Effect of a New β-Sitosterol Analogue on Plasma Lipid Concentrations in Rats[1)]

Yang-Heon Song,*[,a] Soonil Hong,[a] Hongsan Lim,[a] Jinmoo Seo,[a] Sungjoo Chung,[a] Insook No,[a] Kyunghee Lee,[a] and Michung Yoon[b]

[a] *Department of Chemistry, Mokwon University; and* [b] *Department of Biology, Mokwon University; Daejeon 302–729, Korea.* Received August 14, 2003; accepted February 2, 2004

***N*-Substituted succinamic acid β-sitosteryl ester derivatives were prepared and evaluated. Compounds 1 and 2 were prepared in 76—92% yields by the reaction of β-sitosterol and succinic anhydride, followed by the activation of the resulting acid compound 1 by thionyl chloride or methyl chloroformate, and finally by amination with appropriate amines. Compound 2a (DANA87) was also easily obtained in one step by the direct addition of β-sitosterol to dicyclohexylcarbodiimide (DCC) in 80% yield. Administration of the dietary compound DANA87 resulted in significant decreases in total plasma cholesterol (TC) and low-density lipoprotein (LDL) cholesterol concentrations compared with controls in a rat model. High-density lipoprotein cholesterol and plasma triglyceride levels were not affected. These findings indicate that DANA87 functions as TC and LDL cholesterol-reducing agent.**

Key words cholesterol-reducing agent; β-sitosteryl ester derivative; plasma lipid concentration

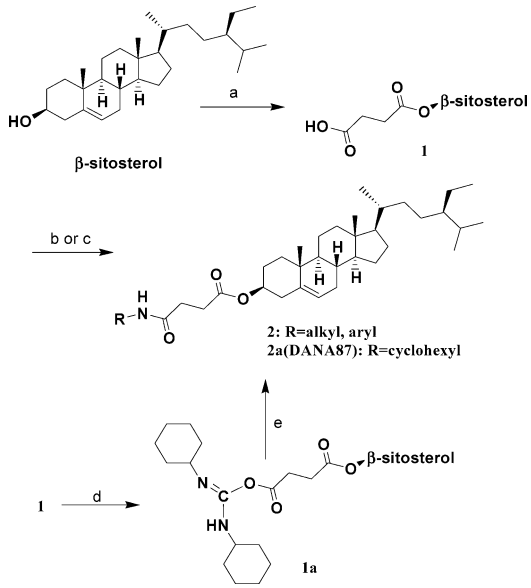
A major risk factor for the development of coronary heart disease (CHD) or atherosclerosis is elevated levels of serum cholesterol. Recent clinical trials and studies have confirmed that lowering of low-density lipoprotein (LDL) cholesterol is related to a reduction in CHD risk.[2,3)] The reduction of serum cholesterol levels has mainly been accomplished by inhibiting cholesterol biosynthesis or by blocking the absorption of dietary cholesterol. Statins, such as pravastatin, that effectively inhibit cholesterol biosynthesis are HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A reductase) inhibitors and are widely prescribed in the treatment of hypercholesterolemia.[4,5)] Several squalene synthesis inhibitors have been also reported.[6,7)] SCH-48461 (a *trans*-azetidinone)[8)] and 4″,6″-bis((2-fluorophenyl)carbamoyl) β-*O*-cellobioside (a steriodal glycoside)[9)] have recently been reported to be cholesterol absorption inhibitors.

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. It has been known that they inhibit the absorption of dietary and endogenously produced cholesterol from the small intestine, reducing blood cholesterol concentrations both in animal models and in humans.[10—13)] β-Sitosterol and β-sitostanol fatty acid esters were recently commercialized as food additives to reduce serum cholesterol levels. A novel hydrophilic phytostanol analogue, FM-VP4, was also developed to reduce serum cholesterol levels.[14)]

However, even with the current therapeutic agents, it is not easy to achieve proper plasma cholesterol levels without side effect. Therefore the discovery of more effective, well-tolerated agents that reduce high cholesterol levels is needed. We here report the synthesis and interesting pharmacologic properties of novel succinamic acid ester derivatives that are β-sitosterol analogues, compounds **1** and **2**, that have the ability to lower plasma cholesterol levels and that can be more effective cholesterol-lowering alternatives to β-sitosterol and β-sitostanol fatty acid esters.

