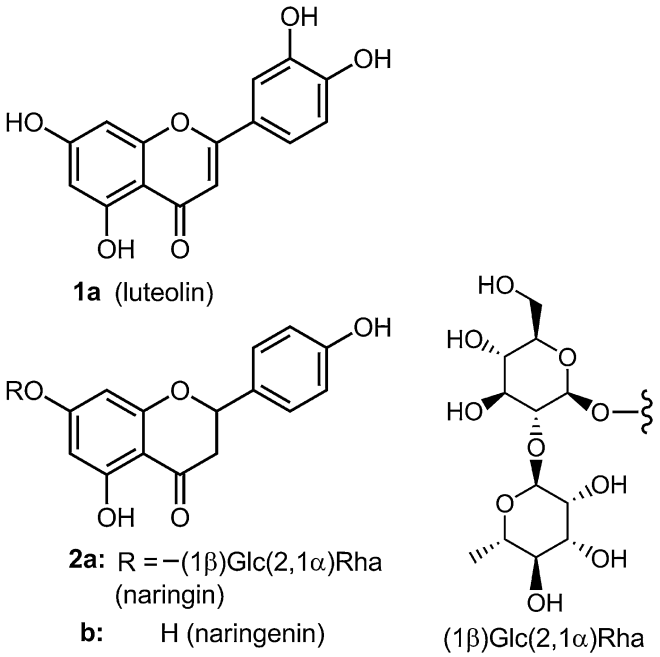


Fig. 1. Luteolin (**1a**), naringin (**2a**), and its aglycone, naringenin (**2b**).

2.1. 4′,5-Diacetoxy-7-[hexa-O-acetyl-(2-O-α-L-rhamnopyranoxyl-β-D-glucopyranosyl)oxy]flavan-4-one (**2c**)

To a solution of naringin dihydrate (**2a**, 5.80 g, 10.0 mmol) in pyridine (50 mL) were added Ac_2O (50 mL) and 4-*N*,*N*-dimethylaminopyridine (DMAP, 122 mg, 1.00 mmol, 0.1 equiv.) under argon atmosphere. The mixture was stirred for 2 h at room temperature, then the reaction was quenched by adding water. The organic materials were extracted with AcOEt, and the combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (150 g). Elution with hexane/AcOEt = 1:1 afforded **2c** (9.08 g, 99%) as colorless amorphous solid. $[\alpha]_D^{25}$ −32 (*c* 1.00, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, *J* = 6.3 Hz, 3H, rhamnose-6-CH_3), 1.93, 1.94, 1.95, 1.99, 2.06, 2.09 (each s, total 18H, sugar-OAc), 2.27 (s, 3H, 4′-OAc), 2.33 (s, 3H, 5-OAc), 2.70 (dd, *J* = 2.7, 16.8 Hz, 0.7H, H-3a), 2.71 (dd, *J* = 2.7, 16.8 Hz, 0.3H, H-3a), 2.98 (dd, *J* = 13.5, 16.8 Hz, 0.7H, H-3b), 3.00 (dd, *J* = 13.5, 16.8 Hz, 0.3H, H-3b), 3.80–4.20 (total 5H), 4.94–5.09 (total 5H), 5.10 (d, *J* = 2.9 Hz, 0.5H, rhamnose-H-1), 5.13 (d, *J* = 2.9 Hz, 0.5H, rhamnose-H-1), 5.27 (d, *J* = 9.6 Hz, 0.13H, glucose-H-1), 5.28 (d, *J* = 9.6 Hz, 0.37H, glucose-H-1), 5.295 (d, *J* = 9.6 Hz, 0.13H, glucose-H-1), 5.299 (d, *J* = 9.6 Hz, 0.37H, glucose-H-1), 5.42 (dd, *J* = 2.7, 13.5 Hz, 1H, H-2), 6.30 (d, *J* = 2.4 Hz, 1H, H-8), 6.51 (d, *J* = 2.4 Hz, 1H, H-6), 7.107 (d, *J* = 8.6 Hz, 0.6H, H-2′, H-6′), 7.112 (d, *J* = 8.6 Hz, 1.4H, H-2′, H-6′), 7.41 (d, *J* = 8.6 Hz, 0.6H, H-3′, H-5′), 7.42 (d, *J* = 8.6 Hz, 1.4H, H-3′, H-5′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.4, 20.38, 20.46, 20.48, 20.57, 20.59, 20.77, 20.95, 20.99, 44.9, 61.8, 66.8, 68.15, 68.24, 69.9, 70.7, 72.2, 74.0, 76.4, 79.1, 98.0, 98.1, 102.2, 105.9, 109.5, 122.0, 127.3, 135.5, 150.9, 151.8, 161.7, 163.8, 169.03, 169.17, 169.51, 169.62, 169.88, 169.92, 169.97, 170.31, 188.5; the signals 122.0 and 127.3 included two carbons. The ^{1}H and ^{13}C NMR spectra were very complex, as the stereochemistry at C-2 is prone to epimerize upon acetylation of phenolic hydroxy groups. In the following sections from 2.2 to 2.5, compounds **2d**, **3a**, **3b** and **3c** were also diastereomeric mixtures. IR: 1743, 1686, 1616, 1439, 1365, 1219, 1037, 910, 748 cm^{-1}.

