The Tomato Saponin, Esculeoside A

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Esculeoside A (**2**), a spirosolane steroidal glycoside, is a major constituent isolated from *Solanum lycopersicum*, a commercial strain of mini tomatoes. The content variability of esculeoside A (**2**) was examined in mini, midi, and Momotaro tomatoes and various processed tomato products. In the green immature tomato fruit, tomatine (**1**) is oxidized at C-23 and C-27 to produce esculeoside A (**2**) in the ripe fruit. Further, esculeoside A (**2**) is partly converted to 3β-hydroxy-5α-pregn-16-en-20-one 3-*O*-β-lycotetraoside (**6**), a pregnane glycoside, in the overripe fruit. Esculeogenin A (**3**), the sapogenol of **2**, is easily converted into 3β,16β-dihydroxy-5α-pregn-20-one (**17**). Metabolic studies showed excretion of androstane derivatives in the urine of human volunteer subjects after tomato consumption. Esculeogenin A (**3**) inhibited the accumulation of cholesterol esters in macrophages through its effects on acyl-CoA:cholesterol acyl transferase (ACAT). Oral administration of esculeoside A (**2**) to apoE-deficient mice significantly reduced serum levels of cholesterol, triglycerides, and LDL-cholesterol and ameliorated the severity of atherosclerotic lesions.

Introduction

The tomato [*Solanum lycopersicum* L. (Solanaceae)] is a convenient model for investigating the metabolic pathways of steroidal glycosides because its aerial parts and immature fruits are rich sources of tomatine (**1**) (Figure 1). The fruits of *S. lycopersicum* are widely available for consumption either fresh or cooked. The types of commercially available tomatoes may be roughly classified as pink (e.g., Momotaro) and red (e.g., Italian San Marzano). The Momotaro cultivar is commonly eaten fresh, while the Italian San Marzano tomato is more often used in cooking.

Tomatoes have attracted considerable attention because they contain the red carotenoid lycopene, a potent antioxidant. Recent studies have discovered several tomato constituents, including the bitter compound TFI, isolated from tomato seeds,[1] as well as steroidal alkaloid glycosides, such as tomatine (**1**), and several spirosolane glycosides obtained from the stems and leaves.[2] Moreover, lactone,[3] pregnane,[4] and several spirosolane derivatives[5] have been identified in tomato plant roots.

Tomato Saponins

Esculeosides A (2) and B (4). Mini tomatoes (719 g) were homogenized in water using a mixer, and the homogenate was filtered. The filtrate was subjected to column chromatography over a highly porous polystyrene gel (Diaion HP-20) and separated on reversed-phase silica gel to yield a major tomato saponin, esculeoside A (**2**), in the form of colorless needle-shaped crystals. Altogether, 312 mg of esculeoside A (**2**) crystals (0.043% of the total starting weight) was obtained. From 475 g of Momotaro tomatoes, 80.4 mg (0.017%) of the same compound was afforded.[6,7]

Esculeoside A (**2**) exhibited mp 225 °C (dec), $[\alpha]_D$ −52.5 (MeOH), elemental formula $C_{58}H_{95}NO_{29}$. Acid hydrolysis of compound **2** yielded colorless needles of a single sapogenol, designated as esculeogenin A (**3**), mp 215−220 °C (dec), $[\alpha]_D$ −99.6 (pyridine), $C_{27}H_{45}NO_4$. EIMS, ^{1}H NMR, ^{13}C NMR, FG-COSY, HMQC, and HMBC analysis of **3** revealed the fundamental framework of a 5α-spirosolane derivative with two oxygen functions at C-23 and C-27, with the configurations at C-22, C-23, and C-25 characterized as *S*, *S*, and *S*, respectively. Thus, the structure of **3** was assigned as (22*S*,23*S*,25*S*)-3β,23,27-trihydroxy-5α-spirosolane. The absolute configurations of the sugar mixture of the hydrolysate of **2** were determined by GLC.[8] In turn, the positions of the sugar linkages were determined from the HMBC NMR data and ^{1}H NMR glycosylation shifts of the prosapogenin after enzymatic hydrolysis of **2** with β-glucosidase or tomatinase.[9] Consequently, the structure of esculeoside A (**2**) was elucidated as 3-*O*-β-lycotetraosyl (22*S*,23*S*,25*S*)-23-acetoxy-3β,27-dihydroxy-5α-spirosolane 27-*O*-β-D-glucopyranoside (Figure 2).[7,10]

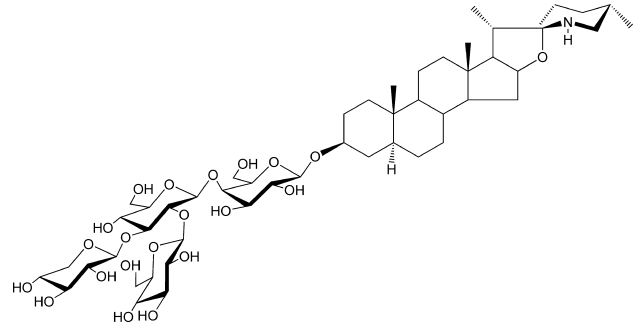

Figure 1. Structure of tomatine (**1**).

Another steroidal alkaloid glycoside, esculeoside B (**4**), was obtained as an amorphous powder, $[\alpha]_D$ −49.2 (pyridine), $C_{56}H_{93}NO_{28}$, from the red tomato cultivar "Italian San Marzano". A starting mass of "6.5 kg" of this cultivar was purified to produce 1.21 g of esculeoside B (**4**) furnished as an amorphous powder. The HRFABMS was performed to determine the molecular formula of esculeoside B (**4**). The rare solanocapsine-type skeleton,[11] the presence of two oxygen functions at C-23 and C-27, the configurations at the C-23 hydroxy group and at C-25, and the sugar components and sugar linkages were determined from the 1D- and 2D-NMR spectroscopic data. Esculeoside B (**4**) was subjected to enzymatic hydrolysis with tomatinase,[9] to yield prosapogenin C (**5′**), which was then treated with β-glucosidase to produce esculeogenin B (**5**).[12] The data obtained suggested that the structure of esculeoside B (**4**) can be represented as 3-*O*-β-lycotetraosyl

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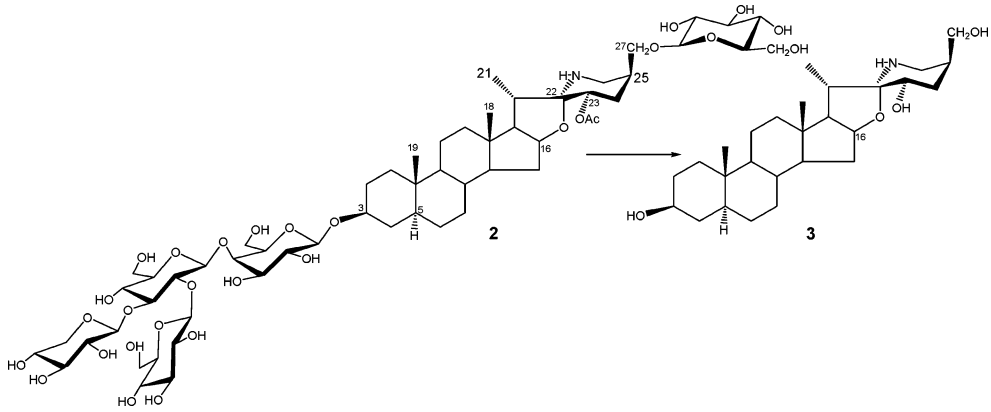

Figure 2. Conversion of esculeoside A (**2**) to esculeogenin A (**3**) by acid hydrolysis.

