SYNTHESIS OF β-SITOSTEROL-QUERCETIN DYADS

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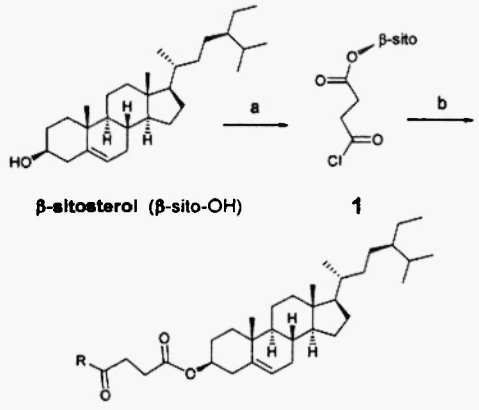
Abstract: Synthesis of novel β-sitosterol-quercetin dyads **6** and **10** as serum cholesterol lowering agent is described. These compounds were prepared in good yields by the reaction of β-sitosterol and succinic anhydride, followed by the activation of the resulting acid compound by thionyl chloride, and finally by esterification with appropriate protected quercetins.

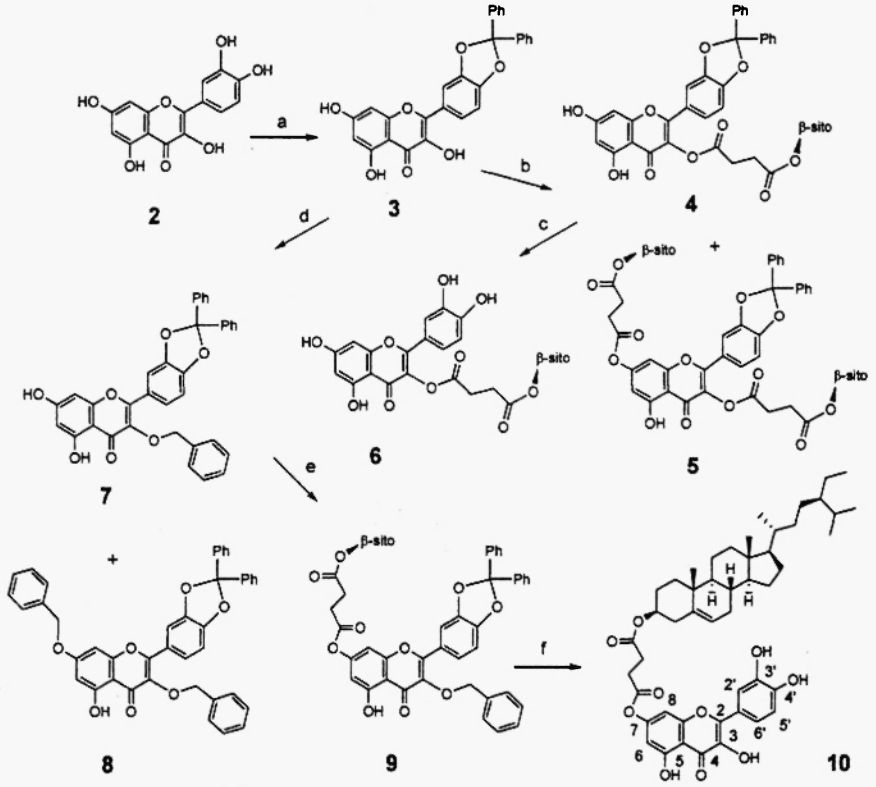
A major risk factor for the development of cardiovascular disease or atherosclerosis is elevated levels of serum cholesterol. Many clinical trials and studies have confirmed that lowering of cholesterol in blood is related to a reduction in cardiovascular disease risk.[1] Since cholesterol biosynthesis and intestinal cholesterol absorption are major regulators of serum cholesterol level, the reduction of serum cholesterol can be usually accomplished by inhibiting them. For instance, statin as HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A reductase) inhibitor is widely prescribed cholesterol biosynthesis inhibitor,[2] and ezetimibe is cholesterol absorption inhibitor to act by decreasing cholesterol absorption in the intestine.[3]

Phytosterols, or plant sterols, are an interesting class of compound due to their application and biological activities. The phytosterols found most frequently in nature are β-sitosterol, campesterol and stigmasterol. The representative β-sitosterol is known to have a hypocholesterolemic effect in both animal and human models by inhibiting the absorption of dietary and endogenously produced cholesterol from the small intestine.[4] The cholesterol lowering mechanism of β-sitosterol is attributed to an inhibitory effect on cholesterol absorption due to the chemical structure similarity of β-sitosterol with cholesterol.

