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Hypocholesterolemic Activity of Hesperetin Derivatives

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Abstract—Hesperetin ester and ether derivatives possessing a long alkyl chain were synthesized for examining their hypocholesterolemic activities in high cholesterol-fed mice. Hesperetin 7-*O*-lauryl ether (**4b**) and hesperetin 7-*O*-oleyl ether (**4e**) exhibited strong cholesterol-lowering effects.

Flavonoids represent an important class of natural products found in fruits, vegetables, tea, and wine. The daily human intake of flavonoids was estimated to be about 2–3 g/day with a high dietary intake of herbs.[1] Flavonoids have shown a broad range of biological activities, including antiallergic,[2] antibacterial,[3] anti-inflammatory,[4] antimutagenic,[5] antioxidant,[6] and anticancer[7] effects. In the course of screening several flavonoids for the development of a hypocholesterolemic agent, hesperetin (**1**), aglycon of hesperidin, was found to show a significant cholesterol-lowering ability in cholesterol fed mice.[8] Our efforts toward the development of a potent hypocholesterolemic agent have focused on examining structure–activity relationship. We envisioned that introduction of a lipophilic group into hesperetin (**1**) may lead to a chance for exploring a new compound with increased activities. Herein, we describe the synthesis and biological evaluation of hesperetin derivatives possessing a long alkyl chain linked to the 7-hydroxyl position of hesperetin.

Ester or ether derivatives of hesperetin differing in length of the lipophilic chain connected to the 7-hydroxyl position of hesperetin (**1**) were synthesized. Ester derivatives were prepared by acylation of hesperetin with appropriate acid chlorides in the presence of triethylamine as shown in Scheme 1. Hesperetin 7-*O*-ester derivatives **2** were obtained as major products together with hesperetin 3′-*O*-ester compounds **3** as minors. On the other hand, ether derivatives of hesperetin were prepared by alkylation with alkyl bromide in *N,N*-dimethylformamide utilizing sodium carbonate as a base. Hesperetin 7-*O*-ether compounds **4** were yielded predominantly. The alkyl halides were formed by mesylation of the corresponding alcohol with methanesulfonyl chloride, followed by replacement of the mesyl group with sodium bromide.[9]

Several analogues of hesperetin were prepared for studying the relationship between the structure and the hypocholesterolemic activity, particularly the importance of the length of the lipophilic chain linked to the 7-hydroxy position of hesperetin. The synthesized compounds were tested for their cholesterol lowering activities in high cholesterol-fed C59BL/6J mice. The plasma total cholesterol levels were measured after feeding a high cholesterol diet supplemented with 0.05% (wt/wt in diet) of the test compounds for 10 days (Table 1).[10] All synthesized compounds except **2a** and **4a**, showed the increased cholesterol-lowering effects in comparison with hesperetin (**1**). The introduction of a lipophilic chain at the 7-hydroxyl position of hesperetin strongly affected the cholesterol lowering activity, and C-12 was the best length for the activity. The hesperetin derivatives possessing a short alkyl chain, **2a** and **4a**, showed a weak activity as compared to those possessing a long alkyl chain. In general, ether derivatives **4** of hesperetin (**1**) showed more potent activities than ester derivatives **2**. Interestingly, it was found that the presence of unsaturation in a lipophilic chain enhanced significantly the

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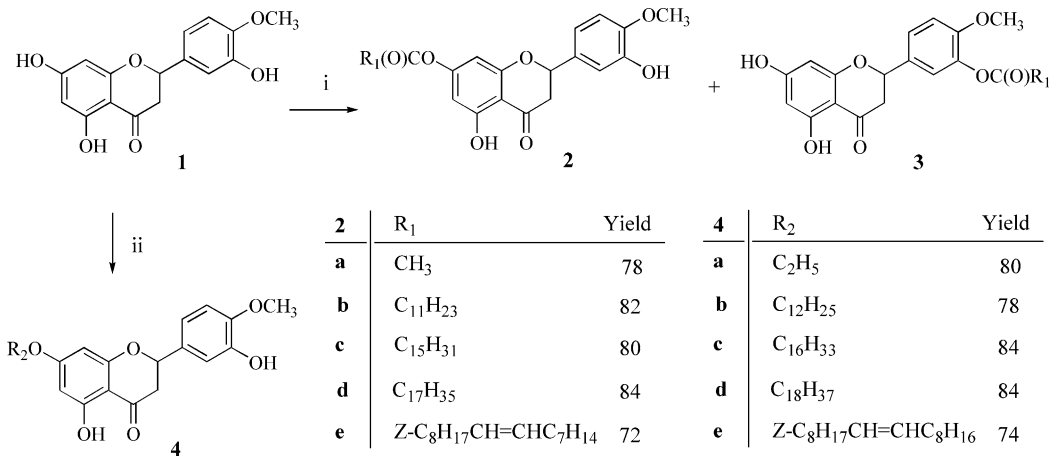

2	R_1	Yield	4	R_2	Yield
a	CH_3	78	a	C_2H_5	80
b	$C_{11}H_{23}$	82	b	$C_{12}H_{25}$	78
c	$C_{15}H_{31}$	80	c	$C_{16}H_{33}$	84
d	$C_{17}H_{35}$	84	d	$C_{18}H_{37}$	84
e	Z-$C_8H_{17}CH{=}CHC_7H_{14}$	72	e	Z-$C_8H_{17}CH{=}CHC_8H_{16}$	74

Scheme 1. Reagents and reaction conditions: (i) R_1COCl, Et_3N, 0 °C; (ii) R_2Br, Na_2CO_3, DMF.