Chemistry

The synthesis of β-sitosterol analogues **1** and **2** are summarized Chart 1. The β-sitosterol-3-hemisuccinate ester **1** was prepared by the reaction of β-sitosterol and succinic anhydride in toluene with a catalytic amount of DMAP.[15)] The chlorination of the resulting acid compound **1** with $SOCl_2$ and then direct amination with appropriate amines afforded *N*-substituted succinamic acid β-sitosteryl ester derivatives **2**. Compounds **2** were also synthesized in 76—92% yields by the reaction of **1** with methyl chloroformate, followed by amination of the activated intermediate. Interestingly, compound **2a** (**DANA87**) was also easily obtained in one step by the direct addition of β-sitosterol to dicyclohexylcarbodiimide (DCC) in 80% yield. It was considered that compound **DANA87** was prepared by slow thermal degradation of the



Reagents and conditions: (a) succinic anhydride, DMAP, toluene, reflux; (b) $SOCl_2$, rt and RNH_2, TEA, THF, reflux; (c) methyl chloroformate, TEA, Et_2O, rt and RNH_2, TEA, THF, reflux; (d) DCC, toluene, reflux, 2 h; (e) toluene, heat, 24 h

Chart 1

* To whom correspondence should be addressed. e-mail: yhsong@mokwon.ac.kr

β-sitosterol-DCC adduct **1a** that was initially formed, followed by the spontaneous formation of the corresponding amide compound. For a large-scale reaction, the procedure using methyl chloroformate gives increased yields compared with the route with $SOCl_2$, because an undesirable side reaction like breakage of the ester bond in **DANA87** could occur in the reaction using $SOCl_2$.

Results and Discussion

In a preliminary biological experiment on compounds **2**, **2a** (**DANA87**) was much more effective than β-sitosterol itself and other compounds **2** and found to lower the increased levels of plasma cholesterol potently in a rat model. Rats were fed a hyperlipidemic diet (1% cholesterol and 0.5% cholic acid) containing 1% of each compounds **2** or β-sitosterol for 1 week. The percentage by which the total plasma cholesterol (TC) level was lowered compared with the control group is shown in Fig. 1. Therefore, to observe the long-term effect of the most potent compound **DANA87** on lowering of the TC level, rats were divided into 4 groups and fed the hyperlipidemic diet (1% cholesterol and 0.5% cholic acid) containing various concentrations (control group 0%; experimental groups 0.1%, 0.5%, 1%) of **DANA87** for 1—4 weeks. The TC levels and the percentage by which cholesterol was lowered in the bloods of rats are shown in Figs. 2 and 3, respectively.

TC levels were reduced in a dose-dependent manner with **DANA87** administration. The TC levels in the 0.1% and 0.5% **DANA87** groups were lowered by about 19% and 1 % at 1 week and by 30% and 35% at 2 weeks, respectively. At 3 and 4 weeks when the TC level was also reduced in the control group fed only the hyperlipidemic diet, it was lowered by 17% and −3%, respectively, in the 0.1% **DANA87** group, and also by 26% and 24% in the 0.5% **DANA87** group, respectively.

The slight decrease in the TCs in the low-dose **DANA87** groups at 3—4 weeks compared with 1—2 weeks might have been due to a physiologic feedback regulation against hypercholesterolemia induced by the hyperlipidemic diet, as shown in control group.

The TC level in the 1% **DANA87** group was markedly and continuously lowered by 42% at 1 week and by 64% at 4 weeks. This suggests strongly that a high blood concentration of **DANA87** is needed to show an efficient pharmacologic effect. The level in the 1% **DANA87** group significantly and

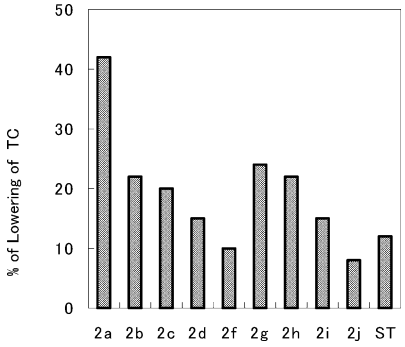


Fig. 1. Lowering (%) of Total Plasma Cholesterol (TC) Levels by Compounds **2** or β-Sitosterol (ST) Compared with Controls

Rats were fed hyperlipidemic powder diets containing 0 (control) or 1% of each of compounds **2** or β-sitosterol (ST), respectively, for 1 week (n=5).

