Simple Synthesis of Sakuranetin and Selinone via a Common Intermediate, Utilizing Complementary Regioselectivity in the Deacetylation of Naringenin Triacetate

Yasunobu Yamashita, Kengo Hanaya, Mitsuru Shoji, and Takeshi Sugai*

Department of Pharmaceutical Sciences, Keio University; 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan.
Received February 26, 2016; accepted March 21, 2016

Sakuranetin and selinone were successfully synthesized utilizing the regioselective deacetylation of naringenin triacetate. Deacetylation of the latter at C-7 with imidazole in 1,4-dioxane at 40°C furnished the corresponding diacetate in 80% yield. Methylation of the obtained free hydroxy group and subsequent removal of the remaining two acetyl groups gave sakuranetin, which was previously isolated as a phytoalexin against rice blast disease fungus, *Pyricularia oryzae*, in 71% overall yield. The same intermediate, naringenin triacetate, was subjected to transesterification with 2-propanol in tetrahydrofuran, catalyzed by *Candida antarctica* lipase B. A contrasting regioselective preference for C-4 deacetylation was observed, giving an isomeric diacetate in 82% yield. Prenylation of the free hydroxy group under Mitsunobu conditions and subsequent deprotection furnished selinone, which was previously isolated from *Monotes engleri* and exhibits antifungal activity against *Candida albicans*, in 55% overall yield.

Key words: flavanone; regioselective deprotection; lipase-catalyzed transesterification

Partially alkylated forms of flavanones are widespread in plants. Among them, sakuranetin (1b) (naringenin (1a) methylated at C-7) was first isolated from cherry tree bark as a sakuranin aglycone by Asahina in 1908,1) and its antifungal activity was reported later.2) Recently, its additional antimutagenic3) and antiinflammatory activities4) were also demonstrated. Agricultural biology and chemistry constitute other fields for the application of 1b. Kodama et al. isolated 1b from UV-irradiated rice leaves as a phytoalexin against the pathogenic fungus *Pyricularia oryzae*, which causes rice blast disease, and also detected 1b in rice leaves infected with *P. oryzae*.5)

Selinone (1c) (naringenin prenylated at C-4) was first isolated by Seshadri and Sood from *Selium vaginatum CLARKE*, an endemic high-altitude Indian medicinal plant of the Umbelliferae family, locally known as “Bhootkeshi.”6) Later, it was also isolated from *Monotes engleri*,7) a plant of the Dipterocarpaceae family in Zimbabwe, and showed antifungal activity against *Candida albicans* (Chart 1).

Sakuranetin (1b) has previously been synthesized by methylation of naringenin (1a), as shown in Chart 2. The formation of 1b in 87% yield by methylation of 1a with dimethyl sulfate in acetone was reported.8) The formation of the regioisomeric monoether 1d under the above condition was also reported.9) These results suggest that sakuranetin (1b) was isolated from a mixture of mono- and dimethylated products by silica gel column chromatography. Furthermore, it was shown that methylation of 1a by treatment with 1.5 eq of methyl iodide in N,N-dimethylformamide (DMF) led to the formation of not only 1b (76%) but also dimethyl ether 1e (3%).9) To selectively methylate the C-7 hydroxy group, which is the most acidic of the three, 1a was treated with diazomethane solution in diethyl ether under almost neutral conditions, to give 1b in 75% yield after silica gel column chromatography.10) This reaction, however, must be carried out at very low concentrations in diethyl ether (1.0 g/400 mL), due to the low solubility of 1a.

In order to address the solubility issue and reduce the amount of regioisomeric by-products, we altered the substrate of methylation from 1a to g, where the C-5 and C-4′ hydroxy groups were protected by acetyl groups. Acetylation of nar-
ingenin (1a) with acetic anhydride and pyridine furnished naringenin triacetate (1f) in 97% yield. Then, the obtained 1f was treated with imidazole\(^{(13)}\) in 1,4-dioxane at 40°C. The most electrophilic acetal at C-7\(^{(13)}\) was predominantly removed to furnish 1g in 80% yield. Treatment of 1g with diazomethane solution in a mixture of diethyl ether and dichloromethane, followed by subsequent short column chromatography, gave 1h in 95% yield. As expected from the greater lipophilicity of 1g compared with 1a, the reaction could be performed at a higher substrate concentration (400 mg/9 mL). Moreover, the methylation under conventional basic conditions by applying methyl iodide in the presence of potassium carbonate in DMF also furnished 1h in 95% yield. Removal of two acetyl groups in 1h by sodium methoxide in methanol gave 1b in 96% yield (Chart 3).

