Synthesis and Characterization of a Series of Novel Monoacylated Ascorbic Acid Derivatives, 6-*O*-Acyl-2-*O*-α-D-glucopyranosyl-L-ascorbic Acids, as Skin Antioxidants

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A series of novel monoacylated vitamin C derivatives were chemically synthesized with a stable ascorbate derivative, 2-*O*-α-D-glucopyranosyl-L-ascorbic acid (AA-2G), and acid anhydrides in pyridine. Their solubility in organic phase, thermal stability, radical scavenging activity, and in vitro skin permeability was evaluated. These monoacylated derivatives were identified as 6-*O*-acyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acids (6-Acyl-AA-2G) by UV spectra, elemental analyses, and nuclear magnetic resonance spectroscopy. The reactions afforded 6-Acyl-AA-2G in high yields (30−60%). 6-Acyl-AA-2G exhibited satisfactory stability in neutral solution comparable to that of a typical stable derivative, AA-2G, and also showed the radical scavenging activity. The lipid solubility of 6-Acyl-AA-2G was increased with increasing length of their acyl group. Increased skin permeability was superior to those of AA-2G and ascorbic acid (AsA). 6-Acyl-AA-2G that is susceptible to enzymatic hydrolysis by tissue esterase and/or α-glucosidase produces AA-2G and AsA, which is in the skin tissues. Thus, these findings indicate that the novel vitamin C derivatives presented here, 6-Acyl-AA-2G, may be effective antioxidants in skin care and medicinal use.

Introduction

The well-known susceptibility of vitamin C to thermal and oxidative degradation has led to interest in derivatives with increased stability. The maintenance of the inherent biological activity in vivo, in particular, the chemical modification of hydroxyl groups of vitamin C at the carbon 2 or 3, is of interest. Numerous stable derivatives of vitamin C have been reported.[1] Among these derivatives, 2-*O*-α-D-glucopyranosyl-L-ascorbic acid (AA-2G)[2−8] is the most promising ascorbic acid (AsA) derivative, because this vitamin C derivative is markedly stable in vitro and exhibits vitamin C activity in vivo after enzymatic degradation to free AsA by α-glucosidase. This stable hydrophilic vitamin C derivative has been admitted as an ingredient in skin care by the Japanese Government. It is already available as an additive in commercial cosmetics.

On the other hand, the rigid lipid lamella structure of the stratum corneum works as a barrier, especially for the absorption of the hydrophilic compounds.[9] Therefore, chemical modification of the hydroxy moiety of vitamin C with a lipophilic molecule is necessary for the development of an efficient transdermal vitamin C derivative. A variety of lipophilic vitamin C derivatives have been developed,[10−12] but no ideal vitamin C derivatives have been available in terms of stability, lipophilicity, and biological activity.

Fatty acid esters of carbohydrates have been used as surfactants and emulsifiers by the food, detergent, and cosmetic industry for a number of years.[13] These compounds are typically nonionic, nontoxic, and biodegradable.[14,15] The chemical acylation of carbohydrates has been previously reported.[16,17] Acylation of carbohydrates can be accomplished efficiently and inexpensively using base-catalyzed transacylation. It is, therefore, expected that the development of acylated AA-2G might be suitable for improved skin penetration of AA-2G.

In this paper, we describe the synthesis of a series of novel acylated vitamin C derivatives, 6-*O*-acyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acids, which exhibit vitamin C activity in vitro and in vivo after enzymatic hydrolysis to free AsA by α-glucosidase and esterase. The stability in neutral solution, radical scavenging activity, solubility in the organic phase, and skin permeation are also described.

Results and Discussion

Synthesis of 6-Acyl-AA-2G. AA-2G was coupled with acid anhydrides with various chain lengths in pyridine to give a series of monoacylated AA-2G derivatives (6-*O*-acyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acids, 6-Acyl-AA-2G). They were identified as 6-butyryl-, 6-hexanoyl-, 6-octanoyl-, 6-decanoyl-, 6-dodecanoyl-, 6-tetradecanoyl-, 6-hexadecanoyl-, and 6-octadecanoyl-ascorbate 2-glucoside (abbreviations: 6-Buty-AA-2G, 6-Hexa-AA-2G, 6-Octa-AA-2G, 6-Deca-AA-2G, 6-Dode-AA-2G, 6-Myri-AA-2G, 6-Palm-AA-2G, and 6-Stea-AA-2G, respectively) by UV spectra, elemental analyses, and nuclear magnetic resonance spectroscopy (Figure 1). The products had superior yields (30−60%) when the molar ratio of AA-2G to acid anhydrides was essentially a 1:1.2 ratio.

Stability of 6-Acyl-AA-2G in Aqueous Solution. The stability of 6-Acyl-AA-2G in a neutral aqueous

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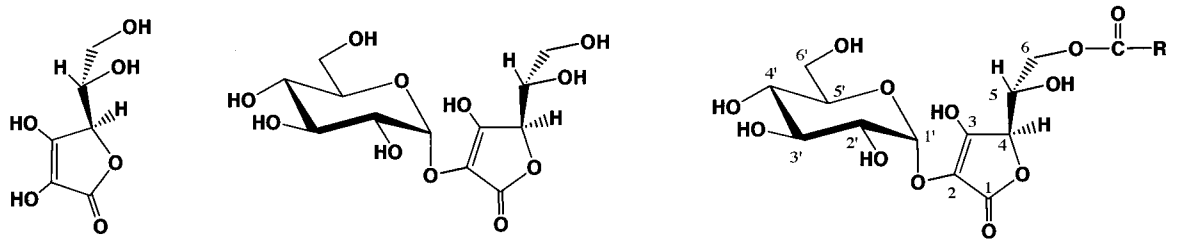

L-Ascorbic acid (AsA) 2-*O*-α-D-Glucopyranosyl-L-ascorbic acid (AA-2G) 6-*O*-Acyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acid (6-Acyl-AA-2G)

