

SYNTHESIS OF THE 3-*O*-RETINOYL-L-ASCORBIC ACID AND RELATED COMPOUNDS: CHARACTERIZATION AND REDUCING ACTIVITY AGAINST DPPH[§]

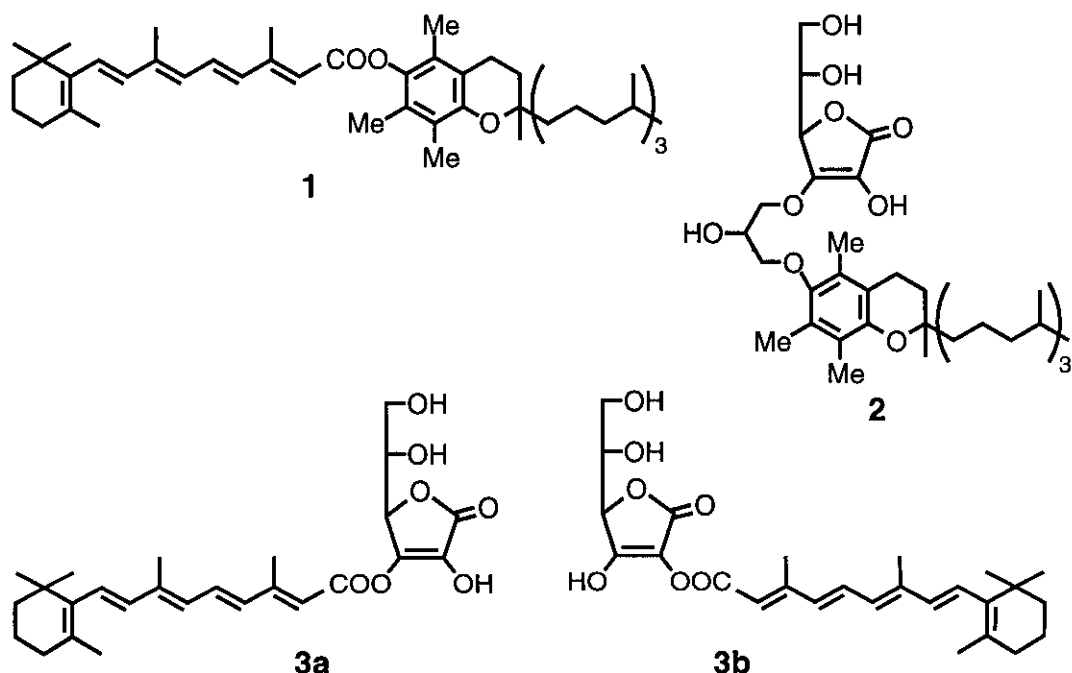
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Abstract—Novel hybrid vitamin, 3-*O*-retinoyl-L-ascorbic acid (**3a**) was conveniently prepared by reaction of sodium L-ascorbate with retinoyl fluoride. The 3-*O*-acylated structure was confirmed by the comparison of spectral data of its methylated compound with those of 3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**20**) prepared from 5,6-*O*-isopropylidene-3-*O*-methyl-L-ascorbic acid. 3-*O*-Retinoyl-L-ascorbic acid (**3a**) showed a reducing activity against the stable radical, α,α -diphenyl- β -picrylhydrazyl (DPPH).

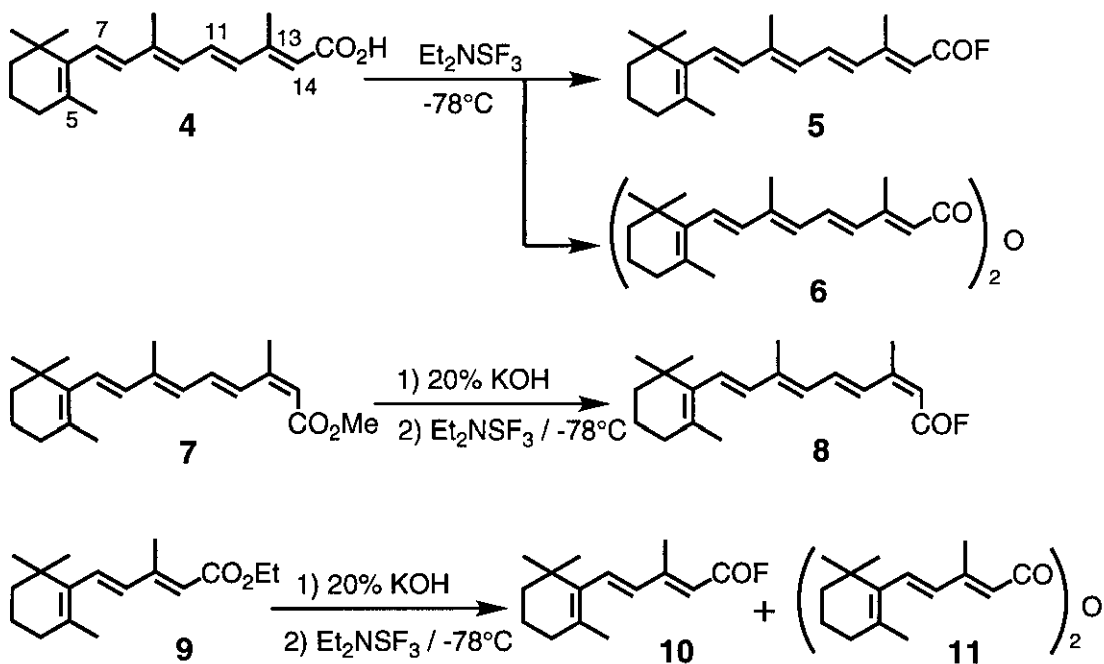
It is well known that L-ascorbic acid (vitamin C) widely distributed in aerobic organisms protects cellular components against oxidative damage by free radicals and oxidants.¹ In addition, there are considerable evidences that vitamin C is important in the prevention of a large number of chronic diseases, such as cancer, heart disease, brain dysfunction and AIDS.² On the other hand, compounds of the vitamin A group (retinoids) play an important role in growth, vision and reproduction.³ The recent characterization of the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), which are members of the steroid/retinoid hormone receptor superfamily of ligand-activated transcriptional regulators, has suggested a molecular explanation for the involvement of retinoids in the control of cell proliferation and the induction of cell differentiation, as well as embryonal morphogenesis.³ Recently, several hybrid vitamin derivatives, such as compounds (**1**) (vitamin A + E)⁴ and (**2**) (vitamin C + E),⁵ have been synthesized and showed to possess important pharmacological properties. In particular, the compound (**1**) (tocoretinate) has been clinically utilized for ulcer therapy for skin. We now designed and synthesized a novel hybrid vitamin (**3**) (retinoyl-L-ascorbic acid) linked lipophilic vitamin A acid to hydrophilic vitamin C at the C-2 or C-3 hydroxyl