2.2. 5-Acetoxy-7-[hexa-O-acetyl-(2-O-α-L-rhamnopyranoxyl-β-D-glucopyranosyl)oxy]-4′-hydroxyflavan-4-one (**2d**)

To a solution of **2c** (9.16 g, 10.0 mmol) in cyclopentanol (180 mL) and cyclopentyl methyl ether (180 mL) were added an immobilized form of *C. antarctica* lipase B (Novozymes, Novozym 435, 9.16 g). The mixture was stirred for 12 h at room temperature. After removal of insoluble materials by filtration with a pad of Celite, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (200 g). Elution with hexane/AcOEt = 1:1 afforded **2d** (8.44 g, 96%) as slightly yellow amorphous solid. $[\alpha]_D^{26}$ −39 (*c* 1.00, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18 (d, *J* = 6.3 Hz, 2.7H, rhamnose-6-CH_3), 1.19 (d, *J* = 6.3 Hz, 0.3H, rhamnose-6-CH_3), 1.95, 1.96, 1.97, 2.01, 2.08, 2.12 (each s, total 18H, sugar-OAc), 2.36 (s, 3H, 5-OAc), 2.68 (dd, *J* = 2.7, 16.8 Hz, 0.7H, H-3a), 2.69 (dd, *J* = 2.7, 16.8 Hz, 0.3H, H-3a), 3.00 (dd, *J* = 13.5, 16.8 Hz, 0.7H, H-3b), 3.02 (dd, *J* = 13.5, 16.8 Hz, 0.3H, H-3b), 3.81–4.21 (total 5H), 4.97–5.09 (total 5H), 5.12 (d, *J* = 2.9 Hz, 0.67H, rhamnose-H-1), 5.15 (d, *J* = 2.9 Hz, 0.33H, rhamnose-H-1), 5.29 (d, *J* = 9.4 Hz, 0.13H, glucose-H-1), 5.30 (d, *J* = 9.4 Hz, 0.37H, glucose-H-1), 5.309 (d, *J* = 9.4 Hz, 0.13H, glucose-H-1), 5.313 (d, *J* = 9.4 Hz, 0.37H, glucose-H-1), 5.37 (dd, *J* = 2.7, 13.5 Hz, 0.25H, H-2), 5.38 (dd, *J* = 2.7, 13.5 Hz, 0.75H, H-2), 6.30 (d, *J* = 2.4 Hz, 1H, H-8), 6.505 (d, *J* = 2.4 Hz, 0.3H, H-6), 6.510 (d, *J* = 2.4 Hz, 0.7H, H-6), 6.84 (d, *J* = 8.4 Hz, 1.2H, H-2′, H-6′), 6.85 (d, *J* = 8.4 Hz, 0.8H, H-2′, H-6′), 7.28 (d, *J* = 8.4 Hz, 2H, H-3′, H-5′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.4, 20.37, 20.40, 20.44, 20.53, 20.55, 20.71, 20.93, 44.6, 61.7, 66.8, 68.1, 68.3, 69.9, 70.7, 72.0, 74.0, 76.4, 79.5, 97.9, 98.0, 102.1, 105.7, 109.3, 115.5, 127.9, 129.3, 151.7, 157.0, 161.7, 164.1, 169.3, 169.6, 169.8, 170.01, 170.05, 170.1, 170.6, 189.4; the signals 115.5 and 127.9 included two carbons. IR: 1743, 1686, 1616, 1520, 1439, 1365, 1211, 1138, 1038, 895, 837 cm^{-1}.

