Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3901–3903

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Naringenin Derivatives as Anti-atherogenic Agents

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Received 3 June 2003; revised 4 September 2003; accepted 6 September 2003

Abstract—Two classes of naringenin derivatives were evaluated for anti-atherogenic activity. Naringenin 7-*O*-oleic ester (**2**) and naringenin 7-*O*-cetyl ether (**3**) inhibited the formation of aortic atherosclerotic lesions in high cholesterol-fed rabbits.

Diverse biochemical properties of flavonoids including naringin, hesperidin, diosmin, and rutin have provoked interest in biology and medicinal chemistry.[1] These compounds have exhibited a broad range of biological and pharmacological activities such as antioxidant,[2] anti-allergic,[3] antibacterial,[4] anti-inflammatory,[5] anti-mutagenic,[6] and anticancer[7] effects. In our efforts to exploit a compound with effective anti-atherogenic activities from citrus flavonoids, naringenin (**1**), aglycon of naringin, was found to inhibit the formation of aortic atherosclerotic lesions in rabbits fed a high cholesterol diet.[8] In addition, our previous studies showed that naringenin and its derivative exhibited hypocholesterolemic activities by reducing cholesterol biosynthesis and cholesterol esterification in high cholesterol-fed rats.[9] In continuation of our studies aimed toward the development of a potent anti-atherogenic agent, two types of naringenin derivatives, ester compound **2** and ether compound **3**, were prepared for further biological evaluation. Herein we describe the anti-atherogenic effects of naringenin 7-*O*-oleic ester (**2**) and naringenin 7-*O*-cetyl ether (**3**) by measuring aortic atherosclerotic lesions in high cholesterol-fed rabbits.

Naringenin 7-*O*-oleic ester (**2**) was prepared by acylation of naringenin (**1**) with oleoyl chloride in the presence of triethylamine. Naringenin 7-*O*-cetyl ether (**3**) was prepared by alkylation of naringenin (**1**) with cetyl bromide in *N*,*N*-dimethylformamide utilizing sodium carbonate as a base (Scheme 1).[10,11]

Atherosclerosis is a progressive disease characterized by the accumulation of occlusive lesions in the large arteries leading to various complications. The anti-atherogenic effects of the compounds **2** and **3** were evaluated by measuring the regression of aortic fatty streak lesions in high cholesterol-fed rabbits. The lesions in the aorta were measured after feeding a high cholesterol diet supplemented with 0.1% (wt/wt in diet) of the test compounds for 8 weeks.[12] The fatty streak lesions of thoracic aorta in each group were easily identified by staining with oil red O. Broad fatty streak lesions were found in control rabbits supplemented with the 1% cholesterol diet alone, whereas small plaques were sparsely observed in the groups supplemented with compounds **2** and **3**. The percentage area of occupied by atherosclerotic lesions on the inner surface between the first and sixth intercostal arteries was significantly reduced in the compound **2**-supplemented rabbits ($21.3 \pm 6.9\%$, $P < 0.001$) and compound **3**-supplemented rabbits ($11.5 \pm 4.9\%$, $P < 0.001$) compared to control group ($62.3 \pm 8.5\%$) (Fig. 1). Compound **3** inhibited the formation of aortic atherosclerotic lesions more effective than compound **2**. In addition, the plasma lipid levels were analyzed after administration of the test compounds **2** and **3** for 8 weeks (Table 1).[12] Lee et al. showed that compound **2** reduced cholesterol biosynthesis through the inhibition of hepatic HMG-CoA reductase in high cholesterol-fed rats, resulting in a decreased plasma total cholesterol and hepatic cholesterol level.[9] In this study, the plasma total cholesterol levels showed no significant difference between groups during the experimental period and had increased to almost 1100 mg/dl in all groups, and so hypocholesterolemic effects of dietary **2** and **3** associated HMG-CoA