Table 1. Effects of hesperetin derivatives on plasma total cholesterols in high cholesterol-fed mice

Group	*N*	Body weight (g)[a]		Total cholesterol (mg/dl)[b]	
		0 day	10 day	0 day	10 day
Control	10	24.8±1.7	24.9±1.5	98±10	223±29
1	6	25.4±1.5	25.6±2.3	103±7	216±13
2a	6	23.4±0.6	23.7±0.5	96±11	220±10
2b	6	22.4±2.4	22.4±3.4	95±4	192±15
2c	6	21.7±1.5	23.4±1.2	97±11	209±11
2d	6	22.3±0.3	23.7±1.2	95±6	214±14
2e	6	22.3±0.3	23.8±0.6	96±7	196±10
4a	6	21.9±1.0	22.5±2.9	102±5	218±15
4b	6	22.0±2.1	22.4±2.6	95±5	189±16
4c	6	22.5±0.7	23.0±1.5	93±8	202±15
4d	6	23.1±1.0	24.4±1.0	101±4	208±20
4e	6	23.4±0.6	23.7±0.5	96±11	192±18

[a]All values are expressed as mean±SD.
[b]Mean±SD; all values are significantly different ($p<0.05$) from control group.

activity as shown in compounds **2e** and **4e** (**2d** vs **2e**, **4d** vs **4e**). Among the tested compounds, hesperetin 7-*O*-ether analogues having lauryl and oleyl moieties (**4b**[11] and **4e**[12]) exhibited strong cholesterol-lowering activities.

In conclusion, hesperetin 7-*O*-ester and hesperetin 7-*O*-ether derivatives possessing a long alkyl chain were synthesized and their hypocholesterolemic activities were evaluated. The lipophilic group linked at the 7-hydroxyl group of hesperetin influenced the activities and the length of C-12 showed the most potent activities. Further detailed evaluations of hesperetin 7-*O*-lauryl ether (**4b**) and hesperetin 7-*O*-oleyl ether (**4e**) showing strong hypocholesterolemic activities are in progress.

Acknowledgements

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8. (a) Hesperetin (1) was reported to show cholesterol-lowering effects in rats fed a high cholesterol diet: Lee, S. H.; Park, Y. B.; Kwon, Y. K.; Choi, M. S.; Bok, S. H.; Jeong, T. S. *Nutr. Res.* **1999**, *19*, 1245. (b) Kim, H. K.; Jeong, T. S.; Lee, M. K.; Park, Y. B.; Choi, M. S. *Clin. Chimi. Acta* **2003**, *327*, 129.
9. All newly synthesized compounds gave satisfactory spectral data.
10. The hypocholesterolemic effects of the synthesized compounds were investigated in male C57BL/6J mice maintained at Korea Research Institute of Bioscience and Biotechnology (Taejon, Korea). The mice were housed in a room with controlled temperature (22±2 °C), relative humidity (55±5%), and lighting (alternating 12 h cycle of light and dark). At 8 weeks of age, six animals were randomly assigned to a group, and fed a high cholesterol diet (CRF-1 supplemented with 15% fat, 1.25% cholesterol, and 0.5% Na-cholate, Oriental Yeast Co. Ltd., Japan) without additional supplement (control), or a high cholesterol diet supplemented with 0.05% (wt/wt in diet) of the test compounds (experimental group). The diet and water were given ad libitum. After treating the test compounds for 10 days, the mice were anesthetized with ethyl ether, and the blood was obtained from the retro-orbital sinus using a heparinized capillary tube. Then, the blood was centrifuged at 8000*g* for 10 min, and the plasma was collected. The concentration of plasma total cholesterol was measured with an automatic blood chemical analyzer (CIBA Corning, OH, USA). To evaluate statistical significance between control and experimental groups, Student's *t*-test was performed, and a *p* value of 0.05 was considered to be statistically significant.
11. **4b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 7.04 (d, J=2.0 Hz, 1H), 6.93 (dd, J=8.4, 2.0 Hz, 1H), 6.88 (d, J=8.0 Hz, 1H), 6.05 (d, J=2.0 Hz, 1H), 6.03 (d, J=2.8 Hz, 1H), 5.32 (dd, J=12.8, 2.8 Hz, 1H), 3.95 (t, J=6.4 Hz, 2H), 3.91 (s, 3H),

3.07 (dd, $J=17.2$, 2.8 Hz, 1H), 2.77 (dd, $J=17.2$, 2.8 Hz, 1H), 1.75 (m, 2H), 1.26 (m, 18H), 0.88 (t, $J=6.4$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.1, 167.8, 164.3, 163.0, 147.2, 146.1, 131.8, 118.4, 112.9, 110.8, 103.2, 95.8, 94.8, 79.1, 68.8, 56.3, 43.4, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.1, 26.1, 22.9, 14.4.

12. **4e**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.00 (s, 1H), 7.02 (d, $J=2.0$ Hz, 1H), 6.91 (dd, $J=8.3$, 2.0 Hz, 1H), 6.89 (d, $J=8.3$ Hz, 1H), 6.03 (d, $J=2.2$ Hz, 1H), 6.01 (d, $J=2.2$ Hz, 1H), 5.70 (s, 1H), 5.32 (m, 3H), 3.93 (t, $J=6.6$ Hz, 2H), 3.90 (s, 3H), 3.05 (dd, $J=17.1$, 3.0 Hz, 1H), 2.76 (dd, $J=17.1$, 3.0 Hz, 1H), 1.98 (m, 3H), 1.72 (m, 2H), 1.27 (m, 23H), 0.86 (t, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.8, 167.6, 164.0, 162.8, 147.0, 145.9, 131.6, 130.0, 129.8, 118.1, 112.6, 110.6, 103.0, 95.5, 94.6, 78.9, 68.5, 56.0, 43.2, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.8, 27.2, 27.1, 25.8, 22.6, 14.1.