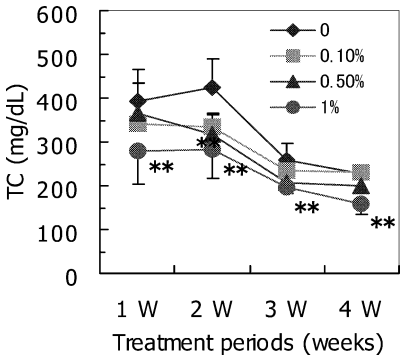


Fig. 2. Effect of **DANA87** on the Total Plasma Cholesterol (TC) Level in the Blood of Rats

Rats were fed hyperlipidemic powder diets containing 0 (control), 0.1, 0.5, or 1% of **DANA87** for 1 to 4 weeks (n=10). ** p< 0.01.

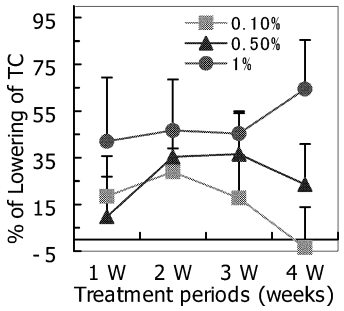


Fig. 3. Lowering (%) of TC by **DANA87** in the Blood of Rats

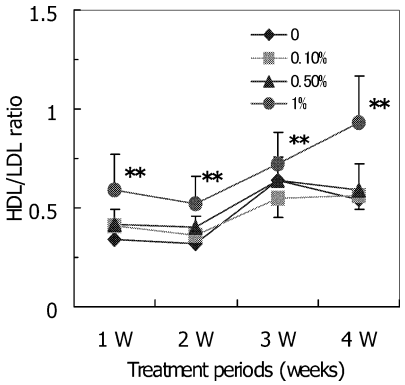


Fig. 4. Effect of **DANA87** on the Ratio of HDL to LDL in the Blood of Rats Fed a Hyperlipidemic Diet Containing Varying Concentrations of **DANA87** for 1 to 4 Weeks

Rats were fed hyperlipidemic powder diets containing 0 (control), 0.1, 0.5, or 1% of **DANA87** for 1 to 4 weeks (n=10). ** p<0.0001.

gradually increased from 1 week to 4 weeks. It was also found that the high-density lipoprotein (HDL) cholesterol level changed little, but the low-density lipoprotein (LDL) cholesterol level was markedly decreased in a dose-dependent manner with **DANA87** administration. The ratio of HDL/LDL cholesterol was also investigated, as shown in Fig. 4.

To examine the effect of **DANA87** in normal rats, another group of rats was fed a normal powder diet containing only 1% **DANA87** for 1 week. The TC, HDL and triglyceride (TG) levels after **DANA87** administration changed little compared with control group, as shown in Fig. 5, but the LDL cholesterol level was decreased, which increased the

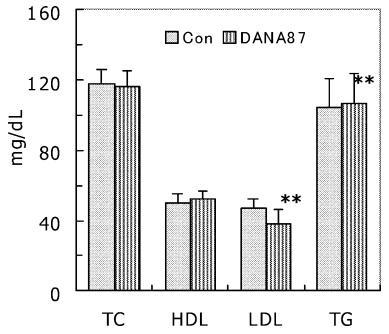


Fig. 5. Effect of **DANA87** on the Levels of TC, HDL, LDL, and TG in Rats Fed a Normal Diet Containing 1% **DANA87** for 1 Week

Rats were fed normal powder diets containing 0 (control) or 1% **DANA87** for 1 week ($n=10$). ** $p<0.01$.

ratio of HDL/LDL cholesterol (from 1.08 to 1.42). Western blotting and RT-PCR analyses showed that the amount of LDL receptor in the liver was also markedly increased (data not shown).