2.3. 5-Acetoxy-7-[hexa-O-acetyl-(2-O-α-L-rhamnopyranoxyl-β-D-glucopyranosyl)oxy]-3′,4′-dihydroxyflavan-4-one (**3a**)

To a solution of **2d** (150 mg, 0.172 mmol) in $CHCl_3$ (1.4 mL) and MeOH (0.35 mL) were added 2-iodoxybenzoic acid (IBX, 62.6 mg, 0.224 mmol, 1.3 equiv.). The mixture was stirred for 1 h at room temperature, then the reaction was quenched by adding aqueous $Na_2S_2O_4$ solution. The organic materials were extracted twice with $CHCl_3$, and the combined extracts were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (4.50 g). Elution with $CHCl_3$/MeOH = 100:1 afforded **3a** (111 mg, 73%) as slightly yellow amorphous solid. $[\alpha]_D^{25}$ −40 (*c* 0.67, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, *J* = 6.3 Hz, 2.4H, rhamnose-6-CH_3), 1.20 (d, *J* = 6.3 Hz, 0.6H, rhamnose-6-CH_3), 1.966, 1.970, 1.98, 2.02, 2.09, 2.12 (each s, total 18H, sugar-OAc), 2.36 (s, 3H, 5-OAc), 2.71 (dd, *J* = 2.9, 16.6 Hz, 0.8H, H-3a), 2.82 (dd, *J* = 3.5, 16.6 Hz, 0.2H, H-3a), 2.96 (dd, *J* = 13.0, 16.6 Hz, 0.8H, H-3b), 3.01 (dd, *J* = 10.5, 16.6 Hz, 0.2H, H-3b), 3.81–4.23 (total 5H), 4.97–5.09 (total 5H), 5.14 (d, *J* = 3.2 Hz, 0.37H, rhamnose-H-1), 5.17 (d, *J* = 3.2 Hz, 0.37H, rhamnose-H-1), 5.19 (d, *J* = 3.2 Hz, 0.13H, rhamnose-H-1), 5.22 (d, *J* = 3.2 Hz, 0.13H, rhamnose-H-1),5.28 (d, *J* = 9.3 Hz, 0.13H, glucose-H-1), 5.30 (d, *J* = 9.3 Hz, 0.37H, glucose-H-1), 5.31 (d, *J* = 9.3 Hz, 0.13H, glucose-H-1), 5.32 (d, *J* = 9.3 Hz, 0.37H, glucose-H-1), 5.33 (dd, *J* = 2.9, 13.0 Hz, 0.8H, H-2), 5.41 (dd, *J* = 3.5, 10.5 Hz, 0.2H, H-2), 5.47 (s, 1H, O*H*), 5.79 (s, 0.8H, O*H*), 6.19 (s, 0.2H, O*H*), 6.31 (d, *J* = 2.4 Hz, 1H, H-8), 6.52 (d, *J* = 2.4 Hz, 0.8H, H-6), 6.55 (d, *J* = 2.4 Hz, 0.2H, H-6), 6.77 (dd, *J* = 1.9, 8.2 Hz, 0.2H, H-6′), 6.81(d, *J* = 1.9, 8.2 Hz, 0.8H, H-6′), 6.83 (d, *J* = 8.2 Hz, 0.2H, H-5′), 6.87 (d, *J* = 8.2 Hz, 0.8H, H-5′), 6.92 (d, *J* = 1.9 Hz, 0.2H, H-2′), 6.96 (d, *J* = 1.9 Hz, 0.8H, H-2′); ^{13}C NMR