This paper is dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday.
§ Retinoids and Related Compounds. 22. For Part 21, see: A. Wada, S. Hiraishi, N. Takamura, K. Aoe, and M. Ito, *J. Org. Chem.*, in press.



group in vitamin C. Its inhibitory effect on free radicals *in vitro* was also evaluated. Retinoyl-L-ascorbic acid was synthesized using retinoyl fluoride (5), a stable acylating agent, which was prepared (Scheme 1) according to the procedure reported by Barua *et al.*⁶ Treatment of all-*E*-retinoic acid (4) with diethylaminosulfur trifluoride (DAST)

Scheme 1.

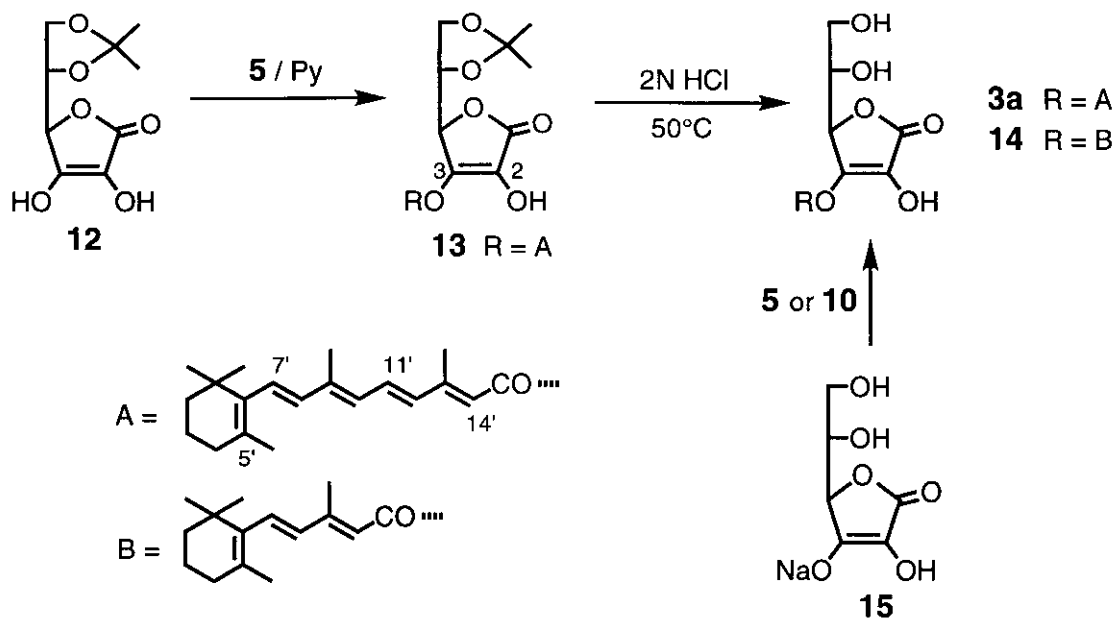


afforded the all-*E*-retinoyl fluoride (**5**) (77%) as a major and less polar product accompanying with all-*E*-retinoyl anhydride (**6**) (9%) as a minor and more polar product. Our results were not identical with those reported by Barua *et al.* They described the all-*E*-retinoyl fluoride (**5**) was obtained (50-70%) as a more polar product accompanying with 13*Z*-isomer (**8**) (10-30%) as a less polar product. The lower chemical shifts of 13-methyl protons on both products (δ 2.39 and δ 2.41) in the ^1H -NMR spectra suggested that 13,14-double bonds in both products were *E* form. Thus, 13*Z*-retinoyl fluoride (**8**) was alternatively synthesized (30%) from 13*Z*-methyl retinoate (**7**)⁷ by hydrolysis followed by treatment with DAST. In its ^1H -NMR spectrum, the 13-methyl signal appeared at δ 2.03 ppm. In proton noise-decoupling ^{13}C -NMR spectrum, carbonyl carbon signals of retinoyl fluorides (**5**) and (**8**) were observed as doublet coupled with a fluorine (J ca. 330 Hz), whereas that of the anhydride (**6**) was observed as singlet. β -Ionylideneacetyl fluoride (**10**) was also prepared (30%) from ethyl β -ionylideneacetate (**9**),⁸ together with anhydride (**11**) (14%).