It should be noted that our present scheme established a more scalable synthesis of 1h. This methyl ether is a reported precursor for 7-methoxyapigenidin (2), which has been synthesized as an antifungal agent against sorghum fungi, *Gloeocercospora sorgi* \(^{11}\).

Selinone (1e) was prepared by a prenyl transferase (NovQ)-catalyzed prenylation of 1a.\(^{14}\) The prenylation, however, occurred mainly in the aromatic ring, at the carbon atom adjacent to C-3' (87%), and the O-prenylated 1e was obtained in as low as 11% yield. An efficient multistep synthesis of 1e was established by Antus and colleagues\(^{(15)}\) (Chart 4). In their first attempt, 3 and 4a were chosen as starting materials for the construction of the carbon skeleton of 1c. It turned out that the pre-installed prenyl group was unstable under the acidic conditions required for the methoxymethyl (MOM) group removal, such as the transformation of 5a to b. To address this issue, the prenyl group in 4a was substituted for a benzyl group in 4b. Furthermore, the MOM group in 1i was replaced with acetyl in 1j. Removal of the benzyl group by hydrogenolysis, prenylation under Mitsunobu conditions, and the final removal of all acetyl groups furnished 1e in 5% total yield over seven steps, starting from 3.

We next focused our interest on intermediate 1k, which has a free hydroxy group at C-4'. In order to prepare 1k effectively, regioselective deacetylation of 1f at C-4' is required, unlike in the case of 1g, where deacetylation occurs at C-7. We have already attempted the regioselective deacetylation of 1f, catalyzed by *Candida antarctica* lipase B (Novozym 435) in the presence of cyclopentanol as nucleophile and cyclopentyl methyl ether (CPME) as solvent. The first step led to the desired deprotection at C-4', however, this reaction was immediately followed by further deprotection at C-7 to give a mixture of 1k and m in 33 and 56% yield, respectively\(^{(46)}\) (Chart 5). The desired 1k was obtained as a minor product.

Our task was the improvement of the yield of 1k. For that purpose, 2-propanol and tetrahydrofuran (THF) were chosen as nucleophile and solvent, respectively,\(^{(77)}\) the nucleophilicity of 2-propanol being less than that of cyclopentanol. In addition, compared with the previous attempt, the catalyst loading was lowered by a factor of 20 [10 wt% vs. 200 wt%]. After incubation for 28 h at 22°C, the desired 1k was isolated in 82% yield. Interestingly, the three factor changes (cyclopentanol to 2-propanol, CPME to THF as solvent, and the lowering of the catalyst loading) cumulatively contributed to regioselectivity enhancement. Based on the present observation, the over-deacetylation at C-7 in our previous case would be rationalized by the swelling of the resin after immersion in CPME, which increased the effective concentration of the enzyme. Although 1f has one chiral center at C-2, kinetic resolution did not occur in the above-mentioned lipase-catalyzed transesterification, and the product 1k did not show any optical rotation.

Finally, according to the slight modification of reported procedure\(^{(45)}\) prenylation of the free hydroxy group at C-4' under Mitsunobu conditions and the subsequent removal of
the remaining acetates furnished 1c in 69% yield over two steps (Chart 6).

In summary, partially alkylated flavanones, namely sakuranetin (1b, 71% over four steps), a phytoalexin, and selinone (1c, 55% over four steps), an antifungal agent, were conveniently synthesized from a common inexpensive starting material, naringenin (1a). Contrasting regioselectivity in the deacetylation of 1f using imidazole (reaction at C-7 to give 1g) and C. antarctica lipase B (reaction at C-4 to give 1k) was a key factor for this success.

**Experimental**

**General** Melting points were measured on a Yanaco MP-J3 micromelting apparatus and are uncorrected. $^1$H-NMR spectra were measured at 400 MHz on a VARIAN 400-MR or at 500 MHz on an Agilent INOVA-500 spectrometers and $^{13}$C-NMR spectra were measured at 125 MHz on an Agilent INOVA-500 spectrometers. IR spectra were measured as at -tenuated total reflectance (ATR) on a Jeol Fourier transform (FT)-IR SPX60 spectrometer. High resolution (HR)-MS electrospray ionization (ESI+) Calcd for C$_{19}$H$_{16}$NaO$_7$ [M$^+$Na]$^+$ 379.0794. Found 379.0788. Its NMR spectrum was identical with that reported previously.$^{13}$