Administration of compound **DANA87** did not show any changes in the levels of functional biochemical indicators such as alanine transaminase (ALT), albumin, creatine, and uric acid in plasma. There was no statistically significant effect on body weight gain.

It has been suggested that phytosterols lower circulating plasma cholesterol concentrations by direct competitive blocking of intestinal cholesterol absorption in animals and humans.[10—13] The potent ability of **DANA87** to reduce plasma cholesterol levels could also be explained by inhibition or displacement of cholesterol by the β-sitosterol part of **DANA87** from cholesterol-containing micelles, formed with bile acids in the small intestine, which are required for cholesterol absorption.[16] It has also been reported recently that an increase in a sitostanol analogue dose decreased the rate of cholesterol absorption and the overall cholesterol exposure to the body.[17] The decrease in absorption rate may be due to a decrease in facilitated cholesterol uptake by enterocyte cholesterol transporters.[18—20] Although such an external intestinal mechanism has been widely cited as responsible for the cholesterol lowering of β-sitosterol, studies in rats have suggested that intraperitoneal and subcutaneous injection of β-sitosterol lowers circulating cholesterol concentrations.[21] It was speculated that β-sitosterol intrinsically affects the circulating cholesterol concentration, possibly by altering enzymes involved in cholesterol metabolism. Decreases in total blood cholesterol levels were due only to decreases in LDL cholesterol concentrations, as HDL cholesterol levels did not change with the **DANA87** dose. This suggests that **DANA87** may further decrease total blood cholesterol by inhibiting the formation of LDL cholesterol. The decrease in LDL cholesterol is consistent with previously reported results that apoE-deficient mice develop severe hypercholesterolemia and atherosclerotic lesions similar to those observed in humans, and that phytosterol therapy is associated with a significant decrease in hepatic and lipoprotein lipase activities in apoE-deficient mice.[22—24] However, further investigations are required to elucidate the mechanism involved and to explain why **DANA87** has more potent cholesterol-lowering ability than β-sitosterol and β-sitostanol fatty acid esters.

In conclusion, we have shown that the succinamic acid ester β-sitosterol analogue **DANA87** functions as a total plasma cholesterol- and LDL cholesterol-reducing agent. Further pharmacologic evaluation and the structure–activity relationship study of **DANA87** and related compounds are in progress.

Experimental

Chemistry ^{1}H-NMR (300 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained with a Varian Unity NMR spectrophotometer. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as internal standard. IR spectra were recorded on a JASCO FT/IR 300E infrared spectrophotometer. Elemental analyses (C, H, N) and MS analyses were performed at the Korea Research Institute of Chemical Technology. Melting points were obtained on a MEL-TEMPII (Laboratory Devices, U.S.A.) apparatus and are uncorrected.

β-Sitosterol-3-hemisuccinate Ester (1) (Succinic Acid Mono-[17-(4-ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl] Ester) To a stirred solution of β-sitosterol (1.5 g, 3.6 mmol) dissolved in distilled toluene (20 ml), succinic anhydride (0.36 g, 3.6 mmol) and DMAP (10 mg, 0.073 mmol) were added and the solution was heated at reflux for 24 h. The mixture was left at room temperature for 4 h, then the precipitated solid was filtered off, and the filtrate was washed with water, hydrochloric acid solution (0.1 M), and again with water. The organic layer was dried over $MgSO_4$, evaporated, and the residue was recrystallized from hexane–ethyl acetate. Yield 88%, mp 150—151 °C. IR (KBr) cm^{-1}: 2945, 1735, 1715. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—2.05 (m, 45H), 2.33 (d, 2H), 2.44 (t, 2H), 2.63 (t, 2H), 4.60 (m, 1H), 5.36 (d, 1H), 11.05 (s, 1H). *Anal.* Calcd for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57%. Found: C, 76.21; H, 11.08%. MS (FAB) *m/z* 514 (M^+).