Reaction of 5,6-*O*-isopropylidene-L-ascorbic acid (**12**)⁹ with retinoyl fluoride (**5**) in CH_2Cl_2 using pyridine as a base (Scheme 2) furnished 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (**13**) (59%), which was deprotected by acid hydrolysis to give 3-*O*-retinoyl-L-ascorbic acid (**3a**) (70%). During the course of these studies, it was observed that 3-*O*-acyl derivatives (**3a**) and (**14**) of L-ascorbic acid could be conveniently synthesized in good yield (**3a**: 86%; **14**: 80%) by reacting sodium L-ascorbate (**15**) with acyl fluorides (**5**) and (**10**) in dimethylformamide (DMF).

It has been known that the acylation of ascorbic acid is fairly sensitive to the reaction conditions. Spectroscopic structural determination of ascorbates was recently reported

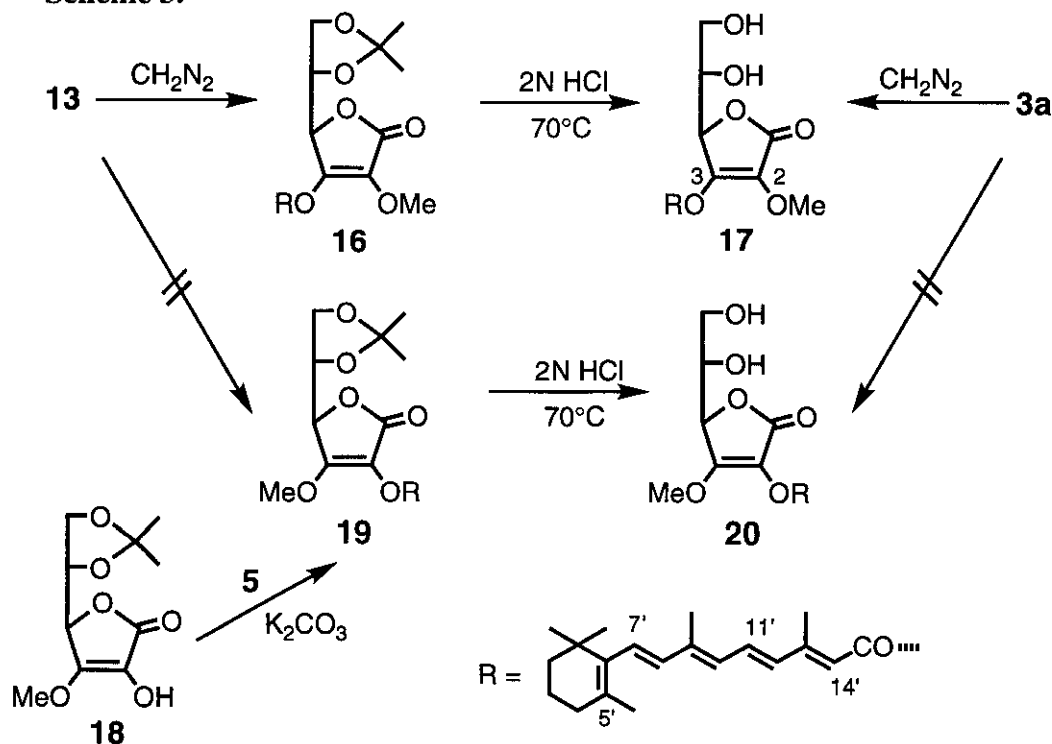
Scheme 2.



by Cabral and Haake.¹⁰ They described that the position of double bond stretching bands and intensity ratio of carbonyl and double bond stretching bands in the IR spectra were useful for distinguishing 2-*O*- and 3-*O*-esters. The double bond bands for 3-*O*-esters are at 1700-1710 cm^{-1} but the bands for 2-*O*-esters are observed at significantly lower frequencies (1680-1690 cm^{-1}). In compounds with an 3-*O*-acyl group, the carbonyl stretching is more intense than the olefinic one but the reverse is true in compounds with a 3-*OH* group. Double bond bands for compounds (**13**), (**3a**) and (**14**) appeared near 1710 cm^{-1} and the carbonyl stretching bands (**13**: 1782 cm^{-1} ; **3a**: 1776 cm^{-1} ; **14**: 1777 cm^{-1}) are slightly more intense than the olefinic stretching bands. Therefore, these three compounds were expected to be 3-*O*-acylated products.

In order to confirm these structures chemically, they were methylated under neutral conditions by diazomethane and then spectral data of the resulting products were compared with those of 3-*O*-methylated compounds (**19**) and (**20**) prepared from 5,6-*O*-isopropylidene-3-*O*-methyl-L-ascorbic acid (**18**)¹¹ (Scheme 3). Compound (**18**) was treated with retinoyl fluoride (**5**) in the presence of K_2CO_3 to afford 5,6-*O*-isopropylidene-3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**19**) (76%), which was further converted into 3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**20**) (64%) by acid hydrolysis. Methylated compounds (**16**) and (**17**) obtained from retinoyl-L-ascorbic acid derivatives (**13**) and (**3a**) by treatment of diazomethane (**16**: 59%; **17**: 83%) were not identical with compounds (**19**) and (**20**) derived from the 3-*O*-methylated compound (**18**).

