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Research Article

^{125}I-Labeled 2-*O*- and 3-*O*-*m*-iodobenzyl, and 6-*O*-*m*-iodophenyl derivatives of L-ascorbic acid: synthesis and preliminary tissue distribution

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Summary

Three ^{125}I-labeled 2-*O*- and 3-*O*-*m*-iodobenzyl, and 6-*O*-*m*-iodophenyl derivatives of L-ascorbic acid were prepared by melt exchange procedures in isolated radiochemical yields of 12–60% after HPLC purification. Biodistribution studies in tumor-bearing mice showed very different *in vivo* tissue uptake properties from previous results obtained with ^{14}C-labeled ascorbic acid and 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid. None of these seems to be suitable radioiodinated analogs of L-ascorbic acid for imaging study of its *in vivo* biochemistry. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: iodine-125; exchange radiolabeling; ascorbic acid; tissue distribution

Introduction

L-Ascorbic acid is an essential nutrient required for an array of biological functions, including antioxidation and enzymatic reactions. In addition, it has been reported that L-ascorbic acid has anti-tumor

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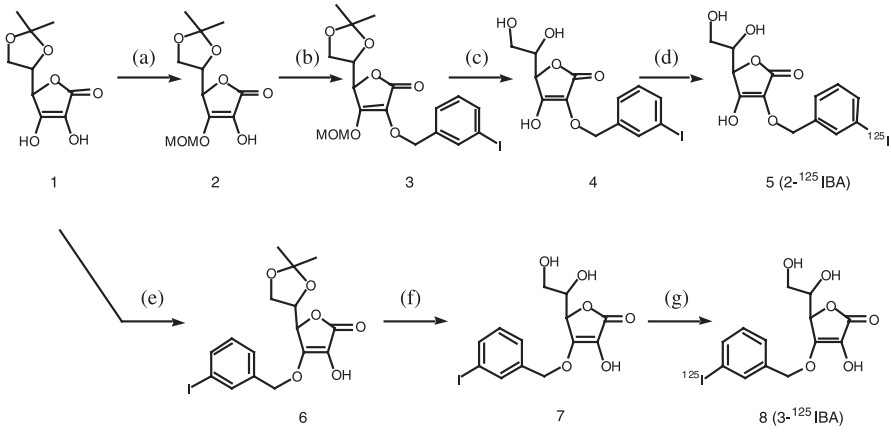
activity and certain human tumors contain increased concentrations of L-ascorbic acid compared with normal tissue, although the precise mechanisms are still unknown.[1] Various derivatives of L- ascorbic acid have been synthesized and studied for their multifold biological and pharmacological interest.[2] We developed 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid (^{18}F-DFA) as a positron emitter-labeled analog of L-ascorbic acid for studying the *in vivo* biochemistry of ascorbic acid by positron emission tomography.[3] Previous tissue distribution studies in rats and tumor-bearing mice[3] demonstrated that the uptake and distribution pattern of ^{18}F-DFA, showing the highest uptake of radioactivity in the adrenal glands with moderate capability to accumulate in tumors, has a remarkable resemblance to that reported for ^{14}C-labeled ascorbic acid.[4,5]

The present study was undertaken to develop ascorbic acid analogs labeled with radionuclides of iodine with the intention of potential application for use in single photon emission tomography. The iodophenyl group was chosen as a supplementary unit for iodine radiolabeling in our initial development work, because of the known *in vivo* stability of the iodine bound to the phenyl ring.[6] This paper describes the synthesis and radiolabeling with ^{125}I of *m*-iodobenzyl ether and *m*-iodophenyl ether derivatives of L-ascorbic acid, and their biodistribution in tumor-bearing mice is also reported.