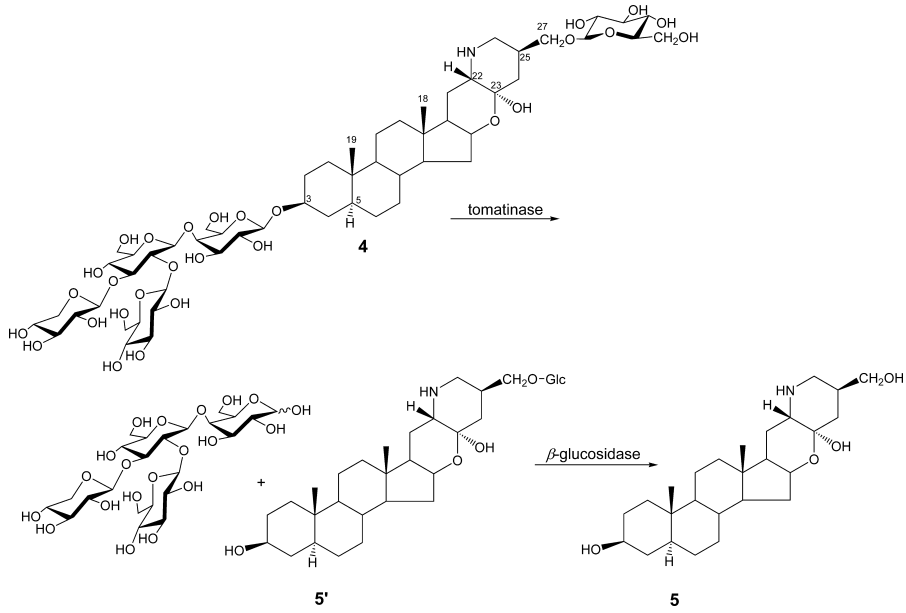

Figure 3. Production of prosapogenin C (**5′**) by reaction of esculeoside B (**4**) with tomatinase, leading to esculeogenin B (**5**) using β-glucosidase.

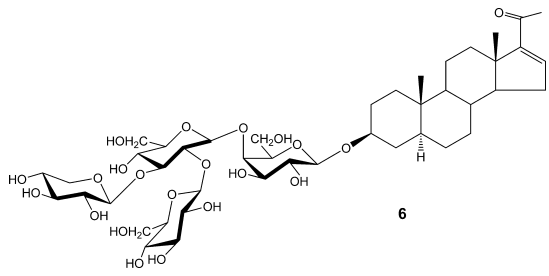

Figure 4. Structure of the pregnane glycoside 3β-hydroxy-5α-pregn-16-en-20-one 3-*O*-β-lycotetraoside (**6**), isolated from overripe tomatoes.

(22*S*,23*R*,25*S*)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxy-5α-cholestane 27-*O*-β-D-glucopyranoside (Figure 3).[7]

A Tomato Pregnane Glycoside. The compound 3β-hydroxy-5α-pregn-16-en-20-one 3-*O*-β-lycotetraoside (**6**) was furnished as a minor component isolated from overripe mini tomato fruits (Figure 4).[13] The presence of this compound indicates that the steroidal glycoside content varies as the tomato matures. Accordingly, tomatine (**1**) found in the green immature fruit is oxidized at C-23 and C-27 to yield esculeoside A (**2**) in the ripe fruit, and **2** is converted into this pregnane glycoside (**6**) in the overripe fruit (Figure 5).

Other Minor Constituents in Tomatoes. Ripe fruits of mini tomatoes were crushed and centrifuged. The supernatant was subjected successively to Diaion HP-20, Chromatorex NH, and silica gel column chromatography, as well as HPLC on ODS, to afford two new steroidal alkaloid glycosides, esculeosides C (**7**) and D (**8**), along with the three previously identified steroidal alkaloid glycosides, esculeosides A (**2**) and B (**4**), and lycoperoside G (**9**) (Figure 6).[14]

The ripe fruits of the miniature tomato cultivar "Chika" were crushed and extracted with MeOH. The extract was subjected successively to passage over Diaion HP-20, Sephadex LH-20, Chromatorex ODS, and silica gel and subjected to HPLC on C_{18}, C_8, and polyamine phases to afford the new steroidal glycoside 3-*O*-β-lycotetraosyl 3β,26-dihydroxycholestan-16,22-dione 26-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**10**) and a new phenyl glycoside, 4-hydroxyphenyl β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**11**), along with two steroidal alkaloid glycosides, esculeosides A (**2**) and B (**4**), and five known aromatic compounds, zizibeoside I (**12**), benzyl alcohol β-gentiobioside (**13**), rutin (**14**), methyl caffeate (**15**), and phenylalanine (**16**) (Figure 6).[15]

Content Variation of Esculeoside A (2) in Various Processed Tomato Samples.[16] Variations in the glycoside content of fresh tomatoes were compared with the levels when various processing procedures were applied. As listed in Table S1 (Supporting Information), all test specimens consisting of commercial

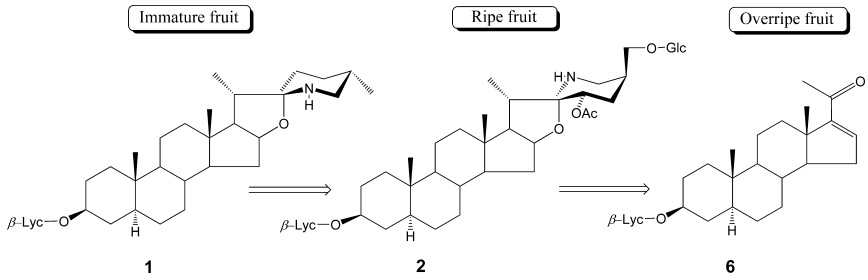

Figure 5. Seasonal variation of steroidal glycosides in tomatoes.