Scheme 3.



Deprotection of 5,6-*O*-acetonide moiety of compound (16) gave the diol (45%), whose spectral data were in good agreement with those of compound (17) obtained from compound (3a). From these results, compounds (13) and (3a) were found to be 3-*O*-acylated products.

According to the procedure developed by Blois,¹² the reducing activity of 3a, 13 and 14 was measured by use of a stable radical, α,α -diphenyl- β -picrylhydrazyl (DPPH), *in vitro*, and the results are shown in Table. Although the most active 3-hydroxyl group of vitamin C moiety against free radicals of these three compounds was shielded, they exhibited almost half reducing activity of L-ascorbic acid. Other biological activity of these compounds is currently under investigation.

Table Reducing Activity against DPPH

Compounds	50%-Inhibitory concentration (M)
L-ascorbic acid	2.3×10^{-5}
12	2.5×10^{-5}
13	4.5×10^{-5}
14	4.5×10^{-5}
3a	4.6×10^{-5}
α -tocopherol	2.0×10^{-5}

DPPH; 1.0×10^{-4} M

ACKNOWLEDGMENT

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EXPERIMENTAL

General. UV spectra were recorded on a JASCO Ubest-55 instrument. IR spectra were measured on a Shimadzu IR-27G spectrophotometer or on a Perkin Elmer FT-IR spectrophotometer, Paragon 1000 in chloroform solutions. ^1H -NMR spectra at 200, 300 or 500 MHz were taken on Varian Gemini-200 or 300, or a Varian VXR-500 superconducting FT-NMR spectrometer, respectively, for deuteriochloroform solutions (tetramethylsilane as internal reference). ^{13}C -NMR spectra at 75 MHz were determined on a Varian Gemini-300 superconducting FT-NMR spectrometer in deuteriochloroform solutions using tetramethylsilane as internal reference. High resolution mass spectra (HRMS) were taken on a Hitachi M-4100 spectrometer. Column chromatography (CC) was performed on silica gel (Merck Art. 7734) unless otherwise stated. Short CC was carried out on silica gel (Merck Art. 7739) under reduced pressure. Preparative TLC (PTLC) was performed on pre-coated plates RP-18 F_{254S} (Merck Art. 15389, 0.25 mm thickness).

Extracts from the reaction mixture were dried over anhydrous sodium sulfate and evaporation of the extract was carried out under reduced pressure. Ether refers to diethyl ether and hexane to *n*-hexane. NMR assignments are given using the retinoid numbering system.

Reaction of all-*E*-retinoic acid (4) with DAST

A solution of all-*E*-retinoic acid (4) (2.00 g, 6.67 mmol) in dry THF (40 mL) was added dropwise to a stirred solution of DAST (0.91 mL, 6.86 mmol) in dry ether (20 mL) at -78 °C. After the reaction mixture was allowed to warm to rt under stirring, the solvent was evaporated. The residue was purified by CC (ether-hexane, 1:19 to 1:9) to give all-*E*-retinoyl fluoride (5) (less polar: 1.55 g, 77%) and all-*E*-retinoic anhydride (6) (polar: 70 mg, 9%), as orange solids, respectively.

Compound (5): UV (MeOH), 376 nm; UV (hexane), 371 nm; IR, 1785 and 1570 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz), δ 1.04 (6H, s, 1-*gem*-Me), 1.72 (3H, s, 5-Me), 2.04 (3H, s, 9-Me), 2.39 (3H, s, 13-Me), 5.67 (1H, br s, 14-H), 6.17 (1H, d, $J=16$ Hz, 8-H), 6.17 (1H, br d, $J=11.5$ Hz, 10-H), 6.33 (1H, d, $J=15$ Hz, 12-H), 6.36 (1H, br d, $J=16$ Hz, 7-H) and 7.17 (1H, dd, $J=15$ and 11.5 Hz, 11-H); $^{13}\text{C-NMR}$ (75 MHz), δ 13.04 (9- CH_3), 14.59 (13- CH_3), 19.20 (C-3), 21.76 (5- CH_3), 28.97 (1-*gem*- CH_3), 33.17 (C-4), 34.28 (C-1), 39.62 (C-2), 111.47 (d, $J=75$ Hz, C-14), 129.00 (C-7), 130.16 (C-10), 130.66 (C-5), 133.41 (d, $J=3.5$ Hz, C-12), 134.16 (C-11), 136.95 (C-8), 137.60 (C-6), 142.23 (C-9), 156.82 (d, $J=328$ Hz, CO) and 161.08 (d, $J=18$ Hz, C-13); HRMS calcd for $\text{C}_{20}\text{H}_{27}\text{OF}$ (M^+), 302.203, found m/z 302.204.