(125 MHz, $CDCl_3$): δ 17.5, 20.5, 20.57, 20.62, 20.70, 20.73, 20.9, 21.1, 44.7, 61.8, 66.9, 68.2, 68.5, 70.0, 70.8, 72.2, 74.1, 76.4, 79.4, 98.0, 98.1, 102.1, 106.0, 109.5, 113.5, 115.4, 119.0, 130.6, 144.2, 144.6, 151.7, 161.8, 164.1, 169.6, 169.7, 170.08, 170.11, 170.2, 170.3, 170.7, 189.3. IR: 3435, 1741, 1686, 1616, 1570, 1520, 1439, 1369, 1217, 1034 cm^{-1}.

2.4. 5-Acetoxy-7-[hexa-O-acetyl-(2-O-α-L-rhamnopyranoxyl-β-D-glucopyranosyl)oxy]-3′,4′-bis(methoxymethoxy)flavan-4-one (**3b**)

To a solution of **3a** (70.0 mg, 0.0786 mmol) in CH_2Cl_2 (0.8 mL) were added methoxymethyl (MOM) chloride (18 μL, 0.236 mmol, 3 equiv.) and diisopropylethylamine (DIPEA, 55 μL, 0.314 mmol, 4 equiv.). The mixture was stirred for 1 h at room temperature, then the reaction was quenched by saturated aqueous NH_4Cl solution. The organic materials were extracted with AcOEt twice and the combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford **3b** (77.0 mg) as yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, J = 6.2 Hz, 3H, rhamnose-6-CH_3), 1.95, 1.976, 1.978, 2.02, 2.08, 2.12 (each s, total 18H, sugar-OAc), 2.35 (s, 3H, 5-OAc), 2.68 (dd, J = 2.7, 16.8 Hz, 0.7H, H-3a), 2.69 (dd, J = 2.7, 16.8 Hz, 0.3H, H-3a), 3.03 (dd, J = 13.6, 16.8 Hz, 0.7H, H-3b), 3.04 (dd, J = 13.6, 16.8 Hz, 0.3H, H-3b), 3.50 (s, 3H, OCH_3), 3.51 (s, 3H, OCH_3), 3.83–4.24 (total 5H), 4.97–5.11 (total 5H), 5.127 (d, J = 3.0 Hz, 0.37H, rhamnose-H-1), 5.131 (d, J = 3.0 Hz, 0.13H, rhamnose-H-1), 5.15 (d, J = 3.0 Hz, 0.37H, rhamnose-H-1), 5.16 (d, J = 3.0 Hz, 0.13H, rhamnose-H-1), 5.21–5.25 (total 4H, O—CH_2—O), 5.29 (d, J = 9.4 Hz, 0.13H, glucose-H-1), 5.30 (d, J = 9.4 Hz, 0.37H, glucose-H-1), 5.31 (d, J = 9.4 Hz, 0.13H, glucose-H-1), 5.32 (d, J = 9.4 Hz, 0.37H, glucose-H-1), 5.37 (dd, J = 2.7, 13.6 Hz, 0.7H, H-2), 5.38 (dd, J = 2.7, 13.6 Hz, 0.3H, H-2), 6.307 (d, J = 2.3 Hz, 0.7H, H-8), 6.311 (d, J = 2.3 Hz, 0.3H, H-8), 6.52 (d, J = 2.3 Hz, 1H, H-6), 7.01 (dd, J = 2.1, 8.4 Hz, 0.7H, H-6′), 7.02 (dd, J = 2.1, 8.4 Hz, 0.3H, H-6′), 7.18 (d, J = 8.4 Hz, 1H, H-5′), 7.23 (d, J = 2.1 Hz, 1H, H-2′). This was employed for the next step without further purification.