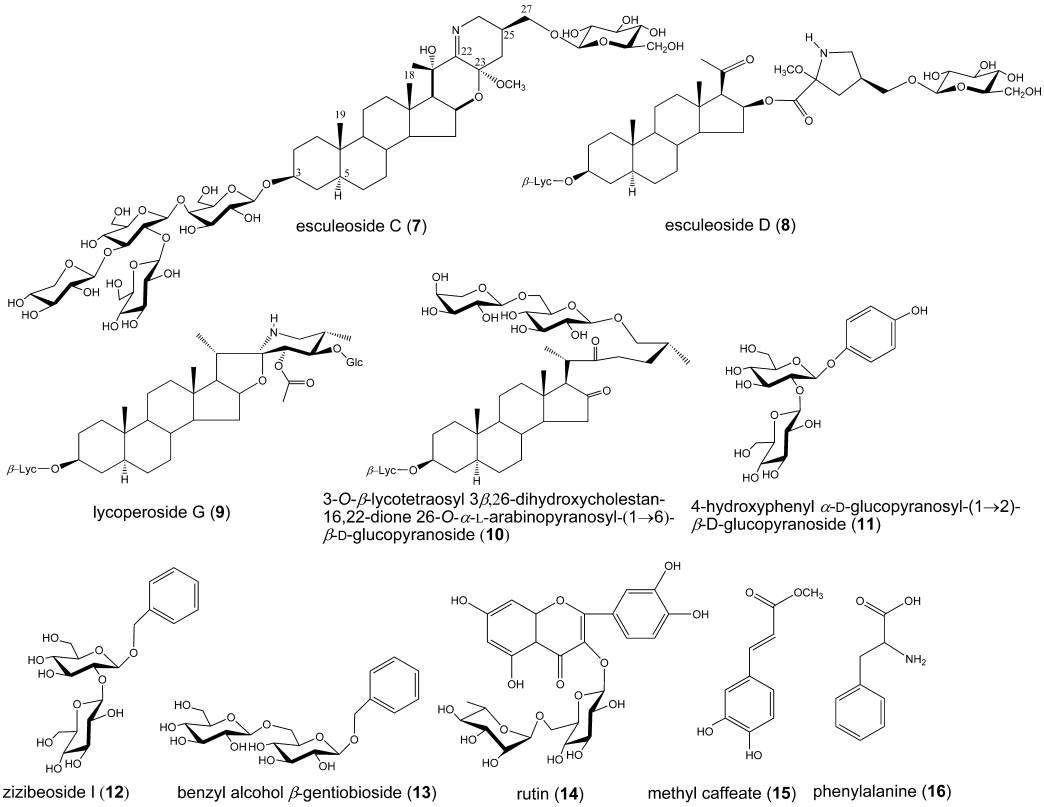

Figure 6. Other identified constituents of the mini tomato.

Momotaro, mini, and midi tomatoes could be classified into four groups: water-blended (group A; specimens 1−6, with specimen 4 specially treated as described below); freeze-dried (group B; specimens 7, 8); heated (group C; specimens 9−12); and processed groups (group D; specimens 13−19).

In the case of group A, specimens 1−3 were individually and briefly (10−20 s) homogenized in water using a mixer, and the homogenate was filtered. The filtrate was then passed through Diaion HP-20 and initially eluted with water. The water eluate was discarded, elution was then carried out using MeOH, and this eluate was evaporated to obtain a residue. The residue was subjected to ODS reversed-phase silica gel column chromatography, eluted with 60% MeOH, and evaporated to obtain esculeoside A (**2**). Specimen 4 was blended with MeOH and refluxed with MeOH for 2.5 h. The sample was filtered, evaporated to dryness, and subjected to Diaion HP-20 column chromatography eluted with water. A second MeOH eluate was collected and evaporated to obtain a residue. This residue was then subjected to ODS with 60% MeOH to afford **2**. Specimens 5 and 6 were incubated at 38 °C for ca. 33 days or left to stand at room temperature for 10 days, and the action of a tomato enzyme present in these specimens was checked. The specimens were then processed as above. In group B, specimens 7 and 8 were freeze-dried and processed as above. In group C, Momotaro (specimen 9) and mini tomatoes (specimen 11) were boiled in water for approximately 20 min. Momotaro tomatoes (specimen 10) were irradiated with far-infrared light for one day, and mini tomatoes (specimen 12) were heated in a microwave oven at 500 W for 15 min. Subsequently, the treatment of these specimens was carried out in the same manner as above. In group D, commercially available processed specimens 13−19 were analyzed that were contained in PET bottles, jars, and cans. The contents of **2** were investigated as described above.

Lycopene was isolated from mini tomatoes (719 g) blended with water in a mixer, and the mixture was filtered through filter paper to obtain 19 g of a dried residue. The residue was then refluxed with $CHCl_3$ for 130 min, and the $CHCl_3$-soluble fraction was subjected to silica gel chromatography with *n*-hexane−AcOEt (100:1−50:1) to purify 132 mg of lycopene.[17]

As a result of performing this work, a number of observation can be made. First, by analyzing specimens 1−12, belonging to the four groups mentioned above, when tomatoes were homogenized in water, the yields of the tomato saponin esculeoside A (**2**) in the mini and midi tomatoes were found to be 0.043% and 0.046%, respectively, as listed in Table S1 (Supporting Information). Thin-layer chromatograms ($CHCl_3$−MeOH−water, 7:3:0.5) of the MeOH eluate from each Diaion HP-20 column showed almost always a single spot of **2**. The yields of esculeoside A (**2**) were approximately 4 times that of lycopene. On the other hand, the yield of **2** in

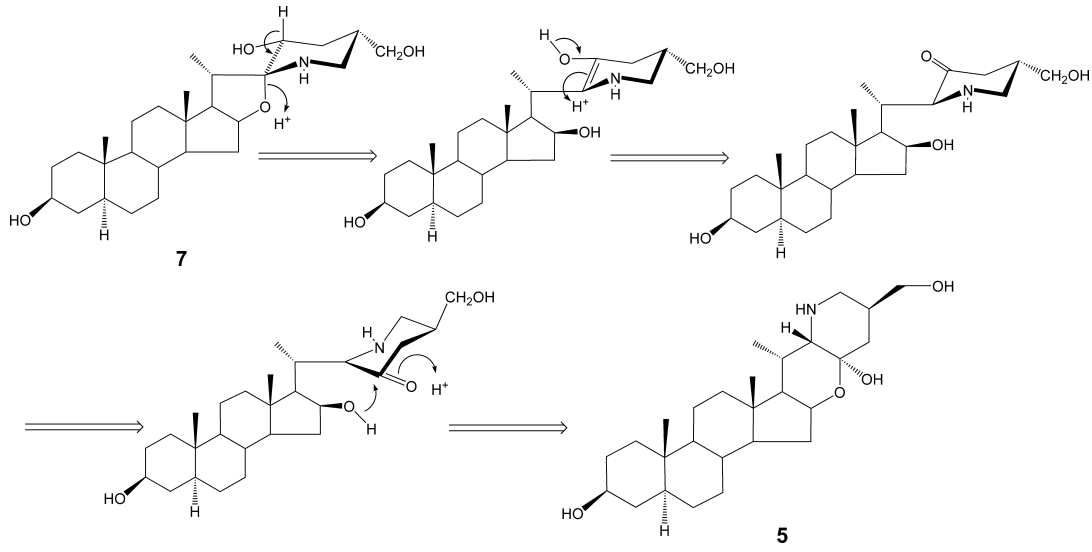

Figure 7. Isomerization of isoesculeogenin A (**7**) into esculeogenin B (**5**).