On the other hand, flavonoids, such as flavanones and flavonols, are a large group of phenolic constituents. Among naturally occurring flavonoids quercetin has been shown to have a wide range of biological effects, including antioxidant, anti-bacterial, anti-inflammatory and anti-carcinogens.[5] Interestingly, it has been also reported recently to exhibit hypocholesterolemic activities by reducing serum triglycerides and cholesterol levels in high cholesterol-fed animals.[6] Based on the beneficial ability of naturally occurring β-sitosterol and quercetin to reduce cholesterol, we now report the synthesis of β-sitosterol-quercetin dyads aiming to get compounds that may behave as serum cholesterol lowering agent with low toxicity. Recently, we reported the synthesis and interesting pharmacological property of novel β-sitosterol succinamide derivatives that have the ability to lower serum cholesterol level in a rat model.[7] The β-sitosterol derivative **1** was prepared as shown Scheme 1 by the reaction of β-sitosterol and succinic anhydride in toluene with a catalytic amount of DMAP, followed by the activation of the resulting acid compound by thionyl chloride. The compound **1** was coupled with appropriate protected quercetin via esterification reaction. The synthetic steps for β-sitosterol-quercetin dyads are described in Scheme 2 in detail.



Scheme-1: Reagents and conditions: (a) (i) succinic anhydride, DMAP (cat.), toluene, reflux; (ii) $SOCl_2$, Et_3N, THF, rt; (b) RH (protected quercetin), pyridine, THF, -15 °C to rt.



Scheme-2: Reagents and conditions: (a) Ph_2CCl_2 (4 equiv), 190 °C; (b) **1**, pyridine, THF, -15 °C to 0 °C; (c) H_2, Pd/C, THF; (d) $PhCH_2Br$, pyridine, THF, -10 °C to rt; (e) **1**, pyridine, THF, rt; (f) H_2, Pd/C, THF.

Since quercetin **2** has five free hydroxyl groups, the reaction of quercetin with activated β-sitosterol **1** in the presence of pyridine in THF leaded, as expected, to the formation of a mixture of many products. For the synthesis of 3- and 7-*O*-β-sitostery quercetins, **6** and **10**, selective protection of the *O*-dihydroxyphenyl group in quercetin was investigated with a few protective reagents under various reaction conditions. Dichlorodiphenylmethane was the most effective protective group for the vicinal hydroxyl group, and protected quercetin **3** having

diphenylmethylene ketal was obtained by heating quercetin at 190°C for 15 minutes with 4 equiv. of dichlorodiphenylmethane without solvent.[8] The treatment of protected quercetin **3** with compound **1** at room temperature in the presence of pyridine in THF afforded a mixture of products, protected 3-*O*-β-sitosteryl quercetin **4** and 3,7-*O*-*di*-β-sitosteryl quercetin **5** in 45 and 33% yields, respectively. The ratio of two products was changed by the reaction temperature: at –15°C to 0°C, the ratio of **4** and **5** was 8:2, whereas, at reflux, the ratio was reversed by 3:7. Thus, product **4** could be synthesized selectively as a major product at the lower reaction temperature. The deprotection of purified compound **4** with hydrogen on Pd/C gave the final dyad product 3-*O*-β-sitosteryl hesperetin 6 in a 92% yield. For the synthesis of 7-*O*-β-sitosteryl quercetin **10**, additional protection of OH of C-3 position in protected quercetin **3** was needed. Selective benzylation of **3** with benzyl bromide at -10°C to room temperature gave protected 3-*O*-benzyl quercetin 7 in a 65% yield as a major product with 3,7-*O*-*di*-benzyl quercetin **8** of 8%. Protected 3-*O*-benzyl-7-*O*-β-sitosteryl hesperetin **9** could be obtained in a 54% yield by esterification of protected 3-*O*-benzyl quercetin **7** with **1** at room temperature in the presence of pyridine in THF. Finally, the deprotection of **9** with hydrogen on Pd/C also gave the target dyad product 7-*O*-β-sitosteryl quercetin **10** in a 90% yield.