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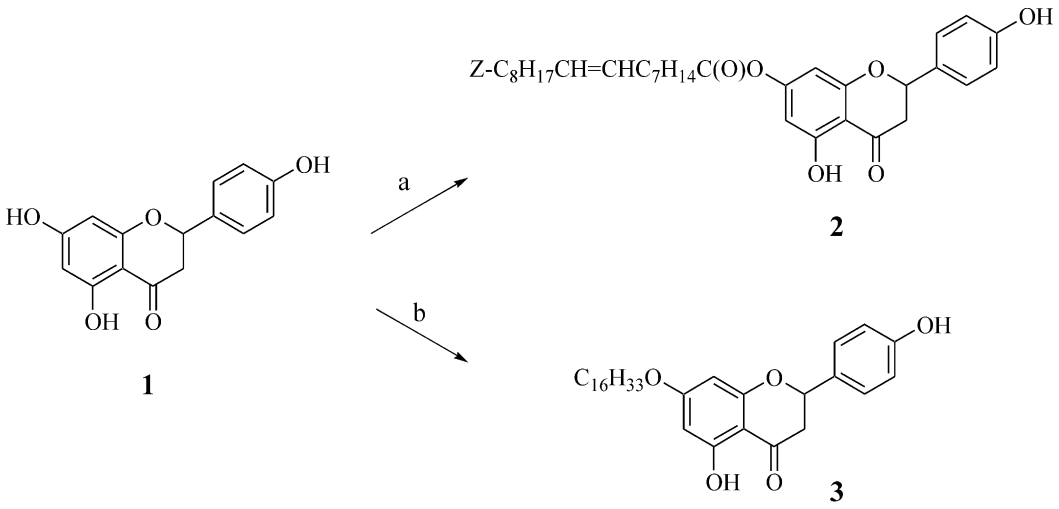
doi:10.1016/j.bmcl.2003.09.009



Scheme 1. (a) Z-$C_8H_{17}CH{=}CHC_7H_{14}COCl$, Et_3N, CH_2Cl_2; (b) $C_{16}H_{33}Br$, Na_2CO_3, DMF, 80 °C.

Table 1. Effects of compounds **2** and **3** on the plasma lipids in high cholesterol-fed rabbits

Groups	*N*	Plasma lipids (mg/dl)[a]					
		Total cholesterol		HDL-cholesterol		Triglyceride	
		0 weeks	8 weeks	0 weeks	8 weeks	0 weeks	8 weeks
Control	10	49±12	1087±182	29±10	92±23	47±15	117±343
Compound **2** (0.1%, wt/wt diet)	10	51±10	1035±175	31±12	78±26	51±13	105±26
Compound **3** (0.1%, wt/wt diet)	10	53±9	1057±217	34±10	93±34	50±12	85±19

[a]All values are expressed as mean±SD (mg/dl). No significant differences were observed among groups.

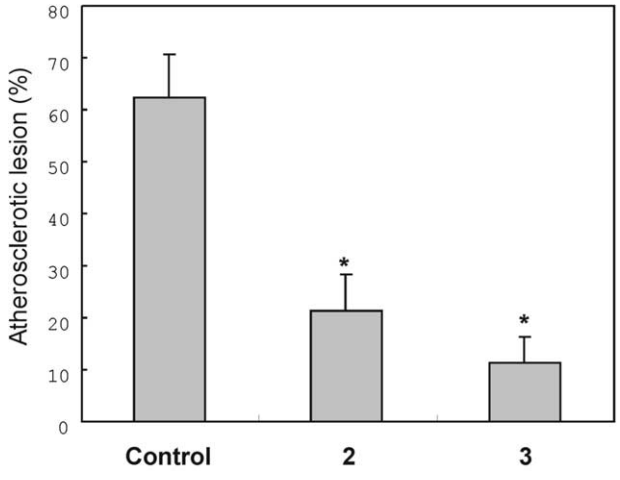


Figure 1. Effects of compounds **2** and **3** on the aortic fatty streak formations in rabbit model fed a high cholesterol diet for 8 weeks. A graph of atherosclerotic lesion size expressed as a percentage of the oil red-O positive area/measured internal surface in each group. Bars represent standard deviations. * is significantly different ($p<0.001$) from control group.

reductase could not observed in 1% high cholesterol-fed rabbits. The triglyceride and HDL cholesterol levels of groups supplemented with compounds **2** and **3** were also not significantly different from those of control group.

In conclusion, naringenin 7-*O*-oleic ester (**2**) and naringenin 7-*O*-cetyl ether (**3**) significantly inhibited the formation of aortic fatty streak in high cholesterol-fed rabbits. This anti-atherogenic effect of **2** and **3** may not associated with plasma lipid levels. Further studies on mechanistic aspects for anti-atherogenic effects of **2** and **3** are underway.