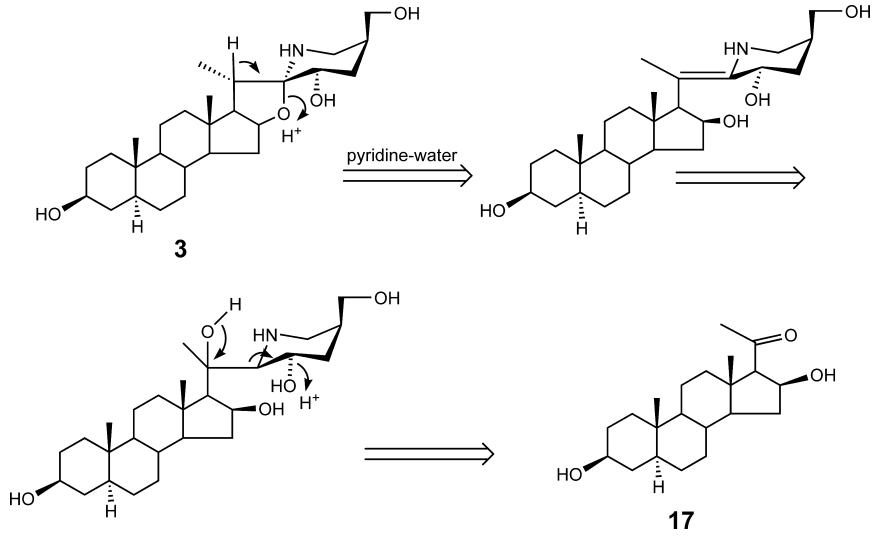

Figure 8. Facile conversion of esculeogenin A (**3**) to the pregnane derivative 3β,16β-dihydroxy-5α-pregn-20-one (**17**).

Momotaro tomatoes was only 0.015%. Using a mixer to homogenize tomato juice with water and incubation at 38 °C for 787 h, or leaving the samples to stand at room temperature for 247 h, did not affect the esculeoside A (**2**) content. Boiling specimens of Momotaro and mini tomatoes in water for approximately 20 min also did not affect the resultant yield of esculeoside A (**2**), indicating that this steroidal alkaloid is not heat labile. Finally, no changes in the yield of **2** were found after heating under far-infrared light or using microwave irradiation. However, it must be pointed out that esculeoside A (**2**) has not been found in commercial specimens analyzed, such as tomato juice and canned tomato products.

Chemical Conversions of Tomato Sapogenols. The chemical correlation between esculeosides A (**2**) and B (**4**) was determined by refluxing the minor component isoesculeogenin A (**7**) with pyridine and water (Figure 7).[18] In this reaction, the hydroxy group at C-23 was converted to an enol by E-ring fission, followed by attack of the C-16-oxygen on C-23, to produce esculeogenin B (**5**).[18]

Next, esculeogenin A (**3**) was converted to a pregnane derivative by refluxing with pyridine (Figure 8). This reaction was unexpected and suggests that the presence of a hydroxy group at C-23 makes the E and F rings very labile, leading to bond fission between C-20 and C-22, to produce the pregnane derivative 3β,16β-dihydroxy-5α-pregn-20-one (**17**).[18] The proposed mechanism for this reaction is as follows. Protonation at the C-16 oxygen causes double-bond formation between C-20 and C-22 and opening of the E ring, followed by hydration of this double bond at C-20(22). Protonation of the C-23 hydroxy group then causes dehydration and leads to bond fission between C-20 and C-22.

Metabolic Experiments on Tomato Glycosides. Our studies on the constituents of *Solanum* plants have shown that pregnane glycosides are accompanied by normal spirostanol and furostanol glycosides.[19−27] In addition, esculeogenin A (**3**) is easily converted to a pregnane derivative by refluxing with aqueous pyridine,[18] and overripe tomato fruits contain a pregnane glycoside.[13] The above findings suggest strongly that orally administered steroidal glycosides may be metabolized to pregnane derivatives.

When eight males consumed tomatoes in an amount of 2 kg per adult over a period of two days, their combined urine samples were collected after 48 h and passed through a polystyrene gel (Diaion HP-20). The initial water eluate was discarded, and the MeOH eluate following was collected. The methanol residue (3.71 g) was subjected to Sephadex LH-20, silica gel, and ODS column chromatography to separate three androstane derivatives: 3-*O*-β-D-glucuronopyranosyl androsterol, 3α-hydroxy-5-α-androstan-17-one (androsterone) β-D-glucuronopyranoside, and 3α-hydroxy-5-β-androstan-17-one (etiocholanolone) β-D-glucuronopyranoside.[28] These androsterone analogues are commonly excreted.[28] However, none of these metabolites were detected

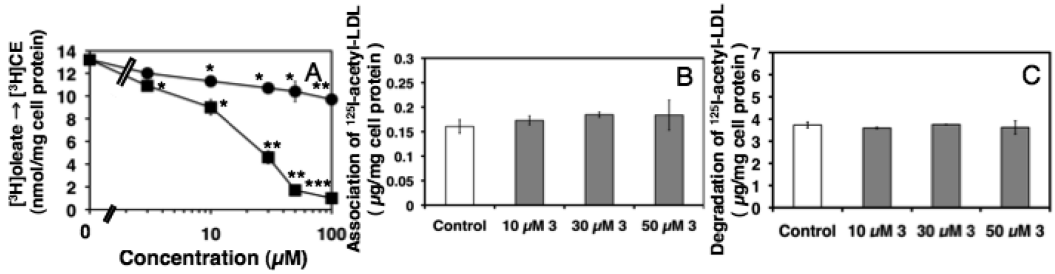

Figure 9. Inhibitory effect of esculeogenin A (**3**) on cholesterol ester (CE) accumulation by acetyl-LDL uptake in HMDM. HMDM were incubated with 50 μg/mL acetyl-LDL and 0.1 mM [^{3}H]oleate conjugated with BSA in the absence or presence of **3** (■) and esculeoside A (**2**) (●). After 24 h incubation, [^{3}H]CE (A) was separated by TLC, and radioactivity was measured with a radioscanner. HMDM were incubated for 5 h with the indicated concentrations of **3** and with 50 μg/mL ^{125}I-acetyl-LDL, followed by determination of cell association (B) and cell degradation (C) of ^{125}I-acetyl-LDL. Experiments were repeated three times with almost identical results. Data are means ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (**3** or **2**, vs without test compounds).