6-Acyl-AA-2G R = $(CH_2)_2CH_3$: 6-*O*-Butyryl-2-*O*-α-D-glucopyranosyl-L-ascorbic acid (6-Buty-AA-2G)
$(CH_2)_4CH_3$: 2-*O*-α-D-Glucopyranosyl-6-*O*-hexanoyl-L-ascorbic acid (6-Hexa-AA-2G)
$(CH_2)_6CH_3$: 2-*O*-α-D-Glucopyranosyl-6-*O*-octanoyl-L-ascorbic acid (6-Octa-AA-2G)
$(CH_2)_8CH_3$: 6-*O*-Decanoyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acid (6-Deca-AA-2G)
$(CH_2)_{10}CH_3$: 6-*O*-Dodecanoyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acid (6-Dode-AA-2G)
$(CH_2)_{12}CH_3$: 2-*O*-α-D-Glucopyranosyl-6-*O*-tetradecanoyl-L-ascorbic acid (6-Myri-AA-2G)
$(CH_2)_{14}CH_3$: 2-*O*-α-D-Glucopyranosyl-6-*O*-hexadecanoyl-L-ascorbic acid (6-Palm-AA-2G)
$(CH_2)_{16}CH_3$: 2-*O*-α-D-Glucopyranosyl-6-*O*-octadecanoyl-L-ascorbic acid (6-Stea-AA-2G)

AA-2G: a stable hydrophilic ascorbic acid derivative

Figure 1. Structure and nomenclature of a series of novel amphipathic AsA derivatives, 6-*O*-acyl-2-*O*-α-D-glucopyranosyl-L-ascorbic acid (6-Acyl-AA-2G).

Table 1. Partition of 6-Acyl-AA-2G between *n*-Octanol and Water (pH 7.0)[a]

sample	distribution percent in octanol	sample	distribution percent in octanol
AsA	0.7 ± 0.8	6-Deca-AA-2G	76.6 ± 5.7
AA-2G	0	6-Dode-AA-2G	83.8 ± 6.5
6-Buty-AA-2G	4.0 ± 0.1	6-Myri-AA-2G	87.0 ± 6.0
6-Hexa-AA-2G	8.7 ± 1.1	6-Palm-AA-2G	87.3 ± 6.3
6-Octa-AA-2G	43.4 ± 2.0	6-Stea-AA-2G	84.6 ± 2.8

[a] Five milliliters of 50 mM potassium phosphate buffer in which 1 mM 6-Acyl-AA-2G was dissolved and 5 mL of *n*-octanol was mixed and partitioned. The amount of 6-Acyl-AA-2G in octanol was analyzed by HPLC. Each value represents the mean ± SD ($n = 6$).

solution (1 mM) at 37 °C was evaluated on the basis of remaining ratio measured by high-performance liquid chromatography (HPLC; Figure 2). It was found that 82−93% of 6-Acyl-AA-2G remained intact after 5 days (Figure 2a,b). The difference in stability among their derivatives was not observed. A major degradation product of 6-Acyl-AA-2G was found to be AA-2G. AA-2G was also scarcely degraded during the tested period, while AsA disappeared within 2 days (Figure 2c). These results indicated that 6-Acyl-AA-2G was approximately stable under the nonenzymatic condition, suggesting the potency of 6-Acyl-AA-2G as an AsA supply source.

Partition of 6-Acyl-AA-2G between *n*-Octanol and Water. The partition between *n*-octanol and water is generally accepted to be a suitable model for studying affinity on cell membranes or on the corneal layer of the epidermis.[9] The lipophilicity of 6-Acyl-AA-2G was investigated with an *n*-octanol/50 mM potassium phosphate buffer (pH 7.0) system. The distribution percent of 6-Acyl-AA-2G in octanol increased with increasing length of their acyl group (Table 1). It was found that 6-Myri-, 6-Palm-, and 6-Stea-AA-2G with the longer acyl group completely reached the maximal distribution in octanol. The distribution percent of 6-Dode-, 6-Deca-, 6-Octa-, 6-Hexa-, and 6-Buty-AA-2G was found to be 83.8, 76.6, 43.4, 8.7, and 4.0%, respectively. In contrast, AsA and AA-2G were not distributed into octanol. These results indicated that the affinity of 6-Acyl-AA-2G for the organic layer mainly reflected the length of their acyl group as a lipophilic group.

Table 2. Radical Scavenging Activity of AA-2G and 6-Acyl-AA-2G against DPPH[a]

antioxidant	scavenging activity (%) (at 10^{-4} M)	EC_{50} (10^{-5} M)
AsA	94.3 ± 1.7	2.2
ascorbyl 6-palmitate	95.7 ± 0.1	2.9
ascorbyl 2,6-dipalmitate	23.7 ± 7.2	
AsA 2-phosphate	1.5 ± 0.7	
AsA 2-sulfate	0.0 ± 0.8	
AA-2G	68.9 ± 1.1	6.1
6-Buty-AA-2G	69.2 ± 3.6	5.9
6-Hexa-AA-2G	73.8 ± 1.2[b]	5.4
6-Octa-AA-2G	76.6 ± 0.7[c,e]	5.7
6-Deca-AA-2G	73.6 ± 3.7	4.2
6-Dode-AA-2G	76.2 ± 0.8[c]	4.6
6-Myri-AA-2G	74.1 ± 1.2[c]	4.4
6-Palm-AA-2G	76.4 ± 1.4[c]	4.1
6-Stea-AA-2G	77.5 ± 0.1[d,e]	4.4
vitamin E	90.9 ± 0.7	1.9

[a] After 20 min of reaction, the free radical scavenging activity of each antioxidant was quantified by the decolorization of DPPH (10^{-4} M) at 516 nm. The EC_{50} value was determined as the concentration of each sample required to give 50% of the absorbance shown by a blank test. Each value is the average ± SD of triplicate determinations. The statistical significance of differences in the mean of each data was calculated with Student's *t* test. [b] $p < 0.05$. [c] $p < 0.01$. [d] $p < 0.001$ as compared with AA-2G. [e] $p < 0.05$ as compared with 6-Buty-AA-2G.