Acknowledgements

This work was supported by a grant from the Ministry of Science and Technology (M1-0302-00-0033), Korea.

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10. Compound **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.90 (s, 1H), 7.28 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.28 (d, J = 2.0 Hz, 1H), 6.26 (d, J = 2.0 Hz, 1H), 5.38–5.31 (m, 3H), 3.11 (dd, J = 17.2, 13.2 Hz, 1H), 2.80 (dd, J = 17.2, 2.8 Hz, 1H), 2.53 (t, J = 7.6 Hz, 2H), 2.00 (m, 4H), 1.71 (quint, J = 7.2 Hz, 2H), 1.40–1.20 (m, 22H), 0.87 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.2, 171.4, 163.1, 162.3, 158.3, 156.3, 130.0, 129.7, 129.6, 127.8, 115.6, 106.1, 103.1, 101.7, 79.0, 43.3, 34.4, 31.9, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 29.0, 28.9, 27.2, 27.1, 24.8, 22.7, 14.2; 1D NOESY: NOE contacts were observed between H (6.28 and 6.26 ppm) and H (2.53 ppm).

11. Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.0 (s, –OH), 7.32 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.04 (d, J = 2.0 Hz, 1H), 6.02 (d, J = 2.0 Hz, 1H), 5.33 (dd, J = 13.2, 2.8 Hz, 1H), 5.09 (s, –OH), 3.95 (t, J = 6.8 Hz, 2H), 3.07 (dd, J = 17.2, 13.2 Hz, 1H), 2.77 (dd, J = 17.2, 2.8 Hz, 1H), 1.75 (quint, J = 6.8 Hz, 2H), 1.44–1.36 (m, 2H), 1.34–1.22 (m, 24H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.8, 167.6, 164.0, 162.8, 156.0, 130.6, 127.9 (2C), 115.6 (2C), 103.0, 95.6, 94.6, 78.9, 68.6, 43.2, 32.0, 29.75–29.69 (six carbons, $-(CH_2)_6-$), 29.62, 29.57, 29.4, 29.3, 28.9, 25.9, 22.7, 14.2; 1D NOESY/NOE contacts were observed between H (6.04 and 6.02 ppms) and H (3.95 ppm); FABMS m/z 497 $[M+H]^+$ (100), 495 (44), 153 (85), 147 (39); HRFABMS m/z found for 497.3267 (calcd for $C_{31}H_{45}O_5$ 497.3267).

12. Male New Zealand White (NZW) rabbits weighing between 2.4 and 2.5 kg at the age of 3 months were used in the experiment. The rabbits were divided into three groups, which were supplemented with a 1% cholesterol diet (RC4, Oriental Yeast Co. Ltd, Tokyo, Japan; n = 10), or a 1% cholesterol diet containing either 0.1% **2** (n = 10) or 0.1% **3** (n = 10) for 8 weeks. All rabbits were individually caged and maintained in a controlled facility at 20 ± 2 °C , relative humidity (55 ± 5%) and a strict 12 h light/dark cycle. First, for the analysis of plasma lipids, the blood samples (3 mL), with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, were obtained from the marginal vein of the ear, and centrifuged at 8000g for 10 min. Collected plasma was analyzed in an automatic blood chemical analyzer (Hitachi 7020, Japan) and the plasma concentrations of total cholesterol, HDL-cholesterol, and triglyceride obtained (Table 1). Then, for the evaluation of aortic fatty streak lesions, all rabbits were anesthetized with thiopental sodium (Choongwae Pharma Co., Seoul, Korea) and sacrificed by exsanguinations from the femoral artery. Immediately after opening the thoracic cavity, the aorta was excised, and adventitial tissue grossly adhering to the aorta removed. The aorta was then dissected longitudinally. The portion, a segment between the first and the sixth intercostal arteries, was fixed in 10% neutral buffered formalin for 1 day. The aorta was then placed in absolute propylene glycol for 2 min and stained with oil red O for 4 h. After washing, the extent of the oil red O-positive area was measured and expressed as a percentage of the internal surface using a computer-assisted morphometry system (Image Pro Plus, MD, USA).