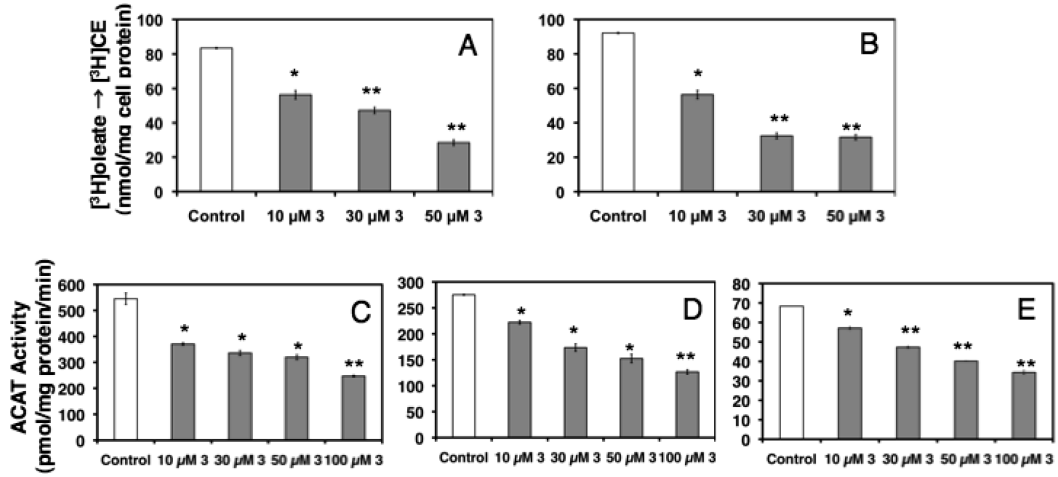

Figure 10. Inhibitory effect of esculeogenin A (**3**) on CE accumulation and ACAT activity in hACAT CHO cells and HMDM. hACAT-1 CHO cells (A) and hACAT-2 CHO cells (B) were incubated with medium containing 10% fetal calf serum in the presence of 0.1 mM [^{3}H]oleate conjugated BSA and the indicated concentration of esculeogenin A (**3**). [^{3}H]CE was separated by TLC, and radioactivity was measured. HMDM (C), hACAT-1 CHO cells (D), or hACAT-2 CHO cells (E) were homogenized with buffer A and reconstituted with sodium PC mixed micelles together with **3**. The ACAT activity was measured. Experiments were repeated three times with almost identical results. Data are means ± SD. *, $p < 0.005$; **, $p < 0.001$ [esculeogenin A (**3**) vs without test compounds].

in the urine of control subjects, who did not consume tomatoes, suggesting that these compounds are the end products of progesterone production induced by tomato consumption (Figure S2, Supporting Information). Thus, the steroidal glycosides in tomatoes might stimulate progesterone secretion or may themselves be metabolized into pregnanes. Generally, it is possible that hydroxy groups may be added at C-23 of orally adminstered steroidal glycosides, such as spirostanol and furostanol glycosides, and these intermediates may then be converted to pregnane derivatives with various pharmacological activities. The final metabolites are excreted as androsterone analogues in the urine. Yoshikawa and colleagues[29] proposed that a portion of an orally administered dose of steroidal glycoside remains in the small intestine and affects the receptors involved in regulating the rise in blood sugar levels. The rest of the glycoside is metabolized to a pregnane hormone that may affect conditions such as osteoporosis and has the same effects as progesterone.[30,31] Furthermore, steroidal glycosides absorbed through the skin may have inhibitory activities against the herpes virus and skin tumors.[32]

Biological Activity of Esculeoside A (2) and Esculeogenin A (3)[33]

Effect on Hyperlipidaemia and Atherosclerosis in ApoE-Deficient Mice. The presence of large clusters of macrophage-derived foam cells in subendothelial spaces is a characteristic feature of early stage atherosclerotic lesions.[34] Macrophages take up chemically modified low-density lipoproteins (LDLs), including oxidized LDLs (ox-LDLs) and acetylated LDLs (acetyl-LDLs), through the scavenger receptors[35] such as class A scavenger receptor (SR-A),[36] class B scavenger receptor (CD36),[37] and class B scavenger receptor type-I (SR-BI).[38] Since free cholesterol, which is incorporated into the cells through the scavenger receptors along with modified LDL, is toxic, it is esterified to cholesterol esters (CEs) by acyl coenzyme A:cholesterol acyl-transferase (ACAT), an intracellular enzyme located in the rough endoplasmic reticulum.[39] These reactions convert the macrophages to foam cells, characterized by intracellular CE accumulation. Two human ACAT isozymes, ACAT-1 and ACAT-2, exist.[40,41] ACAT-1 is highly expressed in foam cells in atherosclerotic lesions and is up-regulated during monocytic differentiation into macrophages.[42] ACAT-1 is found in Kuppfer cells in the liver, the kidneys, and adrenal cortical cells, while ACAT-2 is mainly expressed in hepatocytes and intestinal mucosal cells.[42] These findings are consistent with the concept that ACAT-1 plays a critical role in foam cell formation in macrophages, whereas ACAT-2 is responsible for cholesterol absorption in intestinal mucosal cells.[42] Since foam cell formation is believed to play an essential role in the progression of early atherosclerotic lesions, preventing foam cell formation is a major consideration in the treatment of atherosclerosis. Thus, many investigators have examined the usefulness of various antiathero-

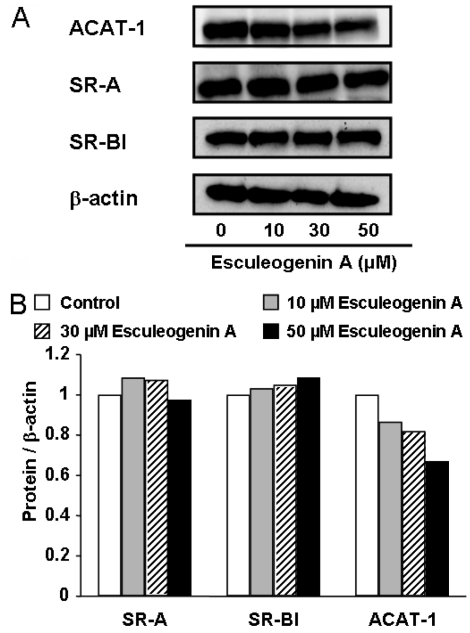

Figure 11. Inhibitory effect of esculeogenin A (**3**) on SR-A, SR-BI, and ACAT-1 expression in HMDM. HMDMs were incubated with **3** for 24 h. (A) Cells were harvested and subjected to immunoblot analyses using antibodies against human ACAT-1, human SR-A, and human SR-BI. (B) Densitometric analysis of ACAT-1, SR-A, and SR-BI immunoblot, which was normalized by β-actin.

sclerotic agents aimed at preventing LDL oxidation[43] and inhibiting scavenger receptor expression[44] and ACAT activity.[45]

Since lifestyle-related diseases, such as atherosclerosis and diabetes, progress gradually due to poor dietary habits, improvement of daily nutrition should prevent the pathogenesis of these diseases. For this reason, the inhibitory effects of esculeoside A (**2**) and esculeogenin A (**3**) on foam cell formation were investigated in human monocyte-derived macrophages (HMDMs).

Treating HMDMs for 24 h with 50 μg/mL acetyl-LDLs increased CE accumulation, while esculeoside A (**2**) and esculeogenin A (**3**) inhibited CE accumulation significantly (Figure 9A), with no cytotoxic effects, even at 100 μM (data not shown). Esculeogenin A (**3**) showed a strong dose-dependent inhibition of CE accumulation (Figure 9A). When HMDMs were treated with 50 μg/mL ^{125}I-acetyl-LDL at 37 °C for 5 h, significant amounts of ^{125}I-acetyl-LDL were found to associate with the cells (Figure 9B) and were lysosomally degraded as a result (Figure 9C). Esculeogenin A (**3**) did not inhibit these cellular responses.