General Procedure for the Preparation of *N*-Substituted Succinamic Acid β-Sitosteryl Ester Derivatives (2): Method A (Using Thionyl Chloride) To a stirred solution of β-sitosterol-3-hemisuccinate ester **4** (1.0 g, 1.94 mmol) and DMF (2 drops) dissolved in THF (20 ml), thionyl chloride (0.17 ml, 2.33 mmol) was added dropwise under cooling with an ice bath. The reaction mixture was stirred at room temperature for 24 h. The solvent and excess thionyl chloride were removed at reduced pressure, and the crude chlorinated β-sitosterol-3-hemisuccinate ester (3-chlorocarbonyl-propionic acid 17-(4-ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl) ester) was used for further reaction without purification. The residue was redissolved in THF (15 ml) and cooled in an ice bath. An amine (2.05 mmol) and TEA (0.7 ml, 5.02 mmol) were added dropwise, and the mixture was heated slowly. After heating at reflux for 24 h, the reaction mixture was poured into ice-water. The solid obtained was filtered, washed with water, and dried, and the residue was purified by recrystallization or column chromatography on silica gel.

Method B (Using Methyl Chloroformate) To a stirred solution of β-sitosterol-3-hemisuccinate ester **1** (1.0 g, 1.94 mmol) dissolved in dry ether (20 ml), methyl chloroformate (0.25 ml, 2.0 mmol) and TEA (0.7 ml, 5.02 mmol) were added dropwise under cooling with an ice bath. The reaction mixture was stirred at room temperature for 24 h. The solution was filtered, washed with water, dried over $MgSO_4$, and evaporated, and the residue was recrystallized from hexane–ethyl acetate to give 4-methoxycarbonyloxy-4-oxo-butyric acid 17-(4-ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl ester. Yield 85%, mp 157—158 °C. IR (KBr) cm^{-1}: 2934, 1735, 1705. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—2.05 (m, 45H), 2.33 (d, 2H), 2.61 (t, 2H), 2.79 (t, 2H), 3.73 (s, 3H), 4.62 (m, 1H), 5.38 (d, 1H). *Anal.* Calcd for $C_{35}H_{56}O_6$: C, 73.38; H, 9.85%. Found: C, 72.76; H, 10.15%. To a stirred solution of 4-methoxycarbonyloxy-4-oxo-butyric acid 17-(4-ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl ester (1.14 g, 2.0 mmol) in dry ether (15 ml) an amine (2.05 mmol) and TEA (0.3 ml, 2.15 mmol) were added, and the mixture was heated slowly. After heating at reflux for 24 h, the reaction mixture was poured into ice-water. The solid obtained was filtered, washed with water, and dried, and the residue was purified by recrystallization or column chromatography on silica gel.

***N*-Cyclohexyl-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2a) (DANA 87): Method B** Yield 88%, mp 162—163 °C, IR (KBr) cm^{-1}: 3305, 2930, 1730, 1645. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.65—2.05 (m, 55H), 2.33 (d, 2H, $J=7.5$ Hz), 2.44 (t, 2H, $J=7.0$ Hz), 2.63 (t, 2H, $J=6.9$ Hz), 3.70 (m, 1H), 4.60 (m, 1H), 5.36 (d,

1H, J=5.7 Hz), 5.64 (d, 1H, J=7.5 Hz), ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 122.63, 139.56 (double bond of β-sitosterol), 170.43 (–CONH–), 172.45 (–COO–). *Anal.* Calcd for $C_{39}H_{65}NO_3$: C, 78.60; H, 10.99; N, 2.35%. Found: C, 78.02; H, 11.15; N, 2.59%. MS (FAB) m/z 596 (M^++1).