Free Radical Scavenging Activity of 6-Acyl-AA-2G. The radical scavenging activity of 6-Acyl-AA-2G was measured by use of a relatively stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the results are shown in Table 2. At 10^{-4} M, which corresponds to an equal molar amount of DPPH, AA-2G and 6-Acyl-AA-2G exhibited the radical scavenging activity, although their activities (68.9−77.5%) were lower than that of AsA (94.3%), a conventional lipophilic ascorbate derivative, ascorbyl 6-palmitate (95.7%), and a typical lipophilic antioxidant, vitamin E (90.9%). It was also found that the activity of each 6-Acyl-AA-2G was slightly superior to AA-2G. Furthermore, 6-Acyl-AA-2G tended to increase the scavenging activity with increasing length of their acyl group. In contrast, ascorbyl 2,6-dipalmitate, AsA 2-phosphate, and AsA 2-sulfate, which

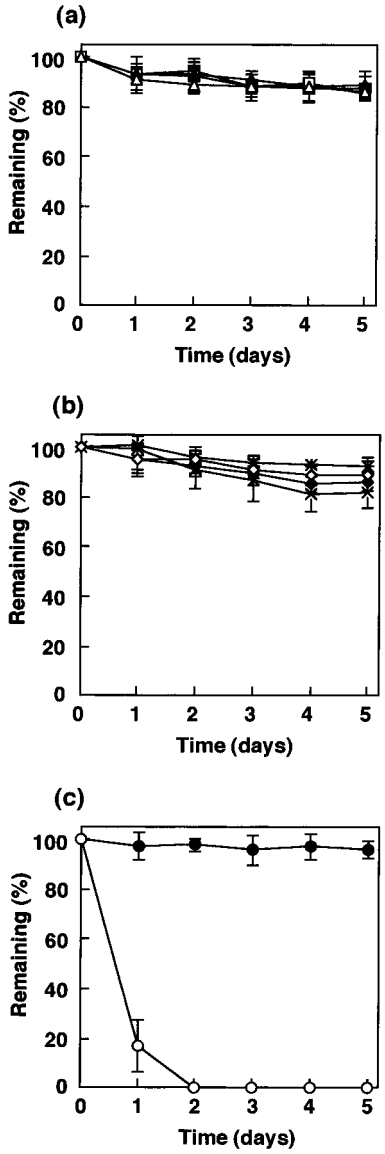

Figure 2. Stability of 6-Acyl-AA-2G in aqueous solution. AsA (○), AA-2G (●), 6-Buty-AA-2G (△), 6-Hexa-AA-2G (▲), 6-Octa-AA-2G (□), 6-Deca-AA-2G (■), 6-Dode-AA-2G (◇), 6-Myri-AA-2G (◆), 6-Palm-AA-2G (×), and 6-Stea-AA-2G (*) were dissolved in 50 mM potassium phosphate buffer (pH 7.0) to give 1 mM solution and then incubated at 37 °C for the indicated time. The concentration of the test compounds was analyzed by HPLC. Each value represents the mean ± SD ($n = 6$).

were stable 2-*O*-substituted ascorbate derivatives such as AA-2G and 6-Acyl-AA-2G, exhibited only limited activity. Especially, the radical scavenging activity was not observed in AsA 2-sulfate.

On the other hand, the 50% effect concentration (EC_{50}) was calculated from the dose response curve of radical scavenging activity, which describes the characteristic profile of a radical scavenger. The EC_{50} of each 6-Acyl-AA-2G ($4.1-5.9 \times 10^{-5}$ M) was smaller than that of AA-2G (6.1×10^{-5} M), indicating that 6-Acyl-AA-2G is more efficient than AA-2G in terms of the radical scavenging potency. As mentioned above, the EC_{50} values also suggested that 6-Acyl-AA-2G tended to increase the scavenging activity with increasing length of their acyl group. In addition, the EC_{50} of ascorbyl 6-palmitate (2.9×10^{-5} M) was almost the same as that of AsA (2.2×10^{-5} M). In contrast, the EC_{50} of ascorbyl 2,6-dipalmitate, AsA 2-phosphate, and AsA 2-sulfate could not be determined, indicating that the radical scavenging activities are extremely weak.

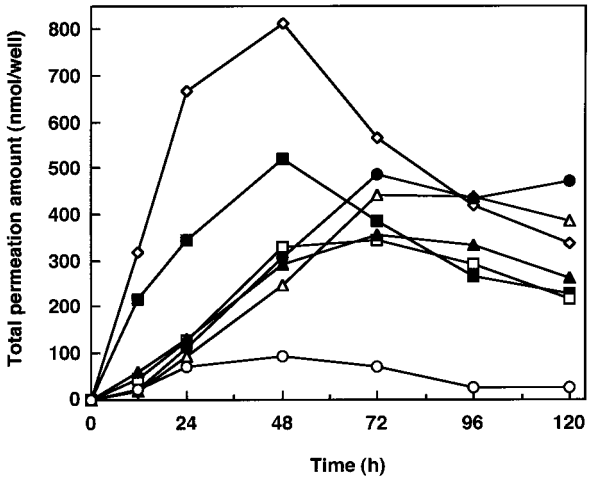

Figure 3. Permeability of 6-Acyl-AA-2G in TESTSKIN, an organotypic model of human skin. AsA (○), AA-2G (●), 6-Buty-AA-2G (△), 6-Hexa-AA-2G (▲), 6-Octa-AA-2G (□), 6-Deca-AA-2G (■), and 6-Dode-AA-2G (◇) were applied to TESTSKIN equipped in the assay plate and then incubated for 120 h at 37 °C in a CO_2 incubator. The permeation level was expressed as a total amount of 6-Acyl-AA-2G and its metabolites (AA-2G and AsA). Each value is the average of triplicate experiments.

These results suggested that the difference in the radical scavenging activity of 2-*O*-substituted ascorbate derivatives was attributed to the linkage type of the substituent group to the enediol−lactone resonant system. Kato et al.[11] have shown that 2-*O*- and 3-*O*-monoalkylascorbic acid had the same electron-donating potency as AsA under similar assay conditions. AA-2G and 6-Acyl-AA-2G are the same as the above *O*-alkyl type and thus have ether linkages, while ascorbyl 2,6-dipalmitate, AsA 2-phosphate, and AsA 2-sulfate possess ester linkages. Thus, introduction of an electron-attracting group into the enediol−lactone resonant system may hinder dehydrogenation at C-3 of AsA, resulting in the reduced radical scavenging activity. In addition, it seems that the difference of the radical scavenging activity between AA-2G and 6-Acyl-AA-2G was caused by an interaction between the enediol−lactone system and the acyl group. In the UV absorption spectra (see Experimental Section) under acidic conditions, AA-2G and each 6-Acyl-AA-2G exhibited characteristic absorptions at 236 and 233 nm, respectively. Under basic conditions, however, they showed the same absorption maximum at 261 nm. These results indicated that 6-Acyl-AA-2G might form an intramolecular hydrogen bond between the C-3 hydroxyl group of AsA and the carboxylic group of the acyl chain. In the actual, 6-Acyl-AA-2G seems to be enzymatically metabolized to AA-2G and then AsA and to play an important part in antioxidation as an AsA source in vivo.