(±)-5,4′-Diacetoxy-7-methoxyflavan-4-one (5,4′-Diacetyl-7-methylnaringenin), 1h An in-situ formed solution of diazomethane in diethyl ether (6.5 mL), which was prepared from N-methyl-N-nitroso-p-toluene sulfonamide (1.125 g, 5.25 mmol), potassium hydroxide (KOH) (0.25 g), water (0.4 mL) and ethanol (1.25 mL) in a special distilling apparatus, was directly added dropwise to a solution of 1g (400.2 mg, 1.12 mmol) in CH$_2$Cl$_2$ (9 mL) at 0°C. The mixture was stirred for 20h at room temperature, and then concentrated in vacuo. The residue was purified by short column chroma-
tography to afford (±)-1h as a yellow solid (395.0 mg, 95%). mp 98.6–99.5°C. 1H-NMR (400 MHz, CDCl₃) δ: 2.30 (3H, s), 2.36 (3H, s), 2.71 (1H, dd, J=2.9, 16.8 Hz), 2.98 (1H, dd, J=13.3, 16.8 Hz), 3.81 (3H, s), 5.45 (1H, dd, J=2.9, 13.3 Hz), 6.27 (1H, d, J=2.7 Hz), 6.40 (1H, d, J=2.7 Hz), 7.13 (2H, d, J=8.4 Hz), 7.44 (2H, d, J=8.4 Hz). 13C-NMR (125 MHz, CDCl₃) δ: 21.1, 21.1, 45.0, 55.8, 79.0, 99.5, 104.8, 107.9, 122.1, 127.4, 136.0, 150.9, 151.9, 164.1, 165.5, 169.3, 169.5, 188.6. IR cm⁻¹: 2977, 1772, 1685, 1616, 1193, 1153. HR-MS (ESI⁺) Calcd for C₂₀H₁₈NaO₂ [M+Na⁺] 393.0950. Found 393.0951. Its NMR spectrum was identical with that reported previously.²⁵

Alternatively, to a solution of 1g (200.1 mg, 0.56 mmol) in DMF (2 mL) were added methyl iodide (42 µL, 0.67 mmol) and potassium carbonate (77.4 mg, 0.56 mmol) and the mixture was stirred for 17h at room temperature. The mixture was quenched with saturated aqueous NH₄Cl (2 mL) and the organic materials were extracted with AcOEt. The combined organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (50 g, hexane–AcOEt=3:1) to afford (±)-1h as a yellow solid (97.4 mg, 95%).

(±)-5,4'-Dihydroxy-7-methoxyflavan-4-one (Sakuranetin), 1b To a solution of 1f (50.0 mg, 0.14 mmol) in methanol (2 mL) was added 28% NaOMe in methanol (50 µL, 0.28 mmol) and the mixture was stirred for 2h at room temperature. The mixture was quenched with saturated aqueous NH₄Cl (5 mL), and the organic materials were extracted with AcOEt. The combined organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (1.0 g, hexane–AcOEt=3:1) to afford (±)-1b as a colorless solid (37.2 mg, 96%). mp 135.2–136.1°C. 1H-NMR (400 MHz, CDCl₃) δ: 2.76 (1H, dd, J=2.9, 17.0 Hz), 3.07 (1H, dd, J=13.1, 17.0 Hz), 3.79 (3H, s), 5.35 (1H, dd, J=2.9, 13.1 Hz), 6.02 (1H, d, J=2.4 Hz), 6.05 (1H, d, J=2.4 Hz), 6.86 (2H, d, J=8.6 Hz), 7.30 (2H, d, J=8.6 Hz). 13C-NMR (125 MHz, CDCl₃) δ: 43.1, 55.8, 79.0, 94.2, 95.0, 103.1, 115.6, 127.8, 130.5, 156.1, 162.9, 164.1, 168.0, 196.0. IR cm⁻¹: 3326, 1648, 1515, 1454, 1205, 1149, 1029. HR-MS (ESI⁺) Calcd for C₁₅H₁₄O₄Na [M+Na⁺] 358.0975. Found 358.0974. Its NMR spectrum was identical with that reported previously.⁵⁰