2.5. 5-Hydroxy-7-[(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]-3′,4′-bis(methoxymethoxy)flavan-4-one (**3c**)

To a solution of **3b** (77.0 mg) in MeOH (1.0 mL) were added K_2CO_3 (33 mg, 0.236 mmol). The mixture was stirred for 19 h at room temperature, then the mixture was acidified with Amberlite IR-120B (H^+ form) to pH 5 and then filtered. The filtrate was concentrated *in vacuo* to afford **3c** (50.0 mg) as yellow solid. ^{1}H NMR (600 MHz, CD_3OD): δ 1.28 (d, J = 6.1 Hz, 3H, rhamnose-6-CH_3), 2.79 (dd, J = 3.0, 17.1 Hz, 0.5H, H-3a), 2.80 (dd, J = 3.0, 17.1 Hz, 0.5H, H-3a), 3.13 (dd, J = 12.7, 17.1 Hz, 0.5H, H-3b), 3.14 (dd, J = 12.7, 17.1 Hz, 0.5H, H-3b), 3.34–3.93 (total 16H), 5.08 (d, J = 7.6 Hz, 0.5H, glucose-H-1), 5.10 (d, J = 7.6 Hz, 0.5H, glucose-H-1), 5.197 (s, 2H, O—CH_2—O), 5.203 (s, 2H, O—CH_2—O), 5.24 (d, J = 2.0 Hz, 0.5H, rhamnose-H-1), 5.25 (d, J = 2.0 Hz, 0.5H, rhamnose-H-1), 5.39 (dd, J = 3.0, 12.7 Hz, 0.5H, H-2), 5.41 (dd, J = 3.0, 12.7 Hz, 0.5H, H-2), 6.15 (d, J = 2.2 Hz, 1H, H-8), 6.198 (d, J = 2.2 Hz, 0.5H, H-6), 6.202 (d, J = 2.2 Hz, 0.5H, H-6), 7.08 (dd, J = 2.2, 8.2 Hz, 0.5H, H-6′), 7.09 (dd, J = 2.2, 8.2 Hz, 0.5H, H-6′), 7.15 (d, J = 8.2 Hz, 0.5H, H-5′), 7.16 (d, J = 8.2 Hz, 0.5H, H-5′), 7.27 (d, J = 2.2 Hz, 1H, H-2′). This was employed for the next step without further purification.

2.6. 5-Hydroxy-7-[(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]-3′,4′-bis(methoxymethoxy)flavone (**1b**)

To a solution of **3c** (50.0 mg) in pyridine (0.8 mL) were added I_2 (22 mg, 0.0869 mmol). The mixture was stirred for 20 h at 100 °C, then the reaction was quenched by saturated aqueous $Na_2S_2O_3$ solution. The organic materials were extracted with $CHCl_3$ three times and the combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford **1b** (26.0 mg) as yellow solid. ^{1}H NMR (600 MHz, CD_3OD): δ 1.32 (d, J = 6.1 Hz, 3H, rhamnose-6-CH_3), 3.38–3.95 (total 16H), 5.20 (d, J = 7.6 Hz, 1H, glucose-H-1), 5.28 (d, J = 1.6 Hz, 1H, rhamnose-H-1), 5.29 (s, 2H, O—CH_2—O), 5.30 (s, 2H, O—CH_2—O), 6.47 (d, J = 2.1 Hz, 1H, H-8), 6.70 (s, 1H, H-3), 6.80 (d, J = 2.1 Hz, 1H, H-6), 7.29 (d, J = 8.7 Hz, 1H, H-5′), 7.67 (dd, J = 2.1, 8.7 Hz, 1H, H-6′), 7.73 (d, J = 2.1 Hz, 1H, H-2′). This was employed for the next step without further purification.

2.7. 3′,4′,5,7-Tetrahydroxyflavone (luteolin, **1a**)