Skin Permeation Assay. Skin penetration of 6-Acyl-AA-2G was evaluated using an organotype model of human skin, living skin equivalent (LSE).[18−20] The time course of permeation amount in the receptor compartment was investigated after 200 μL of 10 mM 6-Acyl-AA-2G solution was applied to LSE, namely, TESTSKIN (Figure 3). To get many of the derivatives into solution,

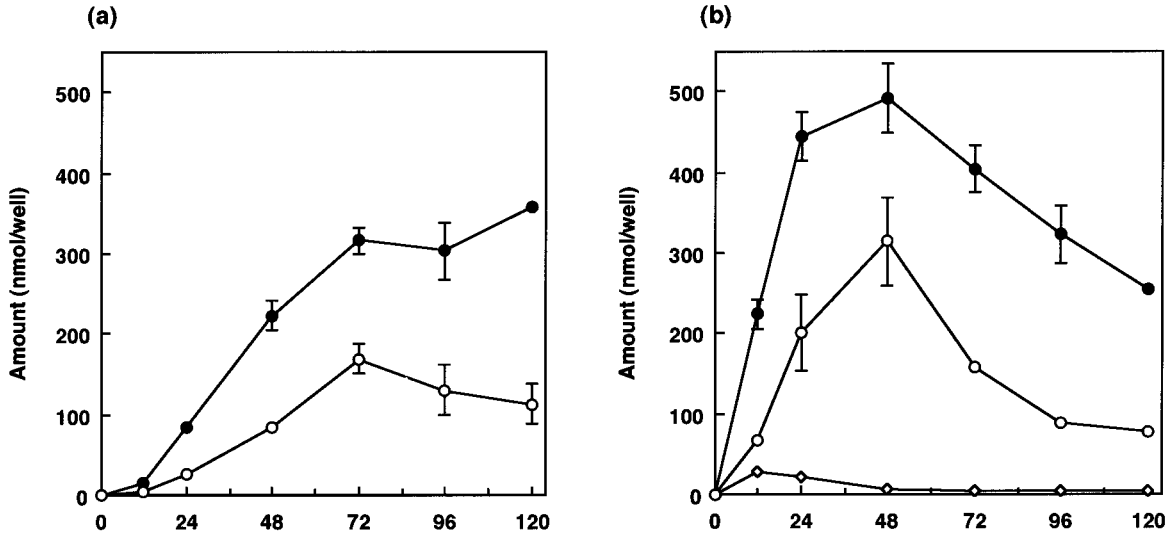

Figure 4. Time course of permeation and metabolism after aplication of AA-2G (a) and 6-Dode-AA-2G (b) to TESTSKIN. AA-2G and 6-Dode-AA-2G were applied to TESTSKIN equipped in the assay plate and then incubated for 120 h at 37 °C. The diffused compounds by AA-2G and 6-Dode-AA-2G application were identified as AsA (○) and AA-2G (●) and as AsA, AA-2G, and 6-Dode-AA-2G (◇), respectively. Each value represents the mean ± SD of three experiments.

dimethyl sulfoxide (DMSO, 5%) was used as a cosolvent in this assay, although it may affect the ability of these compounds to permeate into the skin. Their permeation values were evaluated at a total amount of 6-Acyl-AA-2G and its metabolites, AA-2G and AsA, to compare the ability as an AsA source. It was found that the permeation amount of 6-Deca- and 6-Dode-AA-2G rapidly increased after the beginning, reached a maximum after 48 h, and decreased gradually. Their initial values were much larger than the others and showed skin permeability superior to those of AA-2G and AsA. These results were also consistent with the partition data (Table 1). The maximal penetration dose (ratio) of 6-Deca- and 6-Dode-AA-2G was approximately 500 (25%) and 800 nmol (40%) per well, respectively. It was observed that the permeation amount of AA-2G slowly increased and, after 72 h of incubation, reached the maximum level (490 nmol), which was maintained in the following incubation period. The changes in the permeation amount of 6-Buty-, 6-Hexa-, and 6-Octa-AA-2G were similar to the case of AA-2G. In contrast, the penetration ability of AsA was much less than that of all tested compounds.

Under the nonenzymatic condition, Figure 2 showed that AA-2G and 6-Acyl-AA-2G were approximately stable. In view of the metabolism of the derivatives in the skin, the data of Figure 3 were reevaluated at each amount of the permeated compounds into the receptor compartment. Figure 4 shows the time course of permeation and metabolism after the application of AA-2G and 6-Dode-AA-2G to TESTSKIN. The diffused compounds by AA-2G and 6-Dode-AA-2G application were identified as AsA and AA-2G and as AsA, AA-2G, and 6-Dode-AA-2G, respectively. In the application of AA-2G, it was observed that the permeated compounds, AsA and AA-2G, were gradually increased (Figure 4a). The AA-2G level reached the plateau 72 h after incubation and was maintained, while AsA reached a maximum after 72 h and decreased slightly. The presence of AsA in the receptor compartment indicated that the permeated AA-2G was hydrolyzed with α-glucosidase to give AsA in the skin tissue. These results suggested that AA-2G was slowly supplied to the skin, and the supply of AA-2G after 72 h was the same level as the metabolism. In the application of 6-Dode-AA-2G, it was found that both AsA and AA-2G levels were rapidly increased, reached a maximum after 48 h, and decreased gradually (Figure 4b). In contrast, the 6-Dode-AA-2G level was observed in the trace amounts. The profiles suggested that the permeation rate of 6-Dode-AA-2G was much faster than that of AA-2G. Thus, it seems that 6-Dode-AA-2G was rapidly permeated into TESTSKIN, hydrolyzed with esterase and/or α-glucosidase in the tissue, and then released AsA and AA-2G to the receptor compartment. A similar result was observed in the case of 6-Deca-AA-2G (data not shown). After the permeation maximum, the decrease of AA-2G and AsA in the receptor compartment seemed to be caused by the decreased supply of 6-Dode-AA-2G to the skin. Furthermore, the decrease of the AA-2G level may be attributed to the hydrolysis by α-glucosidase leaked from the skin and AsA to the unstable characteristics as shown in Figure 2. These results indicated that 6-Acyl-AA-2G was superior to AA-2G as a vitamin C supplement in the skin model.