Compound (6): UV (EtOH), 380 nm; UV (hexane), 376 nm; IR, 1760, 1690 and 1570 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz), δ 1.03 (12H, s, 1-*gem*-Me), 1.72 (6H, s, 5-Me), 2.02 (6H, s, 9-Me), 2.41 (6H, s, 13-Me), 5.79 (2H, br s, 14-H), 6.15 (2H, d, $J=16$ Hz, 8-H), 6.16 (2H, br d, $J=11$ Hz, 10-H), 6.32 (2H, d, $J=15$ Hz, 12-H), 6.33 (2H, br d, $J=16$ Hz, 7-H) and 7.11 (2H, dd, $J=15$ and 11 Hz, 11-H); $^{13}\text{C-NMR}$ (75 MHz), δ 12.99 (9- CH_3), 14.37 (13- CH_3), 19.21 (C-3), 21.75 (5- CH_3), 28.97 (1-*gem*- CH_3), 33.14 (C-4), 34.28 (C-1), 39.62 (C-2), 116.89 (C-14), 129.33 and 129.47 (C-7 and C-10), 130.34 (C-5), 132.87 (C-11), 134.48 (C-12), 137.12 (C-8), 137.65 (C-6), 141.05 (C-9), 157.69 (C-13) and 162.71 (CO); HRMS calcd for $\text{C}_{40}\text{H}_{54}\text{O}_3$ (M^+), 582.407, found m/z 582.406.

13*Z*-Retinoyl fluoride (8)

Aqueous 20% KOH (2.5 mL, 5.0 mmol) was added to a solution of 13*Z*-methyl retinoate (7)⁷ (233 mg, 0.74 mmol) in MeOH (2.5 mL) and the mixture was stirred at 50 °C for 2 h. After cooling, the reaction mixture was acidified by addition of aqueous 5% sulfuric acid and extracted with ethyl acetate. The extracts were washed with brine, dried and evaporated to give crude 13*Z*-retinoic acid. Without purification, a solution of this acid (212 mg, 0.71 mmol) in dry THF (3 mL) was added dropwise to a stirred solution of DAST (100 μL , 0.76 mmol) in dry THF (3 mL) at -78 °C. After the reaction mixture was warmed to rt, the solvent was evaporated. The residue was purified by CC (ether-hexane, 1:19) to give 13*Z*-retinoyl fluoride (8) (67 mg, 30% from 7) as orange

solids: UV (EtOH), 378 nm; UV (hexane), 374 nm; IR, 1780 and 1573 cm^{-1} ; $^1\text{H-NMR}$ (200 MHz), δ 1.04 (6H, s, 1-*gem*-Me), 1.73 (3H, s, 5-Me), 2.03 (3H, s, 9-Me), 2.17 (3H, s, 13-Me), 5.55 (1H, br s, 14-H), 6.18 (1H, d, $J=16$ Hz, 8-H), 6.29 (1H, br d, $J=11.5$ Hz, 10-H), 6.37 (1H, br d, $J=16$ Hz, 7-H), 7.17 (1H, dd, $J=15$ and 11.5 Hz, 7-H) and 7.59 (1H, d, $J=15$ Hz, 12-H); $^{13}\text{C-NMR}$ (75 MHz), δ 13.01 (9- CH_3), 19.19 (C-3), 21.20 (13- CH_3), 21.76 (5- CH_3), 28.97 (1-*gem*- CH_3), 33.18 (C-4), 34.27 (C-1), 39.64 (C-2), 109.41 (d, $J=74$ Hz, C-14), 128.01 (C-12), 129.75 (7-C), 130.07 (C-10), 130.73 (C-5), 135.48 (C-11), 137.07 (C-8), 137.55 (C-6), 142.47 (C-9), 155.79 (d, $J=329$ Hz, CO) and 159.49 (d, $J=19$ Hz, C-13); HRMS calcd for $\text{C}_{20}\text{H}_{27}\text{OF}$ (M^+), 302.203, found m/z 302.205.

β -Ionylideneacetyl fluoride (10)

Aqueous 20% KOH (10 mL, 20.0 mmol) was added to a solution of ethyl β -ionylideneacetate (**9**)⁸ (1.73 g, 6.60 mmol) in MeOH (15 mL) and the mixture was stirred at 50 °C for 2 h. After cooling, the reaction mixture was acidified by addition of aqueous 5% sulfuric acid and extracted with ethyl acetate. The extracts were washed with brine, dried and evaporated to give a crude acid. Without purification, to a solution of this acid (1.28 g, 5.47 mmol) in dry ether (100 mL), DAST (0.74 mL, 5.60 mmol) was added dropwise at -78 °C. After the reaction mixture was warmed to rt, the solvent was evaporated. The residue was purified by CC (ether-hexane, 1:19 to 1:9) to give β -ionylideneacetyl fluoride (**10**) (less polar: 460 mg, 30% from **9**) and β -ionylideneacetic anhydride (**11**) (polar: 225 mg, 14% from **9**), as pale yellow oils, respectively. Compound (**10**): UV (EtOH), 322 and 263 nm; IR, 1790 and 1595 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz), δ 1.05 (6H, s, 1-*gem*-Me), 1.73 (3H, s, 5-Me), 2.38 (3H, s, 9-Me), 5.68 (1H, br s, 10-H), 6.19 (1H, d, $J=16$ Hz, 8-H) and 6.79 (1H, br d, $J=16$ Hz, 7-H); $^{13}\text{C-NMR}$ (75 MHz), δ 14.36 (9- CH_3), 19.04 (C-3), 21.73 (5- CH_3), 28.91 (1-*gem*- CH_3), 33.27 (C-4), 34.27 (C-1), 39.55 (C-2), 111.40 (d, $J=74$ Hz, C-10), 133.18 (C-5), 134.75 (d, $J=3.4$ Hz, C-8), 136.87 (C-6), 137.41 (C-7), 156.81 (d, $J=329$ Hz, CO) and 161.36 (d, $J=18$ Hz, C-9); HRMS calcd for $\text{C}_{15}\text{H}_{21}\text{OF}$ (M^+), 236.158, found m/z 236.158.