To a solution of **1b** (26.0 mg) in MeOH (0.4 mL) and THF (0.4 mL) were added conc. HCl (0.2 mL). The mixture was stirred for 14 h at 60 °C, then the reaction was quenched by saturated aqueous $NaHCO_3$ solution. The organic materials were extracted with AcOEt twice and the combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2.00 g). Elution with $CHCl_3$ afforded **1a** (11.6 mg, 52% over 4 steps from **3a**). Mp > 300 °C (uncorrected); ^{1}H NMR (500 MHz, acetone-d_6): δ 6.24 (d, J = 1.5 Hz, 1H, H-8), 6.51 (d, J = 1.5 Hz, 1H, H-6), 6.57 (s, 1H, H-3), 6.99 (d, J = 8.3 Hz, 1H, H-5′), 7.47 (dd, J = 2.2, 8.3 Hz, 1H, H-6′), 7.49 (d, J = 2.2 Hz, 1H, H-2′); ^{13}C NMR (125 MHz, acetone-d_6): δ 94.6, 99.6, 104.2, 105.3, 114.1, 116.6, 120.1, 123.8, 146.4, 150.0, 158.8, 163.4, 164.8, 165.1, 183.0. IR: 3421, 1651, 1598, 1498, 1443, 1362, 1265, 1161, 1120, 1030 cm^{-1}. HRMS (FAB+): m/z 287.0579 $(M+H)^+$; calc. for $C_{15}H_{11}O_6$: 287.0556. Elemental analysis suggests the hemihydrate form. Anal. Calcd for $C_{15}H_{10}O_6 + 0.5H_2O$: C 61.02, H 3.76; found: C 61.04, H 3.86. Its NMR spectral data were identical with those of an authentic sample (Tokyo Chemical Industry Co., Ltd., Catalog No. T2682).

2.8. 4′,5,7-Triacetoxyflavan-4-one (**2e**)

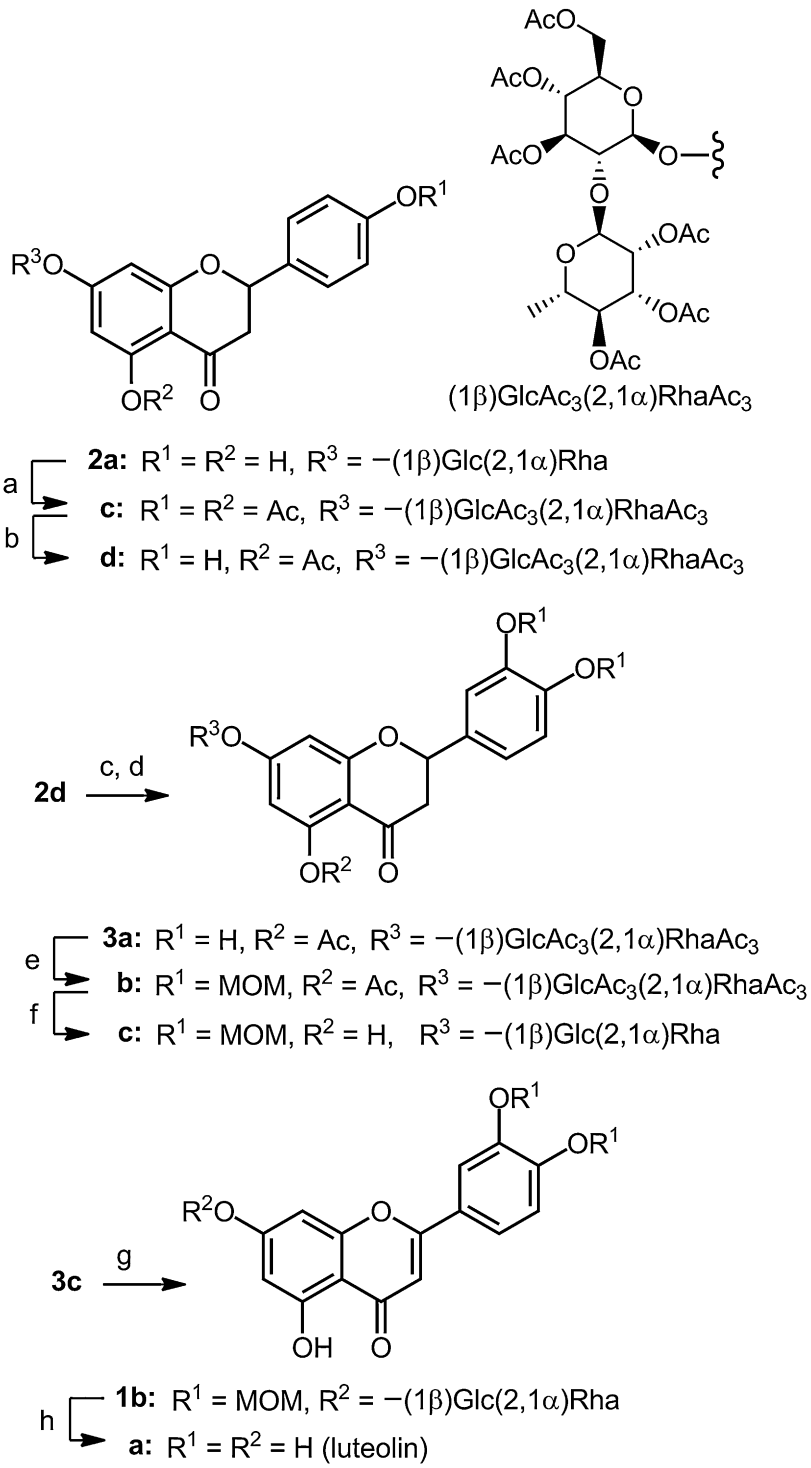
In a similar manner as described for the acetylation of **2a**, treatment of **2b** (100 mg, 0.37 mmol) with Ac_2O (1 mL) and pyridine (1 mL) gave **2e** (129 mg, 88%) as solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, 4′-OAc), 2.30 (s, 3H, 7-OAc), 2.36 (s, 3H, 5-OAc), 2.76 (dd, J = 2.8, 16.8 Hz, 1H, H-3a), 3.01 (dd, J = 13.6, 16.8 Hz, 1H, H-3b), 5.47, (dd, J = 2.8, 13.6 Hz, 1H, H-2), 6.52 (d, J = 2.4 Hz, 1H, H-8), 6.76 (d, J = 2.4 Hz, 1H, H-6), 7.13 (d, J = 8.8 Hz, 2H, H-2′, H-6′), 7.44 (d, J = 8.8 Hz, 2H, H-3′, H-5′).