We have prepared a series of novel monoacylated vitamin C derivatives from a stable ascorbate derivative AA-2G and acid anhydrides in pyridine in 30−60% yields. The monoacylated derivatives were identified as 6-Acyl-AA-2G by UV spectra, elemental analyses, and nuclear magnetic resonance spectroscopy. It was found that 6-Octa-, 6-Deca-, and 6-Dode-AA-2G are amphipathic and exhibit satisfactory thermal stability and improved skin permeability. Of 6-Acyl-AA-2G derivatives, 6-Myri-, 6-Palm-, and 6-Stea-AA-2G were lipophilic and exhibited satisfactory thermal stability and improved skin permeability superior to those of AA-2G and AsA (data not shown). 6-Acyl-AA-2G, which exhibits radical scavenging activity, is susceptible to enzymatic hydrolysis by tissue esterase and/or α-glucosidase to produce AA-2G and AsA. Thus, these findings indicate that the novel vitamin C derivatives presented here, some of 6-Acyl-AA-2G, may be effective antioxidants in skin care and medicinal use.

Experimental Section

General Experimental Procedure. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 Instrument with sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 or tetramethylsilane. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were obtained at JASCO DIP-1000. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. UV spectra were obtained on a Shimadzu UV-1200 spectrophotometer. The HPLC analyses were carried out with a system consisting of a Shimadzu SCL-10A system controller, LC-10AD pump, SPD-10AV UV−vis spectrophotometric detector, SIL-10AD auto injector, CTO-6A column oven, and C-R7A chromatopac.

Chemicals. AA-2G was prepared by the method described previously.[21] ^{1}H NMR (500 MHz, D_2O): δ 3.51 (1H, t, J = 9.8 Hz, 4′-H), 3.68 (1H, dd, J = 3.8, 9.8 Hz, 2′-H), 3.75 (2H, d, J = 7.0 Hz, 6-H_2), 3.78 (2H, d, J = 3.4 Hz, 6′-H_2), 3.86 (1H, t, J = 9.8 Hz, 3′-H), 3.99 (1H, dt, J = 3.4, 9.8 Hz, 5′-H), 4.10 (1H, td, J = 1.4, 7.0 Hz, 5-H), 5.00 (1H, d, J = 1.4 Hz, 4-H), 5.57 (1H, d, J = 3.8 Hz, 1′-H). ^{13}C NMR (125 MHz, D_2O): δ 62.82 (6′-C), 64.73 (6-C), 71.54 (5-C), 71.67 (4′-C), 73.78 (2′-C), 75.22 (3′-C), 75.74 (5′-C), 79.25 (4-C), 101.60 (1′-C), 120.33 (2-C), 165.70 (3-C), 175.03 (1-C). *n*-Butyric anhydride, *n*-hexanoic anhydride, *n*-octanoic anhydride, *n*-capric anhydride, lauric anhydride, myristic anhydride, palmitic anhydride, stearic anhydride, DPPH, ascorbyl 6-palmitate, and ascorbyl 2,6-dipalmitate were purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo. AsA and AsA phosphate magnesium salt *n*-hydrate were obtained from Wako Pure Chemical Industries, Ltd., Osaka. AsA 2-sulfate barium salt (Sigma) was commercially available. α-Tocopherol (vitamin E) was purchased from Nacalai Tesque, Inc., Kyoto.

General Procedure of Monoacylation. 6-*O*-Butyryl-2-*O*-α-D-glucopyranosyl-L-ascorbic Acid (6-Buty-AA-2G). A mixture of AA-2G (5.0 g, 14.8 mmol) and *n*-butyric anhydride (4.3 mL, 26.6 mmol) in pyridine (50 mL) was stirred for 1 h at 60 °C. The reaction mixture was concentrated in vacuo. The residue was dissolved in 100 mL of water and partitioned with EtOAc (2 × 100 mL). The water layer was concentrated to dryness in vacuo, then dissolved in acetone, and allowed to stand until there was no further increase of precipitate. After the mixture was filtered, crude product (2.29 g, 38.0% yield) was obtained as a white powder. The white powder was recrystallized from EtOH to give 6-Buty-AA-2G (1.04 g, 17.2% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 234 (9300). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (13 900). ^{1}H NMR (500 MHz, CD_3OD): δ 0.96 (3H, t, J = 7.3 Hz), 1.66 (2H, sxt, J = 7.3 Hz), 2.35 (2H, t, J = 7.3 Hz), 3.40 (1H, dd, J = 9.4, 10.3 Hz, 4′-H), 3.53 (1H, dd, J = 3.4, 9.4 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.7 Hz, 6′-Ha), 3.78 (1H, t, J = 9.4 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 11.7 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 10.3 Hz, 5′-H), 4.13 (1H, ddd, J = 2.0, 5.9, 7.3 Hz, 5-H), 4.19 (1H, dd, J = 5.9, 11.2 Hz, 6-Ha), 4.26 (1H, dd, J = 7.3, 11.2 Hz, 6-Hb), 4.82 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.4 Hz, 1′-H). ^{13}C NMR (125 MHz, CD_3OD): δ 13.89, 19.22, 36.65, 62.08 (6′-C), 65.43 (6-C), 67.78 (5-C), 71.00 (4′-C), 73.19 (2′-C), 74.32 (3′-C), 74.66 (5′-C), 77.16 (4-C), 101.57 (1′-C), 120.27 (2-C), 161.74 (3-C), 171.90, 174.73 (1-C). Anal. ($C_{16}H_{24}O_{12}$) C, H. $[\alpha]^{24}_D$ +160.7° (c = 0.50, MeOH). mp 163.5−166.5 °C.