Two β-sitosterol-quercetin dyads and other derivatives were purified by column chromatography using hexane/ethyl acetate (5:1 to 2:1) as eluent. They were fully characterized by MALDI-TOF-MS, ^{1}H and ^{13}C NMR spectra and elemental analyses.[9,10]

The position of the β-sitosteryl group in β-sitosterol-quercetin dyads was simply determined by changes in chemical shifts of *O*-β-sitosterylated quercetin compared to ^{13}C NMR spectra of free quercetin. In 7-*O*-β-sitosteryl quercetin **10**, for instance, the chemical shift of C-7 has changed from 164.7 ppm of quercetin to 157.3 ppm, while the signal of C-3 was not affected at 136.2 ppm. It was also unambiguously confirmed using one-dimensional (1D)–nuclear Overhauser effect (NOE) spectroscopy on the *O*-methylated analogue **10a** obtained by permethylation of **10** in mild condition (methyl iodide in DMF and K_2CO_3). The irradiation of methyl peaks showed increase of H-6, H-2' and H-5' resonance frequencies, while H-8 frequency was not affected, indicating that the methyl groups was located on the 3, 5, 3' and 4' positions. Consequently, the β-sitosteryl group was bound on OH of C-7 of quercetin. β-Sitosterol-quercetin dyads, **6** and **10**, were investigated on preliminary biological activity and found to lower the increased levels of total plasma cholesterol in a rat model using high-cholesterol and high-fat diet-feeding experiment.[7] The further study of the effect on plasma cholesterol lowering and the mechanism involved are currently under way.

Acknowledgements

We are grateful to Korea Research Foundation for financial support of this research (2007-C00184).

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9. Selected data for compound 6: pale yellow solid; mp 202-204°C; ^{1}H NMR (300 MHz, $DMSOd_6$) 7.34 (d, 1H, J = 2.1 Hz), 7.28 (dd, 1H, J = 8.3, 1.8 Hz), 6.90 (d, 1H, J = 8.4 Hz), 6.45 (d, 1H, J = 2.1 Hz), 6.22 (d, 1H, J = 2.1 Hz), 5.38 (dd, 1H, J = 4.0, 1.8 Hz), 4.44 (m, 1H), 3.78–0.60 (m, 51H); ^{13}C NMR ($DMSOd_6$) 174.7, 170.5, 169.5, 164.6, 161.1, 156.5, 155.7, 149.3, 145.5, 139.3, 129.5, 121.9, 120.6, 119.5, 115.8, 114.9, 106.9, 103.4, 99.0, 94.0, 73.5, 67.0, 66.6, 56.0, 55.5, 49.4, 45.1, 41.7, 37.5, 36.4, 36.0, 35.5, 33.3, 31.3, 30.4, 28.8, 28.7, 27.8, 27.1, 25.5, 23.5, 22.6, 19.6, 19.0, 18.8, 18.5, 11.7, 11.5; Anal. Calcd for $C_{48}H_{62}O_{10}$: C, 72.16; H, 7.82; O, 20.03. Found: C, 71.82; H, 8.13; MALTI-TOF MS calcd for $C_{48}H_{62}NaO_{10}$ $(M+Na)^+$: 821.4240. Found: 821.4668
10. Selected data for compound 10: pale yellow solid; mp 175-177°C; ^{1}H NMR (300 MHz, $CDCl_3$) 7.47 (d, 1H, J = 2.1 Hz), 7.40 (dd, 1H, J = 8.4, 1.8 Hz), 7.03 (d, 1H, J = 8.4 Hz), 6.84 (d, 1H, J = 1.8 Hz), 6.58 (d, 1H, J = 1.8 Hz), 5.38 (dd, 1H, J = 3.9, 1.8 Hz), 4.66 (m, 1H), 3.10–0.62 (m, 51H); ^{13}C NMR ($CDCl_3$) 175.2, 170.6, 169.2, 161.8, 157.3, 156.5, 155.5, 150.3, 145.4, 139.3, 136.2, 121.9, 120.6, 119.7, 115.8, 115.0, 108.4, 107.1, 105.2, 101.3, 73.5, 67.2, 66.6, 56.3, 55.5, 49.6, 45.4, 41.7, 37.3, 36.4, 36.0, 35.7, 33.5, 31.5, 30.2, 28.9, 28.7, 27.8, 27.3, 25.7, 23.5, 22.4, 19.8, 18.9, 18.6, 18.5, 11.9, 11.3; Anal. Calcd for $C_{48}H_{62}O_{10}$: C, 72.16; H, 7.82; O, 20.03. Found: C, 72.79; H, 8.15; MALTI-TOF MS calcd for $C_{48}H_{62}NaO_{10}$ $(M+Na)^+$: 821.4240. Found: 821.4934

Received on June 16, 2008.