Compound (**11**): UV (EtOH), 324 and 270 nm; IR, 1765, 1700 and 1590 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz), δ 1.04 (12H, s, 1-*gem*-Me), 1.71 (6H, s, 5-Me), 2.39 (6H, s, 9-Me), 5.76 (2H, br s, 10-H), 6.15 (2H, d, $J=16$ Hz, 8-H) and 6.70 (2H, br d, $J=16$ Hz, 7-H); $^{13}\text{C-NMR}$ (75 MHz), δ 14.14 (9- CH_3), 19.08 (C-3), 21.71 (5- CH_3), 28.91 (1-*gem*- CH_3), 33.18 (C-4), 34.24 (C-1), 39.53 (C-2), 116.55 (C-10), 132.25 (C-5), 135.61 and 135.89 (C-7 and C-8), 137.05 (C-6), 157.89 (C-9) and 162.77 (CO); HRMS calcd for $\text{C}_{30}\text{H}_{42}\text{O}_3$ (M^+), 450.313, found m/z 450.313.

5,6-*O*-Isopropylidene-3-*O*-retinoyl-L-ascorbic acid (13)

A solution of retinoyl fluoride (**5**) (290 mg, 0.96 mmol) in CH_2Cl_2 (7 mL) was added to a solution of 5,6-*O*-isopropylidene-L-ascorbic acid (**12**)⁹ (453 mg, 2.1 mmol) in pyridine (3 mL). After being stirred at rt for 15 h, the reaction mixture was diluted with ethyl acetate and washed successively with aqueous 3% HCl and water. Evaporation of the dried solvent gave an oil, which was purified by short CC (ethyl acetate-hexane, 2:3) to provide 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (**13**) (280 mg, 59%) as a

yellow semisolid: UV (EtOH), 365 nm; IR, 3540, 3280, 1780, 1720sh, 1705, 1600 and 1565 cm^{-1} ; $^1\text{H-NMR}$ (200 MHz), δ 1.04 (6H, s, 1'-gem-Me), 1.37 and 1.40 (each 3H, s, OCMe_2O), 1.73 (3H, s, 5'-Me), 2.05 (3H, s, 9'-Me), 2.44 (3H, s, 13'-Me), 4.07 and 4.18 (each 1H, dd, $J=8$ and 7 Hz, 6-H₂), 4.35 (1H, td, $J=7$ and 2.5 Hz, 5-H), 4.75 (1H, d, $J=2.5$ Hz, 4-H), 5.88 (1H, br s, 14'-H), 6.19 (1H, d, $J=16$ Hz, 8'-H), 6.20 (1H, br d, $J=11.5$ Hz, 10'-H), 6.35 (1H, d, $J=15$ Hz, 12'-H), 6.41 (1H, br d, $J=16$ Hz, 7'-H), 7.22 (1H, dd, $J=15$ and 11.5 Hz, 11'-H) and 7.94 (1H, br s, 2-OH); HRMS calcd for $\text{C}_{29}\text{H}_{38}\text{O}_7$ (M^+), 498.262, found m/z 498.263. Anal. Calcd for $\text{C}_{29}\text{H}_{38}\text{O}_7 \cdot 1/3\text{H}_2\text{O}$: C, 69.02; H, 7.72. Found: C, 68.81; H, 7.67.

3-*O*-Retinoyl-L-ascorbic acid (3a) from 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (13)

A solution of 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (13) (280 mg, 0.56 mmol) in THF-MeOH (3:1, 4 mL) and 2N HCl (1.2 mL) was heated at 50 °C for 1.5 h. After cooling, the reaction mixture was diluted with ethyl acetate and washed with water. Evaporation of the dried solvent gave an oil, which was purified by PTLC (THF-MeCN, 1:9) to provide 3-*O*-retinoyl-L-ascorbic acid (3a) (76 mg, 70%) as yellow crystals: mp 90-92°C (CH_2Cl_2 -hexane); UV (EtOH) 365 nm; IR, 3600-3150, 1775, 1720, 1705, 1600 and 1565 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz), δ 1.04 (6H, s, 1'-gem-Me), 1.73 (3H, s, 5'-Me), 2.05 (3H, s, 9'-Me), 2.43 (3H, s, 13'-Me), 3.83 (1H, dd, $J=11$ and 5 Hz, 6-H), 3.89 (1H, dd, $J=11$ and 6 Hz, 6-H), 4.00 (1H, m, 5-H), 4.90 (1H, d, $J=2$ Hz, 4-H), 5.88 (1H, br s, 14'-H), 6.18 (1H, d, $J=16$ Hz, 8'-H), 6.19 (1H, br d, $J=11.5$ Hz, 10'-H), 6.34 (1H, d, $J=15$ Hz, 12'-H), 6.39 (1H, br d, $J=16$ Hz, 7'-H), 7.21 (1H, dd, $J=15$ and 11.5 Hz, 11'-H) and 7.80-8.10 (1H, br s, 2-OH); HRMS calcd for $\text{C}_{26}\text{H}_{34}\text{O}_7$ (M^+), 458.230, found m/z 458.231. Anal. Calcd for $\text{C}_{26}\text{H}_{34}\text{O}_7 \cdot 1/4\text{H}_2\text{O}$: C, 67.44; H, 7.51. Found: C, 67.33; H, 7.63.