2-*O*-α-D-Glucopyranosyl-6-*O*-hexanoyl-L-ascorbic Acid (6-Hexa-AA-2G). The synthetic reaction was essentially the same as 6-Buty-AA-2G. From AA-2G (5.0 g, 14.8 mmol) and *n*-hexanoic anhydride (6.2 mL, 26.6 mmol), 6-Hexa-AA-2G was obtained as a white powder (2.28 g, 35.3% yield) and then as a crystal (1.59 g, 24.6% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (10 300). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (15 300). ^{1}H NMR (500 MHz, CD_3OD): δ 0.91 (3H, t, J = 6.8 Hz), 1.34 (4H, m), 1.64 (2H, qn, J = 7.6 Hz), 2.37 (2H, t, J = 7.6 Hz), 3.39 (1H, dd, J = 9.1, 10.3 Hz, 4′-H), 3.52 (1H, dd, J = 3.7, 9.1 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.7 Hz, 6′-Ha), 3.78 (1H, t, J = 9.1 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 11.7 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 10.3 Hz, 5′-H), 4.13 (1H, ddd, J = 2.0, 6.4, 6.8 Hz, 5-H), 4.18 (1H, dd, J = 6.4, 10.7 Hz, 6-Ha), 4.26 (1H, dd, J = 6.8, 10.7 Hz, 6-Hb), 4.81 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.7 Hz, 1′-H). ^{13}C NMR (125 MHz, CD_3OD): δ 14.19, 23.20, 25.49, 32.22, 34.72, 62.08 (6′-C), 65.43 (6-C), 67.76 (5-C), 71.00 (4′-C), 73.19 (2′-C), 74.31 (3′-C), 74.65 (5′-C), 77.14 (4-C), 101.57 (1′-C), 120.27 (2-C), 161.73 (3-C), 171.88, 174.87 (1-C). Anal. ($C_{18}H_{28}O_{12}$) C, H. $[\alpha]^{24}_D$ +149.3° (c = 0.50, MeOH). mp 175.5−177.5 °C.

2-*O*-α-D-Glucopyranosyl-6-*O*-octanoyl-L-ascorbic Acid (6-Octa-AA-2G). A mixture of AA-2G (3.42 g, 10.1 mmol) and *n*-octanoic anhydride (3.6 mL, 12.1 mmol) in pyridine (40 mL) was stirred for 30 min at 60 °C. The reaction mixture was concentrated in vacuo. Ethyl acetate was added to the oil residue, which immediately gave a white precipitate. After the residue was filtered, the residue was washed with hot EtOAc, then dried, and crystallized from water to give 6-Octa-AA-2G (1.85 g, 39.4% yield) as a white powder. The recrystallization from EtOH gave 6-Octa-AA-2G (1.41 g, 30.0% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (10 200). UV λ_{max} (MeOH + NaOH) nm (ϵ): 260 (15 000). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, J = 7.1 Hz), 1.33 (8H, m), 1.63 (2H, qn, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 3.39 (1H, dd, J = 9.3, 10.3 Hz, 4′-H), 3.52 (1H, dd, J = 3.9, 9.3 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.2 Hz, 6′-Ha), 3.78 (1H, t, J = 9.3 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 11.2 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 10.3 Hz, 5′-H), 4.13 (1H, ddd, J = 2.0, 5.9, 6.8 Hz, 5-H), 4.18 (1H, dd, J = 5.9, 11.2 Hz, 6-Ha), 4.26 (1H, dd, J = 6.8, 11.2 Hz, 6-Hb), 4.82 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.9 Hz, 1′-H). ^{13}C NMR (125 MHz, CD_3OD): δ 14.35, 23.49, 25.82, 29.90, 30.00, 32.68, 34.77, 62.09 (6′-C), 65.44 (6-C), 67.77 (5-C), 71.02 (4′-C), 73.21 (2′-C), 74.32 (3′-C), 74.67 (5′-C), 77.15 (4-C), 101.59 (1′-C), 120.29 (2-C), 161.73 (3-C), 171.90, 174.87 (1-C). Anal. ($C_{20}H_{32}O_{12}$) C, H. $[\alpha]^{24}_D$ +144.2° (c = 0.50, MeOH). mp 170.5−174.0 °C.

6-*O*-Decanoyl-2-*O*-α-D-glucopyranosyl-L-ascorbic Acid (6-Deca-AA-2G). The synthetic reaction was essentially the same as 6-Octa-AA-2G. From AA-2G (5.0 g, 14.8 mmol) and *n*-capric anhydride (5.80 g, 17.8 mmol), 6-Deca-AA-2G was obtained as a white powder (3.64 g, 49.9% yield) and then as a crystal (2.88 g, 39.5% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (9600). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (14 900). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, J = 7.1 Hz), 1.30 (12H, m), 1.63 (2H, qn, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 3.39 (1H, dd, J = 9.3, 9.8 Hz, 4′-H), 3.52 (1H, dd, J = 3.7, 9.3 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.7 Hz, 6′-Ha), 3.78 (1H, t, J = 9.3 Hz, 3′-H), 3.79 (1H, dd, J = 2.4, 11.7 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 9.8 Hz, 5′-H), 4.13 (1H, ddd, J = 1.8, 5.9, 6.8 Hz, 5-H), 4.18 (1H, dd, J = 5.9, 10.7 Hz, 6-Ha), 4.26 (1H, dd, J = 6.8, 10.7 Hz, 6-Hb), 4.81 (1H, d, J = 1.8 Hz, 4-H), 5.37 (1H, d, J = 3.7 Hz, 1′-H). ^{13}C NMR (125 MHz, CD_3OD): δ 14.38, 23.55, 25.81, 30.04, 30.22, 30.24, 30.39, 32.86, 34.76, 62.08 (6′-C), 65.43 (6-C), 67.77 (5-C), 71.01 (4′-C), 73.20 (2′-C), 74.32 (3′-C), 74.66 (5′-C), 77.14 (4-C), 101.58 (1′-C), 120.28 (2-C), 161.73 (3-C), 171.89, 174.85 (1-C). Anal. ($C_{22}H_{36}O_{12}$) C, H. $[\alpha]^{24}_D$ +134.4° (c = 0.50, MeOH). mp 174.5−176.5 °C.