Method C (Using DCC) To a stirred solution of β-sitosterol-3-hemisuccinate ester **1** (3.0 g, 5.82 mmol) dissolved in dry ether (40 ml), methyl chloroformate (0.75 ml, 6.0 mmol) and TEA (0.9 ml, 7.8 mmol) were added dropwise under cooling with an ice bath. The reaction mixture was stirred at room temperature for 24 h. The solution was filtered, washed with water, dried over $MgSO_4$, and evaporated, and the residue was recrystallized from hexane–ethyl acetate to give 4-(*N*,*N*′-dicyclohexyl-carbamimidoyloxy)-4-oxo-butyric acid 17-(4-ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl ester (**1a**). Yield 92%, mp 50—51 °C. IR (KBr) cm^{-1}: 3325, 2934, 1735, 1707, 1660. ^{1}H-NMR ($CDCl_3$, 300 MHz) 0.64—2.04 (m, 65H), 2.34 (d, 2H, J=7.3 Hz), 2.45 (t, 2H, J=6.9 Hz), 2.65 (t, 2H, J=7.0 Hz), 3.69 (m, 1H), 4.05 (m, 1H), 4.60 (m, 1H), 5.36 (d, 1H, J=5.8 Hz), δ: 7.15 (br, 1H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 122.67, 139.57 (double bond of β-sitosterol), 153.99 (–C=N–), 170.94 (–COOC=N–), 172.49 (–COO–). *Anal.* Calcd for $C_{46}H_{76}N_2O_4$: C, 76.56; H, 10.54; N, 3.88%. Found: C, 76.81; H, 10.75; N, 4.03%. MS (FAB) m/z 719 (M^+−1). A solution of **1a** (2.0 g, 2.77 mmol) in toluene (25 ml) was heated with stirring at 80 °C in a water bath for 24 h. The solution was evaporated and the residue was purified by column chromatography on silica gel to afford **DANA87**. Yield 90%. **DANA87** was also easily obtained in 80% yield without isolation of **1a** by the direct addition of β-sitosterol to DCC under the same conditions.

***N*-Phenyl-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2b): Method A** Yield 76%, mp 182—183 °C, IR (KBr) cm^{-1}: 3440, 2965, 1715, 1680. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—1.95 (m, 45H), 2.48 (d, 2H), 2.66 (t, 2H), 2.73 (t, 2H), 4.61 (m, 1H), 5.37 (d, 1H,) 7.09 (t, 1H), 7.30 (m, 2H), 7.50 (m, 2H), 7.72 (s, 1H) *Anal.* Calcd for $C_{39}H_{59}NO_3$: C, 79.40; H, 10.08; N, 2.37%. Found: C, 79.75; H, 10.45; N, 2.72%. MS (FAB) m/z 589 (M^+).

***N*-(4-Fluoro-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2c): Method A** Yield 79%, mp 158—161 °C, IR (KBr) cm^{-1}: 3430, 2975, 1715, 1705. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—1.80 (m, 45H), 1.88 (d, 2H), 2.00 (t, 2H), 2.28 (t, 2H), 3.60 (m, 1H), 5.42 (d, 1H,) 6.63 (m, 2H), 6.86 (m, 2H), 7.35 (s, 1H). *Anal.* Calcd for $C_{39}H_{58}FNO_3$: C, 77.05; H, 9.61; N, 2.30%. Found: C, 76.73; H, 9.83; N, 2.66%. MS (FAB) m/z 607 (M^+).

***N*-(4-Chloro-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2d): Method B** Yield 92%, mp 199—200 °C, IR (KBr) cm^{-1}: 3425, 2985, 1725, 1690. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—1.90 (m, 45H), 2.31 (d, 2H), 2.65 (t, 2H), 2.72 (t, 2H), 4.63 (m, 1H), 5.35 (d, 1H), 7.25 (d, 2H,) 7.46 (d, 2H), 7.98 (s, 1H). *Anal.* Calcd for $C_{39}H_{58}ClNO_3$: C, 75.02; H, 9.36; N, 2.24%. Found: C, 75.62; H, 10.05; N, 2.47%. MS (FAB) m/z 624 (M^++1).

***N*-(3-Bromo-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2e): Method A** Yield 77%, mp 137—138 °C, IR (KBr) cm^{-1}: 3430, 2981, 1713, 1670. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—2.05 (m, 45H), 2.32 (d, 2H), 2.65 (t, 2H), 2.72 (t, 2H), 4.63 (m, 1H), 5.35 (d, 1H), 7.12—7.26 (m, 2H,) 7.39 (d, 1H), 7.77 (s, 1H), 7.91 (s, 1H). *Anal.* Calcd for $C_{39}H_{58}BrNO_3$: C, 70.04; H, 8.74; N, 2.09%. Found: C, 70.82; H, 9.02; N, 1.83%. MS (FAB) m/z 668 (M^+).