Treating CHO cells overexpressing human ACAT-1 (hACAT-1 CHO) or human ACAT-2 (hACAT-2 CHO) for 24 h with medium containing 10% fetal calf serum in the presence of [^{3}H]oleate increased CE accumulation. Esculeogenin A (**3**) inhibited CE accumulation in both hACAT-1 and hACAT-2 CHO cells in a dose-dependent manner (Figure 10A and B). Thus, **3** may inhibit CE accumulation in both HMDMs and hACAT CHO cells by inhibiting ACAT activity and/or ACAT expression. As shown in Figure 8C, esculeogenin A (**3**) inhibited ACAT activity in a dose-dependent manner in HMDMs. Similar results were seen in hACAT-1 and hACAT-2 CHO cells (Figure 10D and E). These data suggest that esculeogenin A (**3**) significantly inhibits foam cell formation by inhibiting ACAT activity.

A 24 h treatment with esculeogenin A (**3**) reduced ACAT-1 expression in a dose-dependent manner in HMDMs, whereas expression of SR-A and SR-BI remained unchanged compared to the control (Figure 11). These results indicate that esculeogenin A (**3**) inhibits both the expression and activity of ACAT.

To examine the effect of esculeoside A (**2**) on atherogenesis, this compound was administered to apoE-deficient mice. As shown in Figure 10A, treatment with **2** over 90 days did not affect mean body weight gain. However, esculeoside A (**2**) reduced total cholesterol levels by approximately 25% (Figure 12B). Furthermore, this compound reduced the serum levels of LDL cholesterol (Figure 12C) and triglycerides (Figure 12D) by approximately 25% (Figure 12C) and 45% (Figure 12D), respectively, without affecting the TC/HDL ratio (data not shown).

Cross sections of the aortic sinus showed a marked thickening of the intima filled with oil red O-positive foam cells in control mice (Figure 13A), while esculeoside A (**2**) treatment greatly reduced such lesions (Figure 13B). The cross-sectional lesion area in the mice treated with **2** was 52% smaller than that of the control mice (214 508 μm^2 vs 444 555 μm^2; $p < 0.005$) (Figure 13A−C). LC-MS/MS analysis was used to detect **3** in the aorta of the esculeoside A-treated apoE-deficient mice, demonstrating that orally administered **2** is converted to **3** by intestinal bacteria.

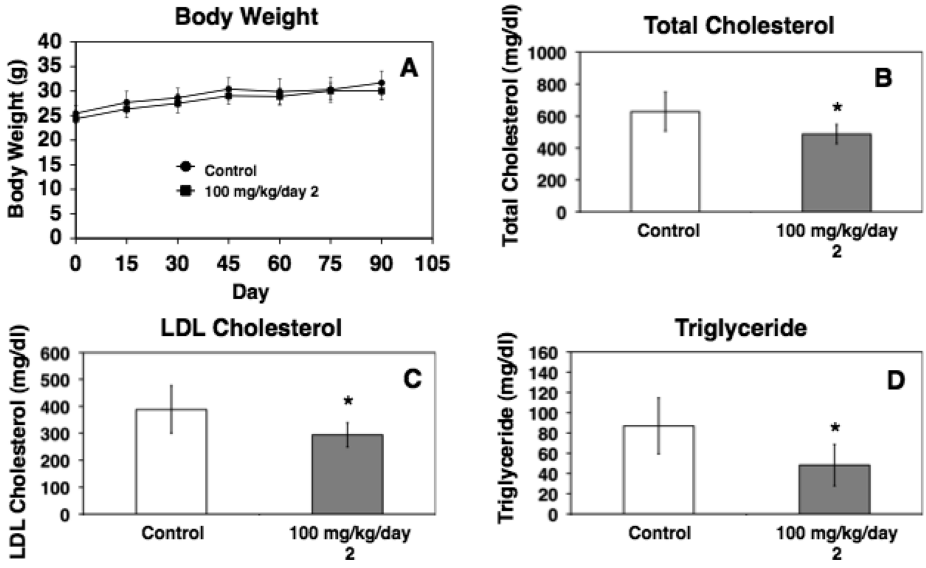

Figure 12. Change in body weight and biochemical data of plasma samples in apoE-deficient mice. ApoE-deficient mice were fed diets with or without esculeoside A (**2**) (100 mg/kg/day) for 90 days ($n = 10$, each group), and body weight (A), plasma total cholesterol (B), LDL cholesterol (C), and TG (D) were measured. Data are means ± SD. *, $p < 0.01$.

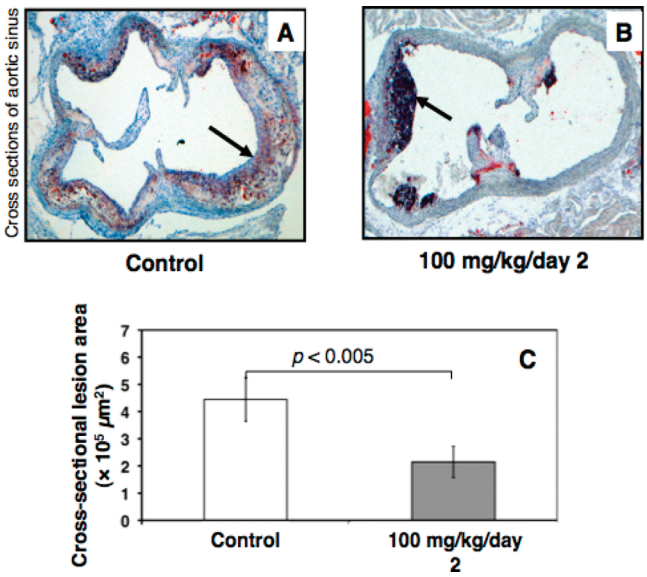

Figure 13. Inhibition of atherogenesis in apoE-deficient mice by esculeoside A (**2**). Representative sections of aortic sinus atherosclerosis stained with oil red O in apoE-deficient mice without treatment with **2** (A) and with (100 mg/kg/day) treatment with **2** (B), magnification ×200. Quantitative evaluations of the cross-sectional lesions of aortic sinus (C).

Overall Analysis of Biological Activity Both ACAT isozymes are expressed in the liver and provide cholesteryl esters for very low density lipoproteins (VLDL),[42] and ACAT-1 inhibitors, such as K-604, significantly decrease serum cholesterol levels.[46] Furthermore, ACAT inhibitors block dietary cholesterol absorption in the intestines of animals fed a high-fat, high-cholesterol diet.[47] Since the apoE-deficient mice in our studies were fed a normal diet, it is possible that esculeoside A (**2**) inhibits ACAT activity in the liver and macrophages rather than in the intestine, and thus decreases endogenous cholesterol production. However, the mechanism by which **2** ameliorates triglyceride levels remains unclear. A similar question has arisen regarding other known ACAT inhibitors, including R-755,[47] U-73482,[48] and CI-976;[47] in rats, these compounds reduce serum triglyceride levels through poorly understood mechanisms. Furthermore, although the HMG Co-A reductase inhibitor atorvastatin decreases the serum levels of not only cholesterol but also of triglycerides,[49] little is known about the mechanism involved. It is likely that the administration of esculeoside A (**2**) decreases endogenous cholesterol production by inhibiting liver ACAT activity and decreasing the serum triglyceride concentrations, similar to ACAT inhibitors and atorvastatin. Taken together, the results of our work indicate that the administration of esculeoside A (**2**) may inhibit atherosclerosis development by inhibiting liver ACAT to decrease serum cholesterol levels and by inhibiting macrophage ACAT to suppress foam cell formation.