2.9. C. antarctica lipase B-catalyzed transesterification of **2e**

In a similar manner as described for the transesterification of **2c**, a solution of **2e** (25.0 mg, 0.063 mmol) in cyclopentanol (2 mL) and cyclopentyl methyl ether (2 mL) was added Novozym 435 (50.0 mg). The mixture was stirred for 24 h at room temperature. After removal of insoluble materials by filtration with a pad of Celite, the filtrate was concentrated *in vacuo* to give a mixture of **2f** and **2g**.

2f: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, 7-OAc), 2.37 (s, 3H, 5-OAc), 2.71 (dd, J = 2.8, 16.8 Hz, 1H, H-3a), 3.02 (dd, J = 13.6, 16.8 Hz, 1H, H-3b), 5.37, (dd, J = 2.8, 13.6 Hz, 1H, H-2), 6.50 (d, J = 2.4 Hz, 1H, H-8), 6.74 (d, J = 2.4 Hz, 1H, H-6), 6.84 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.28 (d, J = 8.8 Hz, 2H, H-3′, H-5′).

2g (400 MHz, $CDCl_3$): ^{1}H NMR: δ 2.37 (s, 3H, 5-OAc), 2.67 (dd, J = 2.8, 16.4 Hz, 1H, H-3a), 2.98 (dd, J = 13.2, 16.4 Hz, 1H, H-3b), 5.35, (dd, J = 2.8, 13.2 Hz, 1H, H-2), 6.17 (d, J = 2.4 Hz, 1H, H-8), 6.29 (d, J = 2.4 Hz, 1H, H-6), 6.84 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.28 (d, J = 8.4 Hz, 2H, H-3′, H-5′).