6-*O*-Dodecanoyl-2-*O*-α-D-glucopyranosyl-L-ascorbic Acid (6-Dode-AA-2G). The synthetic reaction was essentially the same as 6-Octa-AA-2G. From AA-2G (3.7 g, 10.9 mmol) and lauric anhydride (5.0 g, 13.1 mmol), 6-Deca-AA-2G was obtained as a white powder (2.84 g, 50.1% yield) and then as a crystal (2.59 g, 45.7% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (9700). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (14 600). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, J = 6.8 Hz), 1.29 (16H, m), 1.63 (2H, qn, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 3.39 (1H, t, J = 9.6 Hz, 4′-H), 3.52 (1H, dd, J = 3.4, 9.6 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.7 Hz, 6′-Ha), 3.78 (1H, t, J = 9.6 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 11.7 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 9.6 Hz, 5′-H), 4.13 (1H, ddd, J = 2.0, 6.4, 7.3 Hz, 5-H), 4.18 (1H, dd, J = 6.4, 11.2 Hz, 6-Ha), 4.26 (1H, dd, J = 7.3, 11.2 Hz, 6-Hb), 4.81 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.4 Hz, 1′-H). ^{13}C NMR (125 MHz, CD_3OD): δ 14.41, 23.65, 25.91, 30.13, 30.32, 30.37, 30.51, 30.64 (×2), 32.99, 34.84, 62.17 (6′-C), 65.54 (6-C), 67.86 (5-C), 71.12 (4′-C), 73.34 (2′-C), 74.42 (3′-C), 74.77 (5′-C), 77.26 (4-C), 101.69 (1′-C), 120.37 (2-C), 161.86 (3-C), 172.00, 174.99 (1-C). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.84, 22.00, 24.29, 28.40, 28.62 (×2), 28.80, 28.91 (×2), 31.21, 33.32, 60.19 (6′-C), 64.16 (6-C), 65.40

(5-C), 69.43 (4′-C), 71.62 (2′-C), 72.61 (3′-C), 73.23 (5′-C), 75.03 (4-C), 98.36 (1′-C), 117.64 (2-C), 158.92 (3-C), 168.86, 172.55 (1-C). Anal. ($C_{24}H_{40}O_{12}$) C, H. $[\alpha]^{24}_D$ +128.5° (c = 0.50, MeOH). mp 171.5−174.0 °C.

2-*O*-α-D-Glucopyranosyl-6-*O*-tetradecanoyl-L-ascorbic Acid (6-Myri-AA-2G). The synthetic reaction was essentially the same as 6-Octa-AA-2G. From AA-2G (5.0 g, 14.8 mmol) and myristic anhydride (7.79 g, 17.8 mmol), 6-Myri-AA-2G was obtained as a white powder (2.68 g, 33.0% yield) and then as a crystal (2.42 g, 29.8% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (10 200). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (14 800). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, J = 6.8 Hz), 1.29 (20H, m), 1.63 (2H, qn, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 3.39 (1H, t, J = 9.7 Hz, 4′-H), 3.52 (1H, dd, J = 3.4, 9.7 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.7 Hz, 6′-Ha), 3.78 (1H, t, J = 9.7 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 11.7 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 9.7 Hz, 5′-H), 4.13 (1H, m, 5-H), 4.18 (1H, dd, J = 6.4, 11.2 Hz, 6-Ha), 4.26 (1H, dd, J = 6.8, 11.2 Hz, 6-Hb), 4.81 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.4 Hz, 1′-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.86, 22.05, 24.33, 28.47, 28.67 (×2), 28.87, 28.98 (×4), 31.27, 33.36, 60.27 (6′-C), 64.17 (6-C), 65.44 (5-C), 69.49 (4′-C), 71.66 (2′-C), 72.66 (3′-C), 73.27 (5′-C), 75.06 (4-C), 98.42 (1′-C), 117.72 (2-C), 158.92 (3-C), 168.87, 172.53 (1-C). Anal. ($C_{26}H_{44}O_{12}$) C, H. $[\alpha]^{24}_D$ +136.3° (c = 0.50, DMSO). mp 169.5−171.0 °C.

2-*O*-α-D-Glucopyranosyl-6-*O*-hexadecanoyl-L-ascorbic Acid (6-Palm-AA-2G). A mixture of AA-2G (5.0 g, 14.8 mmol) and palmitic anhydride (8.79 g, 17.8 mmol) in pyridine (50 mL) was stirred for 30 min at 60 °C. The reaction mixture was concentrated in vacuo. Ethyl acetate was added to the oil residue, which immediately gave a white precipitate. After it was filtered, the residue was washed with hot EtOAc and hot water (60−70 °C) and then dried to give a white powder (4.58 g, 53.7% yield). The recrystallization from EtOH−H_2O (4:1) gave 6-Palm-AA-2G (4.22 g, 49.5% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (10 000). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (15 100). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, J = 7.1 Hz), 1.29 (24H, m), 1.63 (2H, qn, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 3.39 (1H, dd, J = 9.5, 10.3 Hz, 4′-H), 3.52 (1H, dd, J = 3.7, 9.5 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 12.2 Hz, 6′-Ha), 3.78 (1H, t, J = 9.5 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 12.2 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 10.3 Hz, 5′-H), 4.13 (1H, m, 5-H), 4.18 (1H, dd, J = 5.9, 11.0 Hz, 6-Ha), 4.26 (1H, dd, J = 6.8, 11.0 Hz, 6-Hb), 4.81 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.7 Hz, 1′-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.86, 22.06, 24.34, 28.49, 28.68, 28.72, 28.89, 28.99 (×2), 29.02 (×4), 31.28, 33.37, 60.28 (6′-C), 64.18 (6-C), 65.44 (5-C), 69.51 (4′-C), 71.67 (2′-C), 72.67 (3′-C), 73.28 (5′-C), 75.06 (4-C), 98.44 (1′-C), 117.73 (2-C), 158.93 (3-C), 168.88, 172.52 (1-C). Anal. ($C_{28}H_{48}O_{12}$) C, H. $[\alpha]^{24}_D$ +129.4° (c = 0.50, DMSO). mp 168.0−169.5 °C.