***N*-(3-Iodo-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2f): Method B** Yield 90%, mp 141—142 °C, IR (KBr) cm^{-1}: 3440, 2985, 1714, 1705. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.67—2.10 (m, 45H), 2.32 (d, 2H), 2.64 (t, 2H), 2.70 (t, 2H), 4.61 (m, 1H), 5.36 (d, 1H), 7.01 (s, 1H), 7.40—7.48 (m, 2H) 7.82 (s, 1H), 7.92 (s, 1H). *Anal.* Calcd. for $C_{39}H_{58}INO_3$: C, 65.44; H, 8.16; N, 1.95%. Found: C, 65.62; H, 8.22; N, 2.32%. MS (FAB) m/z 715 (M^+).

***N*-(4-Methoxy-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2g): Method A** Yield 79%, mp 164—165 °C, IR (KBr) cm^{-1}: 3430, 2975, 1715, 1672. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.67—2.05 (m, 45H), 2.33 (d, 2H), 2.64 (t, 2H), 2.72 (t, 2H), 3.78 (s, 3H), 4.63 (m, 1H), 5.37 (d, 1H), 6.85 (d, 2H), 7.40 (d, 2H,) 7.57 (s, 1H). *Anal.* Calcd for $C_{40}H_{61}NO_4$: C, 77.49; H, 9.91; N, 2.25%. Found: C, 77.88; H, 10.21; N, 2.44%. MS (FAB) m/z 619 (M^+).

***N*-(2,6-Diethyl-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2h): Method A** Yield 78%, mp 132—133 °C, IR (KBr) cm^{-1}: 3445, 2975, 1715, 1703. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—2.10 (m, 51H), 2.30 (d, 2H), 2.56 (d, 4H), 2.60—2.72 (m, 4H), 4.63 (m, 1H), 5.35 (d, 1H), 7.01 (s, 1H), 7.05 (d, 1H) 7.20 (d, 1H), 7.25 (dd, 1H). *Anal.* Calcd for $C_{43}H_{67}NO_3$: C, 79.94; H, 10.45; N, 2.16%. Found: C, 80.41; H, 10.66; N, 2.35%. MS (FAB) m/z 645 (M^+).

***N*-(3,4-Dimethyl-phenyl)-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2i): Method B** Yield 88%, mp 129—130 °C, IR (KBr) cm^{-1}: 3450, 2975, 1710, 1685. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.68—2.05 (m, 45H), 2.30 (d, 2H), 2.56 (d, 6H), 2.64 (t, 2H), 2.72 (t, 2H), 4.63 (m, 1H), 5.36 (m, 1H), 7.09—7.25 (m, 3H). *Anal.* Calcd for $C_{41}H_{63}NO_3$: C, 79.69; H, 10.27; N, 2.26%. Found: C, 80.02; H, 11.01; N, 2.43%. MS (FAB) m/z 617 (M^+).

***N*-*o*-Tolyl-succinamic Acid 17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl Ester (2j): Method A** Yield 77%, mp 142—143 °C, IR (KBr) cm^{-1}: 3445, 2978, 1715, 1687. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.67—2.10 (m, 45H), 2.26 (s, 3H), 2.34 (d, 2H), 2.66 (t, 2H), 2.74 (t, 2H), 4.63 (m, 1H), 5.36 (d, 1H), 7.05 (m, 1H), 7.20 (m, 2H), 7.47 (s, 1H), 7.81 (d, 1H). *Anal.* Calcd for $C_{40}H_{61}NO_3$: C, 79.55; H, 10.18; N, 2.31%. Found: C, 79.92; H, 10.33; N, 2.62%. MS (FAB) m/z 603 (M^+).

Pharmacology Four-week-old male Sprague–Dawley rats (160) weighing 100—120 g were acclimatized for 4 d on a pellet diet in an animal chamber and subsequently fed a hyperlipidemic diet (Purina Rodent Chow Special Mix 5001-S) supplemented with 0.5% cholic acid and 1% cholesterol for 3 d, and then the hyperlipidemic diet containing either no **DANA87** (control) or **DANA87** at 0.10, 0.50, and 1% w/w for 4 continuous weeks. Blood was collected from the heart under the ether anesthesia after 1 to 4 weeks, and the serum was separated by centrifugation. The lipoproteins (TC, HDL, LDL, TG) and ALT, albumin, creatine, and uric acid in plasma were analyzed using the Sigma Diagnostics enzymatic kit and the automatic analyzer Hitachi model 747.