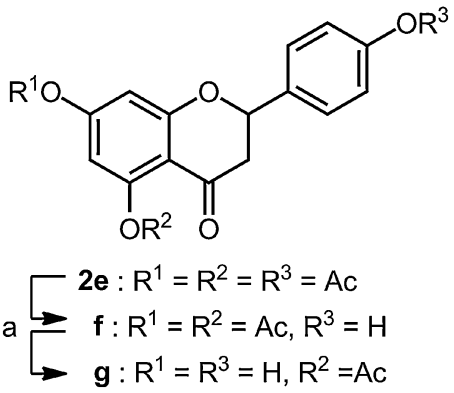


Scheme 1. Chemo-enzymatic transformation of naringin (**2a**) to luteolin (**1a**). Reagents and conditions: (a) Ac_2O, DMAP, pyridine; (b) *Candida antarctica* lipase B (Novozymes, Novozym 435), cyclopentanol, cyclopentyl methyl ether (CPME); (c) 2-iodoxybenzoic acid (IBX), $CHCl_3$—MeOH (4:1); (d) $Na_2S_2O_4$; (e) methoxymethyl (MOM) chloride, diisopropylethylamine (DIPEA), CH_2Cl_2; (f) K_2CO_3, MeOH; (g) I_2, pyridine and (h) conc. HCl, MeOH, THF.

3. Results and discussion

Toward this end, peracetylated form (**2c**) of naringin was treated with *C. antarctica* lipase B (Novozym 435) under transesterification conditions with cyclopentanol as nucleophile. The diminished steric hindrance in cyclopentanol compared with conventional secondary alcohol such as isopropyl alcohol is advantageous to generate higher reaction rate as nucleophile [11]. In contrast, primary alcohols are poor nucleophiles [9], because the transesterification products, primary acetates work as good acyl donors to cause the undesired reverse reaction catalyzed by the same lipase in the reaction mixture.

As expected by so far reported examples [9,10], the reaction was influenced by steric hindrance in the substrate, and proceeded highly regioselectively to give **2d** with only free hydroxy group at the desired C-4′ position (Scheme 1). The same result was obtained by the action of *Burkholderia cepacia* lipase (Amano PS-IM). Interestingly, no transesterification on primary acetate of glucose side chain



Scheme 2. Regioselectivity in lipase-catalyzed transesterification on peracetylated form of naringenin (**2e**). Reagents and conditions: (a) *C. antarctica* lipase B, cyclopentanol, CPME.

was observed, in contrast to the previous observation that primary position showed certain reactivity [12]. The acetylated rhamnopyranosyl group on the glucose was supposed to work as some kind of "cap" to prevent the transesterification on the oligosaccharide side chain.

For the introduction of 3′-OH, free phenol was oxidized with 2-iodoxybenzoic acid (IBX) [13–15] followed by the reductive workup with $Na_2S_2O_4$ to give **3a**. At this stage, the mixture of $CHCl_3$ and MeOH was superior to dimethyl sulfoxide (DMSO) as the solvent. As the yield in the removal of other acetyl protective groups, was unexpectedly low, then the resulting catechol moiety was temporary protected. While the diol withstood the protection as acetonide, the formation of bis(methoxymethyl) (MOM) ether proceeded smoothly to give **3b**. Prior to the dehydrogenation by the treatment with I_2 [16], all of the acetyl protective groups in **3b** were hydrolyzed to free form **3c**. The increase of hydrophilicity [16] was really advantageous for dehydrogenation, as the attempts for the dehydrogenation at the stage of acetate **3b** only resulted in the complex mixture. Finally, the remaining MOM protective groups and glycosidic bonds in **1b** were removed simultaneously under acidic conditions to give **1a** (Scheme 1).

In addition to the low price, the choice of naringin, the glycosylated form, as the starting material had another advantage, judging from the control experiment shown below. Triacetylated form (**2e**) of naringenin (**2b**), the aglycone, was treated under the same transesterification conditions as for **2c**. The cleavage of C-4′ acetate was predominantly occurred, but the cleavage of C-7 was accompanied to a certain extent to give the mixture of **2f** and **2g** (Scheme 2). In contrast, for naringin, the neohesperidosyl [–(1β)Glc(2,1α)Rha] disaccharide side chain worked as the efficient protective group in our approach.

4. Conclusion

Luteolin (**1a**) was efficiently prepared from abundantly available naringin (**2a**) in total 8 steps and 36% yield. "Protective group technology" involving lipase-catalyzed regioselective opening as well as the blocking with inherent disaccharide side chain on the proper hydroxy groups, in a polyphenol, was demonstrated. This approach would be applicable to the synthesis of flavanones, the saturated forms such as eriodictyrol and pinocembrin, from an abundant bioresource.

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