(±)-5,7-Diacectyl-4'-hydroxyflavan-4-one (5,7-Diacetyl naringenin), 1k To a solution of 1f (1.97 g, 4.94 mmol) in a mixture of 2-propanol (10 mL) and THF (20 mL), which was pre-dried with anhydrous Na₂SO₄ at room temperature overnight, was added an immobilized form of C. antarctica lipase B (Novozymes, Novozym 435, 192 mg). The mixture was stirred for 28h at 22°C. The mixture was filtered to remove insoluble materials with a pad of Celite. The precipitates were washed with AcOEt. The combined filtrate and washings were concentrated in vacuo. The residue was purified by silica gel column chromatography (40 g, hexane–AcOEt=1:1) to afford (±)-1k as a colorless solid (1.45 g, 82%). mp 113.9–114.8°C. 1H-NMR (400 MHz, CDCl₃) δ: 2.30 (3H, s), 2.39 (3H, s), 2.70 (1H, dd, J=2.6, 16.7 Hz), 3.00 (1H, dd, J=13.7, 16.7 Hz), 5.32 (1H, dd, J=16.7, 13.7 Hz), 6.52 (1H, d, J=2.4 Hz), 6.75 (1H, d, J=2.4 Hz), 6.82 (2H, d, J=8.7 Hz), 7.25 (2H, d, J=8.7 Hz). 13C-NMR (125 MHz, CDCl₃) δ: 21.1, 21.2, 44.8, 79.3, 109.3, 110.4, 115.6, 128.0, 129.7, 151.1, 155.9, 156.4, 156.6, 163.5, 168.3, 169.9, 190.0. IR cm⁻¹: 3382, 1770, 1683, 1508, 1373, 1157, 1016. HR-MS (ESI⁺) Calcd for C₁₅H₁₂O₄Na [M+Na⁺] 439.0794. Found 439.0790. Its NMR spectrum was identical with that reported previously.²⁵

Chart 6. Regioselectivity Improvement of the Deacetylation of 1f Catalyzed by C. antarctica Lipase B, and Its Application to the Synthesis of Selinone (1e)
Acknowledgments We thank Novozymes Japan and Zeon Corporation for generous gift of Novozym 435 and CPME, respectively. This work was supported by a Grant-in-Aid for Scientific Research (No. 26450143) and Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and is gratefully acknowledged with thanks.

Conflict of Interest The authors declare no conflict of interest.

References
1) Asahina Y., Arch. Pharm., 246, 259–272 (1908).
2) Atkinson P., Blakeman J. P., New Phytol., 92, 63–74 (1982).
3) Miyazawa M., Kinoshita H., Okuno Y., J. Food Sci., 68, 52–56 (2003).
4) Zhang X., Hung T. M., Phuong P. T., Ngoc T. M., Min B.-S., Song K.-S., Seong Y. H., Bae K., Arch. Pharm. Res., 29, 1102–1108 (2006).
5) Kodama O., Miyakawa J., Akatsuka T., Kiyosawa S., Phytochemistry, 31, 3807–3809 (1992).
6) Seshadri T. R., Sood M. S., Tetrahedron Lett., 1967, 853–855 (1967).
7) Garo E., Wolfender J.-L., Hostettmann K., Hiller W., Antus S., Mavi S., Helv. Chim. Acta, 81, 754–763 (1998).
8) Kim J., Park K.-S., Lee C., Chong Y., Bull. Korean Chem. Soc., 28, 2527–2530 (2007).
9) Narasimhachari N., Seshadri T. R., Proc. Ind. Acad. Sci., Sec. A, 30, 151–162 (1949).
10) Oyama K., Kondo T., J. Org. Chem., 69, 5240–5246 (2004).
11) Aida Y., Tamogami S., Kodama O., Tsukiboshi T., Biochim. Biophys. Acta, 60, 1495–1496 (1996).
12) Looker J. H., Holm M. J., Minor J. L., Kagal S. A., J. Heterocycl. Chem., 1, 253–256 (1964).
13) Kawamura T., Hayashi M., Mukai R., Terao J., Nemoto H., Synthesis, 44, 1308–1314 (2012).
14) Ozaki T., Mishima S., Nishiyama M., Kuzuyama T., J. Antibiot., 62, 385–392 (2009).
15) Kenéz A., Juhász L., Antus S., Heterocycl. Commun., 8, 543–548 (2002).
16) Kobayashi R., Itou T., Hanaya K., Shoji M., Hada N., Sugai T., J. Mol. Catal. B: Enzym., 92, 14–18 (2013).
17) Kobayashi R., Hanaya K., Shoji M., Umezawa K., Sugai T., Chem. Pharm. Bull., 60, 1220–1223 (2012).