Previous studies have examined the usefulness of antiatherosclerotic agents that inhibit ACAT activity. Mice lacking ACAT-2 exhibited a restricted capacity to absorb cholesterol and were protected against diet-induced hypercholesterolemia and gallstone formation.[50] Nonspecific ACAT inhibition reduces atherosclerosis in apo E-deficient mice.[51] The nonspecific ACAT inhibitors NTE-122 and F-1394 prevent the progression of atherosclerosis in cholesterol-fed rabbits.[52,53] Moreover, the ACAT inhibitor avasimibe diminishes macrophage and matrix metalloproteinase expression in atherosclerotic lesions of hypercholesterolemic rabbits[54] and reduces atherosclerosis; in addition it also reduces cholesterol in apoE*3-Leiden mice.[55] Sakashita et al.[56] demonstrated that ACAT-2 is also expressed in macrophage-derived foam cells in vitro and in vivo. Therefore, it is likely that esculeogenin A (**3**) prevents foam cell formation in HMDM by inhibiting the activity of both ACAT-1 and ACAT-2. Most known ACAT inhibitors, such as CI976[57] and avasimibe,[58] are highly hydrophobic because of their ring-shaped structures and alkyl chains. However, esculeogenin A (**3**) is considerably less hydrophobic than cholesterol because of its hydroxy and amino groups and the absence of an alkyl chain. Known ACAT inhibitors may be divided roughly into compounds that contain a urea group and compounds that contain an amide derivative. Esculeogenin A (**3**) does not belong to either of these structural classes.

Our group compared CI976,[59] a synthetic ACAT inhibitor, with the effects of esculeogenin A (**3**). Incubating HMDM with 5 μM CI976 inhibited "CE" accumulation by 90%. A dose of 50 μM of **3** was required to inhibit "CE" accumulation by 40% (data not shown), indicating that **3** is less effective in inhibiting foam cell formation and ACAT activity than CI976. However, preventive measures are important in deterring lifestyle-related diseases such as atherosclerosis, so an ACAT inhibitor that can be taken as part of a normal daily diet may be preferable to a potent synthetic compound. Since esculeoside A (**2**) occurs naturally in tomatoes, it can be consumed as a part of a normal diet and thus may play an important role in preventing atherosclerosis despite being less potent than CI976. Furthermore, tomatoes contain not only esculeoside A (**2**) but also lycopene, which inhibits the oxidation of circulating LDL[60] and may also decrease the risk of cardiovascular diseases. Further studies are required to elucidate the mechanism by which esculeogenin A (**3**) inhibits ACAT expression.

Supporting Information Available: Table of various preparation methods and yields of the tomato saponin esculeoside A (**2**), a metabolic scheme for the tomato steroidal glycoside esculeoside A (**2**), and a figure postulating the potential uses of tomato steroidal glycosides. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Sato, H.; Sakamura, S. *Agric. Biol. Chem.* **1973**, *37*, 225–231.
(2) Yahara, S.; Uda, N.; Nohara, T. *Phytochemistry* **1996**, *42*, 169–172.
(3) Nagaoka, T.; Yoshihara, T.; Sakamura, S. *Phytochemistry* **1987**, *26*, 2113–2114.
(4) Yoshihara, T.; Nagaoka, T.; Sakamura, S. *Phytochemistry* **1988**, *27*, 3982–3984.
(5) Nagaoka, T.; Yoshihara, T.; Ohra, J.; Sakamura, S. *Phytochemistry* **1993**, *34*, 1153–1157.
(6) Fujiwara, Y.; Yahara, S.; Ikeda, T.; Ono, M.; Nohara, T. *Chem. Pharm. Bull.* **2003**, *51*, 234–235.
(7) Fujiwara, Y.; Takaki, A.; Uehara, Y.; Ikeda, T.; Okawa, M.; Yamauchi, K.; Ono, M.; Yoshimitsu, H.; Nohara, T. *Tetrahedron* **2004**, *60*, 4915–4920.
(8) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501–507.
(9) Ito, S.; Takahama, H.; Kawaguchi, T.; Tanaka, S.; Iwaki, M. J. *Phytopathology* **2002**, *150*, 474–480.
(10) Hirai, Y.; Konishi, T.; Sanada, S.; Ida, Y.; Shoji, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3176–3184.
(11) Schreiber, K.; Ripperger, H. *Liebig's Ann.* **1962**, *655*, 114–135.
(12) Yoshizaki, M.; Matsushita, S.; Fujiwara, H.; Ono, M.; Nohara, T. *Chem. Pharm. Bull.* **2005**, *53*, 839–840.
(13) Fujiwara, Y.; Yoshizaki, M.; Matsushita, M.; Yahara, S.; Yae, E.; Ikeda, T.; Ono, M.; Nohara, T. *Chem. Pharm. Bull.* **2005**, *53*, 580–585.
(14) Ono, M.; Takara, Y.; Egami, M.; Uranaka, K.; Yoshimitsu, H.; Matsushita, S.; Fujiwara, Y.; Ikeda, T.; Nohara, T. *Chem. Pharm. Bull.* **2006**, *54*, 237–239.
(15) Ono, M.; Shiono, Y.; Yanai, Y.; Fujiwara, Y.; Ikeda, T.; Nohara, T. *Chem. Pharm. Bull.* **2008**, *56*, 1499–1501.
(16) Manabe, H.; Murakami, Y.; El-Aasr, M.; Ikeda, T.; Fujiwara, Y.; Ono, M.; Nohara, T. *J. Nat. Med.*, in press.
(17) Tiziani, S.; Schwatrz, S. J.; Vodovotz, Y. *J. Agric. Food Chem.* **2006**, *54*, 6094–6100.
(18) Matsushita, S.; Yoshizaki, M.; Fujiwara, Y.; Ikeda, T.; Ono, M.; Okawara, T.; Nohara, T. *Tetrahedron Lett.* **2005**, *46*, 3549–3551.
(19) Nohara, T.; Yabuta, H.; Suenobu, M.; Hida, R.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1973**, *21*, 1240–1247.
(20) Zhu, X.; Tsumagari, H.; Honbu, T.; Ikeda, T.; Ono, H.; Nohara, T. *Tetrahedron Lett.* **2001**, *42*, 8043–8046.
(21) Dong, M.; Feng, X.; Wang, B.; Wu, L.; Ikejima, T. *Tetrahedron* **2001**, *57*, 501–506.