References and Notes

1) In memory of the late Professor LeRoy H. Klemm.
2) Jaeger B. R., Seidel D., *Herz*, **26**, 531—544 (2001).
3) Assmann G., Cullen P., Schulte H., *Atherosclerosis*, **130** (Suppl. 1), S22 (1997).
4) Shepherd J., *Lancet*, **359**, 2271—2273 (2002).
5) Jones P., Kafonek S., Laurora I., Hunniganhake D., *Am. J. Cardiol.*, **81**, 582—587 (1998).
6) Miki T., Kori M., Mabuchi H., Tozawa R., Nishimoto T., Sugiyama Y., Teshima K., Yukimasa H., *J. Med. Chem.*, **45**, 4571—4580 (2002).
7) Chan C., Andreotti D., Cox B., Kirk B. E., Lester M. G., McCarthy A. D., Procopiou P. A., Ross B. C., *J. Med. Chem.*, **39**, 207—216 (1996).
8) Burnett D. A., Caplen M. A., Davis H. R., Jr., Burrier R. E., Clader J. W., *J. Med. Chem.*, **37**, 1733—1736 (1994).
9) McCarthy P. A., DeNinno M. P., Morehouse L. A., Chandler C. E., Bangerter F. W., Wilson T. C., Urban F. J., Walinsky S. W., Cosgrove P. G., Duplantier K., Etienne J. B., Fowler M. A., Lambert J. F., O'Donnell J. P., Pezzullo S. L., Watson H. A., Wilkins R. W., Zaccaro L. M., Zawistoski M. P., *J. Med. Chem.*, **39**, 1935—1937 (1996).
10) Ling W. H., Jones P. J. H., *Atherosclerosis*, **118**, 319—331 (1995).
11) Becker M., Staab D., von Bergmann K., *J. Pediatr.*, **122**, 292—296 (1993).
12) Heinemann T., Axtmann G., von Bergmann K., *Eur. J. Clin. Invest.*, **23**, 827—831 (1993).
13) Laraki L., Pelletier X., Debry G., *Ann. Nutr. Metab.*, **35**, 221—225 (1991).
14) Wasan K. M., Najafi S., Peteherych K. D., Pritchard P. H., *J. Pharm. Sci.*, **90**, 1795—1799 (2001).
15) Habib N. S., Khalil M. A., *Arch. Pharm.*, **323**, 401—404 (1990).
16) Heinemann T., Leiss O., von Bergmann K., *Atherosclerosis*, **61**, 219—223 (1986).
17) Wasan K. M., Peteherych K. D., Najafi S., Zamfier C., Pritchard P. H., *J. Pharm. Pharmaceut. Sci.*, **4**, 207—216 (2001).
18) Berge K. E., Tian H., Graf G. A., Yu L., Grishin N. V., Schultz J., Kwitwrrovich P., Shan B., Barnes R., Hobbs H. H., *Science*, **290**, 1771—1775 (2000).
19) Simons K., Ikonen E., *Science*, **290**, 1721—1725 (2000).
20) Repa J. J., Turley S. D., Lobaccaro J.-M. A., Medina J., Li L., Lustig

K., Shan B., Heyman R. A., Dietschy J. M., Mangelsdorf D. J., *Science*, **289**, 1524—1529 (2000).
21) Malini T., Vanithakumari G., *J. Ethnopharm.*, **36**, 51—55 (1992).
22) Moghadasian M. H., MaManus B. M., Godin D. V., Rodrigues B., Frohlich J. J., *Circulation*, **99**, 1733—1739 (1999).
23) Moghadasian M. H., Frohlich J. J., *Am. J. Med.*, **107**, 588—594 (1999).
24) Moghadasian M. H., MaManus B. M., Pritchard P. H., Frohlich J. J., *Arterioscler. Thromb. Vasc. Biol.*, **17**, 119—126 (1997).