3-*O*-Retinoyl-L-ascorbic acid (3a) from sodium L-ascorbate (15)

A suspension of retinoyl fluoride (5) (304 mg, 1.01 mmol) and sodium L-ascorbate (15) (304 mg, 1.54 mmol) in DMF (7 mL) was stirred at rt for 1 h. The reaction mixture was diluted with ethyl acetate, washed with water, dried and evaporated. The resulting residue was purified by CC (Cosmosil 140 C₁₈-OPN, Nacalai tesque INC.; THF-MeCN, 1:9) to afford 3-*O*-retinoyl-L-ascorbic acid (3a) (396 mg, 86%). The spectral properties of this compound were identical with those of the sample prepared from 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (13).

3-*O*- β -Ionylideneacetyl-L-ascorbic acid (14)

A suspension of β -ionylidene-acetyl fluoride (10) (173 mg, 0.73 mmol) and sodium L-ascorbate (15) (218 mg, 1.10 mmol) in DMF (3 mL) was stirred at rt for 1.5 h. The reaction mixture was diluted with ethyl acetate, washed with water, dried and evaporated. The resulting residue was purified by CC (Cosmosil 140 C₁₈-OPN, Nacalai tesque INC.; THF-MeCN, 1:9) to afford 3-*O*- β -ionylideneacetyl-L-ascorbic acid (14) (230 mg, 80%) as a pale yellow foam: UV (EtOH), 314 and 262 nm; IR, 3300, 1775, 1705 and 1590 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz), δ 1.05 (6H, s, 1'-gem-Me), 1.73 (3H, s, 5'-Me), 2.40 (3H, s, 9'-Me), 3.80 (1H, dd, $J=11$ and 5.5 Hz, 6-H), 3.87 (1H, dd, $J=11$ and 6.5 Hz, 6-H), 4.00

(1H, m, 5-H), 4.95 (1H, d, $J=2$ Hz, 4-H), 5.90 (1H, br s, 10'-H), 6.21 (1H, d, $J=16$ Hz, 8'-H) and 6.81 (1H, br d, $J=16$ Hz, 7'-H); HRMS calcd for $C_{21}H_{28}O_7$ (M^+), 392.184, found m/z 392.183.

Methylation of 3-*O*-retinoyl-L-ascorbic acid (3a) by diazomethane

To a stirred solution of 3-*O*-retinoyl-L-ascorbic acid (3a) (105 mg, 0.23 mmol) in THF (0.5 mL) was added dropwise a solution of diazomethane (*ca.* 1.0 mmol) in ether (3 mL) generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide according to the literature.¹³ After being stirred at rt for 20 min, the solvent was evaporated to give a residue, which was purified by PTLC (MeCN-MeOH, 1:2) to provide the 2-*O*-methyl-3-*O*-retinoyl-L-ascorbic acid (17) (90 mg, 83%) as an orange oil: UV (EtOH), 374 and 224 nm; IR, 3500, 1774, 1729, 1700sh, 1600 and 1573 cm^{-1} ; 1H -NMR (500 MHz), δ 1.04 (6H, s, 1'-*gem*-Me), 1.73 (3H, s, 5'-Me), 2.04 (3H, s, 9'-Me), 2.41 (3H, s, 13'-Me), 3.74-3.94 (3H, m, 5-H and 6-H₂), 3.99 (3H, s OMe), 5.16 (1H, d, $J=1.5$ Hz, 4-H), 5.89 (1H, br s, 14'-H), 6.17 (1H, d, $J=16$ Hz, 8'-H), 6.18 (1H, br d, $J=11.5$ Hz, 10'-H), 6.34 (1H, d, $J=15$ Hz, 12'-H), 6.37 (1H, br d, $J=16$ Hz, 7'-H) and 7.18 (1H, dd, $J=15$ and 11.5 Hz, 11'-H); HRMS calcd for $C_{26}H_{34}O_7$ (M^+), 472.246, found m/z 472.247.

Methylation of 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (13) by diazomethane

According to methylation of 3-*O*-retinoyl-L-ascorbic acid (3a) by diazomethane, 5,6-*O*-isopropylidene-3-*O*-retinoyl-L-ascorbic acid (13) (160 mg, 0.32 mmol) was methylated to afford 5,6-*O*-isopropylidene-2-*O*-methyl-3-*O*-retinoyl-L-ascorbic acid (16) (97 mg, 59%) as an orange oil: UV (EtOH), 383 and 223 nm; IR, 1775, 1736, 1703, 1601 and 1573 cm^{-1} ; 1H -NMR (300 MHz), δ 1.04 (6H, s, 1'-*gem*-Me), 1.35 and 1.38 (each 3H, s, OMe₂O), 1.72 (3H, s, 5'-Me), 2.04 (3H, s, 9'-Me), 2.41 (3H, s, 13'-Me), 4.01 (3H, s, OMe), 4.04 and 4.15 (each 1H, dd, $J=8.5$, 6.5, 6-H₂), 4.30 (1H, td, $J=6.5$ and 2.5 Hz, 5-H), 5.25 (1H, d, $J=2.5$ Hz, 4-H), 5.90 (1H, br s, 14'-H), 6.17 (1H, d, $J=16$ Hz, 8'-H), 6.19 (1H, br d, $J=11.5$ Hz, 10'-H), 6.33 (1H, d, $J=15$ Hz, 12'-H), 6.37 (1H, br d, $J=16$ Hz, 7'-H) and 7.16 (1H, dd, $J=15$ and 11.5 Hz, 11'-H); HRMS calcd for $C_{30}H_{40}O_7$ (M^+), 512.277, found m/z 512.277.