2-*O*-α-D-Glucopyranosyl-6-*O*-octadecanoyl-L-ascorbic Acid (6-Stea-AA-2G). The synthetic reaction was essentially the same as 6-Palm-AA-2G. From AA-2G (5.0 g, 14.8 mmol) and stearic anhydride (9.78 g, 17.8 mmol), 6-Stea-AA-2G was obtained as a white powder (5.13 g, 57.3% yield) and then as a crystal (4.73 g, 52.8% yield). UV λ_{max} (MeOH + HCl) nm (ϵ): 233 (9700). UV λ_{max} (MeOH + NaOH) nm (ϵ): 261 (14 000). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, J = 7.1 Hz), 1.29 (28H, m), 1.63 (2H, qn, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 3.39 (1H, dd, J = 9.5, 10.3 Hz, 4′-H), 3.52 (1H, dd, J = 3.9, 9.5 Hz, 2′-H), 3.70 (1H, dd, J = 4.9, 11.7 Hz, 6′-Ha), 3.78 (1H, t, J = 9.5 Hz, 3′-H), 3.80 (1H, dd, J = 2.4, 11.7 Hz, 6′-Hb), 4.02 (1H, ddd, J = 2.4, 4.9, 10.3 Hz, 5′-H), 4.13 (1H, m, 5-H), 4.18 (1H, dd, J = 5.9, 11.2 Hz, 6-Ha), 4.26 (1H, dd, J = 6.8, 11.2 Hz, 6-Hb), 4.82 (1H, d, J = 2.0 Hz, 4-H), 5.37 (1H, d, J = 3.9 Hz, 1′-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.86, 22.08, 24.36, 28.53, 28.71, 28.76, 28.92, 29.05 (×8), 31.23, 33.35, 60.30 (6′-C), 64.19 (6-C), 65.45 (5-C), 69.52 (4′-C), 71.69 (2′-C), 72.68 (3′-C), 73.29 (5′-C), 75.06 (4-C), 98.47 (1′-C), 117.75 (2-C), 158.95 (3-C), 168.88, 172.51 (1-C). Anal. ($C_{30}H_{52}O_{12}$) C, H. $[\alpha]^{24}_D$ +120.9° (c = 0.50, DMSO). mp 161.5−162.5 °C.

Stability of 6-Acyl-AA-2G in Aqueous Solution. The test compounds were dissolved in 50 mM potassium phosphate buffer (pH 7.0) containing 1% DMSO to give 10 mL of 1 mM solution. The resulting solution was stored at 37 °C for periods up to 5 days, and 100 μL samples were periodically taken. The concentration of the test compounds was analyzed by HPLC. The difference from initial concentration was taken as the remaining ratio.

Partition of 6-Acyl-AA-2G between *n*-Octanol and Water. Fifty microliters of 100 mM 6-Acyl-AA-2G in DMSO was diluted to 5 mL with 50 mM potassium phosphate buffer (pH 7.0). Five milliliters of *n*-octanol was added to the resulting solution, vigorously mixed, and partitioned at 37 °C. The amount of 6-Acyl-AA-2G in octanol was analyzed by HPLC. The difference from the total amount of both phases was taken as the distribution percent of 6-Acyl-AA-2G in octanol.

Free Radical Scavenging Activity of 6-Acyl-AA-2G. The free radical scavenging activity of each monoacylated AA-2G was assayed using a relatively stable free radical, DPPH, according to the method of Blois.[22] The reaction mixture contained 1 mL of 0.5 mM DPPH in ethanol and 4 mL of 1.25 × 10^{-6}, 1.25 × 10^{-5}, 6.25 × 10^{-5}, 1.25 × 10^{-4}, and 1.25 × 10^{-3} M antioxidant in ethanol/H_2O (1:1, v/v). After the reaction was carried out at room temperature for 20 min, the free radical scavenging activity of each antioxidant was quantified by the decolorization of DPPH at 516 nm. The EC_{50} value was determined as the concentration of each sample required to give 50% of the absorbance shown by a blank test.

Skin Permeation Assay. LSE, TESTSKIN, with a standard 6 well plate and culture medium, was a generous gift from TOYOBO Co., Ltd., Osaka. The special packaging in which LSE was shipped was opened immediately prior to use. The dermal portion of the LSE was rested on a 3 μm pore size polycarbonate membrane in contact with 1.2 mL of an assay medium (DMEM/Ham's F-12, 1:1) on a 6 well plate, called a receptor compartment. A polyethylene ring (10 mm internal diameter) was affixed to the epidermal surface with silicone sealant, and then, 200 μL of 10 mM 6-Acyl-AA-2G solution dissolved in the assay medium containing 5% DMSO was added to the interior of each ring. Aliquots of 100 μL were withdrawn from the receptor compartment periodically and replaced with equal volumes of the assay medium maintained at 37 °C. The experiments were performed for 120 h at 37 °C in a humidified incubator gassed with 5% CO_2. The resulting samples were directly subjected to HPLC analyses. For the detection of the total AsA, the sample reduced by DTT was used. AsA, AA-2G, and 6-Acyl-AA-2G contents were determined from the peak area of the samples with reference to the calibration of authentic AsA, AA-2G, and 6-Acyl-AA-2G, respectively.

HPLC Conditions. Separation for AsA and AA-2G was achieved by isocratic elution of an Inertsil ODS-3 column (ϕ 4.6 × 250 mm, 5 μm, GL Sciences Inc., Tokyo) kept at 40 °C with 0.1 M potassium phosphate−phosphoric acid buffer (pH 2.0) at a flow rate of 0.7 mL/min. The absorbance at 240 nm was monitored. Separation for 6-Acyl-AA-2G was carried out by isocratic elution of an Inertsil Ph column (ϕ 4.6 × 250 mm, 5 μm, GL Sciences Inc.) kept at 40 °C with 75% MeOH−H_2O containing 1% acetic acid at a flow rate of 0.7 mL/min. The absorbance at 240 nm was monitored.

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