(22) Tran, Q.; Tezuka, Y.; Banskota, A. H.; Tran, Q. K.; Saiki, I.; Kadota, S. *J. Nat. Prod.* **2001**, *64*, 1127–1132.
(23) Yokosuka, A.; Mimaki, Y.; Sashida, Y. *J. Nat. Prod.* **2002**, *65*, 1293–1298.
(24) Mimaki, Y.; Watanabe, K.; Sakagami, H.; Sashida, Y. *J. Nat. Prod.* **2002**, *65*, 1863–1868.
(25) Yin, J.; Kouda, K.; Tezuka, Y.; Tran, Q. L.; Miyahara, T.; Chen, Y.; Kadota, S. *J. Nat. Prod.* **2003**, *66*, 646–650.
(26) Liu, H.; Xiong, Z.; Li, F.; Qu, G.; Kobayashi, H.; Yao, X. *Chem. Pharm. Bull.* **2003**, *51*, 1089–1091.
(27) Ikeda, T.; Tsumagari, H.; Okawa, M.; Nohara, T. *Chem. Pharm. Bull.* **2004**, *52*, 142–145.
(28) Noguchi, E.; Fujiwara, Y.; Matsushita, S.; Ikeda, T.; Ono, M.; Nohara, T. *Chem. Pharm. Bull.* **2006**, *54*, 1312–1314.
(29) Yoshikawa, M. *Kagaku to Seibutsu* **2002**, *40*, 172–178.
(30) Liu, H.; Xiong, Z.; Li., F.; Ou, G.; Kobayashi, H.; Yao, X. *Chem. Pharm. Bull.* **2003**, *51*, 1089–1091.
(31) Yin, J.; Kouda, K.; Tezuka, Y.; Tran, Q. L.; Miyahara, T.; Chen, Y.; Kadota, S. *J. Nat. Prod.* **2003**, *66*, 646–650.
(32) Cham, B. E.; Daunter, B. *Cancer Lett.* **1990**, *55*, 221–225.
(33) Fujiwara, Y.; Kiyota, N.; Hori, M.; Matsushita, S.; Iijima, Y.; Aoki, K.; Shibata, D.; Takeya, M.; Ikeda, T.; Nohara, T.; Nagai, R. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 2400–2406.
(34) Ross, R. *Am. Heart J.* **1999**, *138*, 419–420.
(35) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. *N. Engl. J. Med.* **1989**, *320*, 915–924.
(36) Kodama, T.; Freeman, M.; Rohrer, L.; Zabrecky, J.; Matsudaira, P.; Krieger, M. *Nature* **1990**, *343*, 531–535.
(37) Endemann, G.; Stanton, L. W.; Madden, K. S.; Bryant, C. M.; White, R. T.; Protter, A. A. *J. Biol. Chem.* **1993**, *268*, 11811–11816.
(38) Acton, S. L.; Scherer, P. E.; Lodish, H. F.; Krieger, M. *J. Biol. Chem.* **1994**, *269*, 21003–21009.
(39) Chang, T. Y.; Chang, C. C.; Lin, S.; Yu, C.; Li, B. L.; Miyazaki, A. *Curr. Opin. Lipidol.* **2001**, *12*, 289–296.
(40) Chang, C. C.; Huh, H. Y.; Cadigan, K. M.; Chang, T. Y. *J. Biol. Chem.* **1993**, *268*, 20747–20755.
(41) Oelkers, P.; Behari, A.; Cromley, D.; Billheimer, J. T. *J. Biol. Chem.* **1998**, *273*, 26765–26771.
(42) Rudel, L. L.; Lee, R. G.; Cockman, T. L. *Curr. Opin. Lipidol.* **2001**, *12*, 121–127.
(43) Koyama, N.; Kuribayashi, K.; Seki, T.; Kobayashi, K.; Furuhata, Y.; Suzuki, K.; Arisaka, H.; Nakano, T.; Amino, Y.; Ishii, K. *J. Agric. Food. Chem.* **2006**, *54*, 4970–4976.
(44) Luan, Y.; Griffiths, H. R. *Arch. Biochem. Biophys.* **2006**, *450*, 89–99.
(45) Namatame, I.; Tomoda, H.; Ishibashi, S.; Omura, S. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 737–742.
(46) Yagyu, H.; Ishibashi, S.; Chen, Z.; Osuga, J.; Okazaki, M.; Perrey, S.; Kitamine, T.; Shimada, M.; Ohashi, K.; Harada, K.; Shionoiri, F.; Yahagi, N.; Gotoda, T.; Yazaki, Y.; Yamada, N. *J. Lipid. Res.* **1999**, *40*, 1677–1685.
(47) Matsui, Y.; Horiuchi, K.; Yamamoto, K.; Kanai, K. *Jpn. J. Pharmacol.* **2001**, *85*, 423–433.
(48) Bell, F. P.; Gammill, R. B.; St. John, L. C. *Atherosclerosis* **1992**, *92*, 115–122.
(49) Ozaki, K.; Kubo, T.; Imaki, R.; Shinagawa, H.; Fukaya, H.; Ohtaki, K.; Ozaki, S.; Izumi, T.; Aizawa, Y. *J. Atheroscler. Thromb.* **2006**, *13*, 216–219.
(50) Buhman, K. K.; Accad, M.; Novak, S.; Choi, R. S.; Wong, J. S.; Hamilton, R. L.; Turley, S.; Farese, R. V. *J. Nat. Med.* **2000**, *6*, 1341–1347.
(51) Kusunoki, J.; Hansoty, D. K.; Aragane, K.; Fallon, J. T.; Badimon, J. J.; Fisher, E. A. *Circulation* **2001**, *103*, 2604–2609.
(52) Azuma, Y.; Date, K.; Ohno, K.; Matsushiro, S.; Nobuhara, Y.; Yamada, T. *Jpn. J. Pharmacol.* **2001**, *86*, 120–123.
(53) Aragane, K.; Kojima, K.; Fujinami, K.; Kamei, J.; Kusunoki, J. *Atherosclerosis* **2001**, *158*, 139–145.
(54) Bocan, T. M.; Krause, B. R.; Rosebury, W. S.; Mueller, S. B.; Lu, X.; Dagle, C. *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 70–79.
(55) Delsing, D. J.; Offerman, E. H.; van Duyvenvoorde, W.; van Der Boom, H.; de Wit, E. C.; Gijbels, M. J.; van Der Laarse, A.; Jukema, J. W.; Havekes, L. M.; Princen, H. M. *Circulation* **2001**, *103*, 1778–1786.
(56) Sakashita, N.; Miyazaki, A.; Chang, C. C.; Chang, T. Y.; Kiyota, E.; Satoh, M.; Komohara, Y.; Morganelli, P. M.; Horiuchi, S.; Takeya, M. *Lab. Invest.* **2003**, *83*, 1569–1581.
(57) Krause, B. R.; Anderson, M.; Bisgaier, C. L.; Bocan, T.; Bousley, R.; DeHart, P.; Essenburg, A.; Hamelehle, K.; Homan, R.; Kieft, K. *J. Lipid Res.* **1993**, *34*, 279–294.
(58) Lee, H. T.; Sliskovic, D. R.; Picard, J. A.; Roth, B. D.; Wierenga, W.; Hicks, J. L.; Bousley, R. F.; Hamelehle, K. L.; Homan, R.; Speyer, C.; Stanfield, R. L.; Krause, B. R. *J. Med. Chem.* **1996**, *39*, 5031–5034.
(59) Bocan, T. M.; Mueller, S. B.; Uhlendorf, P. D.; Newton, R. S.; Krause, B. R. *Arterioscler. Thromb.* **1991**, *11*, 1830–1843.
(60) Morris, D. L.; Kritchevsky, S. B.; Davis, C. E. *J. Am. Med. Assoc.* **1994**, *272*, 1439–1441.

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