Acid-treatment of 5,6-*O*-isopropylidene-2-*O*-methyl-3-*O*-retinoyl-L-ascorbic acid (16)

A solution of 5,6-*O*-isopropylidene-2-*O*-methyl-3-*O*-retinoyl-L-ascorbic acid (16) (85 mg, 0.17 mmol) in THF-MeOH (3:1, 5 mL) and 2N HCl (1 mL) was heated at 70 °C for 20 min. After cooling, the reaction mixture was diluted with ethyl acetate and washed with water. Evaporation of the dried solvent gave an oil, which was purified by PTLC (MeCN-MeOH, 1:2) to provide 2-*O*-methyl-3-*O*-retinoyl-L-ascorbic acid (17) (35 mg, 45%) as an orange oil. The spectral properties of this compound were identical with those of the sample prepared by methylation of 3-*O*-retinoyl-L-ascorbic acid (3a).

5,6-*O*-Isopropylidene-3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (19)

A solution of retinoyl fluoride (5) (200 mg, 0.66 mmol) in THF (5 mL) was added to a suspension of 5,6-*O*-isopropylidene-3-*O*-methyl-L-ascorbic acid (18)¹¹ (258 mg, 1.12

mmol) and K_2CO_3 (127 mg, 0.92 mmol) in THF-DMF (1:1, 10 mL). After being stirred at rt for 2.5 h, the reaction mixture was diluted with ethyl acetate and washed with water. Evaporation of the dried solvent gave a residue, which was purified by short CC (ethyl acetate-hexane, 2:3) to provide 5,6-*O*-isopropylidene-3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**19**) (258 mg, 76%) as an orange oil: UV (EtOH), 372 and 223 nm; IR, 1779, 1734, 1693, 1603 and 1576 cm^{-1} ; 1H -NMR (300 MHz), δ 1.04 (6H, s, 1'-*gem*-Me), 1.37 and 1.42 (each 3H, s, OCM₂O), 1.72 (3H, s, 5'-Me), 2.02 (3H, s, 9'-Me), 2.40 (3H, s, 13'-Me), 4.08 (3H, s, OMe), 4.09 and 4.16 (each 1H, dd, $J=8.5$ and 6.5 Hz, 6-H₂), 4.37 (1H, td, $J=6.5$ and 3 Hz, 5-H), 4.68 (1H, d, $J=3$ Hz, 4-H), 5.90 (1H, br s, 14'-H), 6.16 (1H, d, $J=16$ Hz, 8'-H), 6.17 (1H, br d, $J=11.5$ Hz, 10'-H), 6.32 (1H, d, $J=15$ Hz, 12'-H), 6.33 (1H, br d, $J=16$ Hz, 7'-H) and 7.12 (1H, dd, $J=15$ and 11.5 Hz, 11'-H); HRMS calcd for C₃₀H₄₀O₇ (M⁺), 512.277, found m/z 512.276.

Acid-treatment of 5,6-*O*-isopropylidene-3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**19**)

According to the acid-treatment of 5,6-*O*-isopropylidene-2-*O*-methyl-3-*O*-retinoyl-L-ascorbic acid (**16**), 5,6-*O*-isopropylidene-3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**19**) (70 mg, 0.14 mmol) was treated with 2N HCl (1 mL) to give 3-*O*-methyl-2-*O*-retinoyl-L-ascorbic acid (**20**) (41 mg, 64%) as an orange oil: UV (EtOH), 371 and 223 nm; IR, 3566, 1777, 1733, 1690, 1602 and 1576 cm^{-1} ; 1H -NMR (300 MHz), δ 1.04 (6H, s, 1'-*gem*-Me), 1.72 (3H, s, 5'-Me), 2.03 (3H, s, 9'-Me), 2.40 (3H, s, 13'-Me), 2.75-3.04 (2H, br s, OH), 3.79 (1H, dd, $J=11.5$ and 4.5 Hz, 6-H), 3.87 (1H, dd, $J=11.5$ and 5.5 Hz, 6-H), 4.03 (1H, td-like, $J=5$ and 3 Hz, 5-H), 4.07 (3H, s OMe), 4.86 (1H, d, $J=3$ Hz, 4-H), 5.93 (1H, br s, 14'-H), 6.16 (1H, d, $J=16$ Hz, 8'-H), 6.17 (1H, br d, $J=11.5$ Hz, 10'-H), 6.33 (1H, d, $J=15$ Hz, 12'-H), 6.34 (1H, br d, $J=16$ Hz, 7'-H) and 7.13 (1H, dd, $J=15$ and 11.5 Hz, 11'-H); HRMS calcd for C₂₆H₃₄O₇ (M⁺), 472.246, found m/z 472.248.

Measurement of the reducing activity against the stable radical DPPH

The test compound in EtOH [in the case of L-ascorbic acid: in 0.1M sodium acetate buffer solution (pH 5.5)] was added to a solution of DPPH (1×10^{-4} M) in EtOH and sodium acetate buffer (pH 5.5) to prepare four kinds of ($1-5 \times 10^{-5}$ M) sample solution. After 30 min, the absorbance at 517 nm was measured. The difference in absorbance from the control, without test compound, was taken as the reducing activity. The results are shown in Table.

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