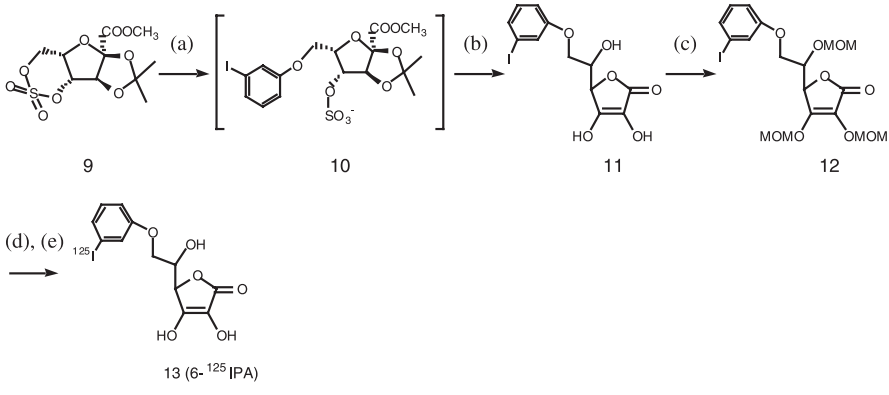
Results and discussion

Synthesis

The synthesis of 2-*O*-(*m*-iodobenzyl)-L-ascorbic acid (2-IBA)(**4**), as depicted in Scheme 1, started with 5,6-*O*-isopropylidene-L-ascorbic acid (**1**). The hydroxy group at the 3-position of (**1**) was protected with a methoxymethyl group by use of chloromethyl methyl ether and *m*-iodobenzylation with *m*-iodobenzyl bromide in the presence of potassium carbonate, followed by acid hydrolysis, gave the required 2-IBA (**4**) in good yield. 3-*O*-(*m*-iodobenzyl)-L-ascorbic acid (3-IBA)(**7**) was obtained from direct *m*-iodobenzylation of (**1**) followed by acid hydrolysis in good yield. Initial attempts to prepare 6-*O*-iodobenzyl-L-ascorbic acid failed to give the desired iodobenzylation product. Therefore, we turned our attention to 6-*O*-(*m*-iodophenyl)-L-ascorbic acid (6-IPA)(**11**), which was prepared by an alternative route involving



Scheme 1. (a) MOMCl, K_2CO_3, rt, 5 h (30%), (b) *m*-iodobenzyl bromide, K_2CO_3, rt, 40 min (57%), (c) 10% HCl, MeOH, rt, 15 h (96%), (d) Na^{125}I, 160° C, 9 min, (e) *m*-iodobenzyl bromide, K_2CO_3, rt, 3 hr (49%), (f) TsOH, MeOH, rt, 15 h (89%), (g) Na ^{125}I, 150° C, 13 min.



Scheme 2. (a) potassium *m*-iodophenoxide CH_3CN, reflux, 1 h, (b), HCl/H_2O, reflux, 2 h (76%), (c) methylal, P_2O_5, $CHCl_3$, rt, 18h (64%), (d) Na ^{125}I, 150° C, 10 min, (e) HCl/MeOH, 60° C, 30 min.

the nucleophilic ring opening of the cyclic sulfate (**9**)[3] with potassium *m*-iodophenoxide, followed by acid hydrolysis (Scheme 2). The structures were determined on the basis of analysis in ^{1}H- and ^{13}C-NMR spectra, consistent with those reported for related ascorbic acid derivatives.[7,8] The structures of regioisomers 2-IBA and 3-IBA were also confirmed by ^{13}C-NMR chemical shifts depending on pH (Table 1). In the ^{13}C-NMR

Table 1. ^{13}C-NMR chemical shifts of 4 and 7 depending on pH

	^{13}C chemical shifts[a] δ (ppm)					
	Compound 4			Compound 7		
Carbon position	pH = 3.5	pH > 10[b]	Δ[c]	pH = 6	pH > 10[b]	Δ[c]
1	172.61	179.70	+7.09	172.72	179.58	+6.86
2	121.52	118.06	−3.46	121.36	132.35	+10.99
3	161.86	177.93	+16.07	151.17	145.63	−5.54
4	76.94	80.18	+3.24	76.72	76.89	+0.17
5	70.63	72.05	+1.42	70.51	71.58	+1.07
6	63.42	64.20	+0.78	63.42	64.25	+0.83

[a]Spectra recorded in CD_3OD. [b]Two drops of 20% $NaOH/D_2O$ was added to adjust pH. [c]Δ = δ(pH 3.5 or 6)−δ(pH > 10).

Table 2. Reducing activity and partition coefficient

Compounds	Reducing activity (%)[a]	log P (octanol/water)[b]
2-IBA	92	−1.16
3-IBA	60	+0.78
6-IPA	72	−0.42
Ascorbic acid	96	−1.27

[a]Reducing activity of 1×10^{-4} M antioxidants against α,α-diphenyl-β-picrylhydrazyl (DPPH). [b]Values were determined by shaking solutions of compounds in 2.5 ml 1-octanol with 2.5 ml potassium phosphate buffer (pH 7.4) for 20 min and the data are the average of three separate experiments.

spectrum of 2-IBA, the signal of the carbon at the 3-position moved most downfield when the pH of the solution was changed from 3.5 to > 10. On the other hand, the ^{13}C-NMR spectrum of 3-IBA revealed an 11 ppm downfield shift of the carbon at the 2-position with a similar increase in pH. These observations are in accord with those found earlier.[7,8]

The reducing abilities of the three iodinated ascorbic acid analogs were determined by use of a stable radical α,α-diphenyl-β-picrylhydrazyl (Table 2). 2-IBA exhibited almost the same reducing potency as ascorbic acid while the activities of 3-IBA and 6-IPA were lower than that of ascorbic acid. The octanol/water distribution (logP value, Table 2) showed that, as expected, the attachment of a iodobenzyl or iodophenyl group to the ascorbic acid molecule decreased the hydrophilicity to some extent; 3-IBA exhibited a somewhat higher partition coefficient compared to the two other iodinated derivatives, likely due to the predominantly uncharged character of the 3-*O*-substituted derivative at a physiological pH.

Radiolabeling

A number of different methods of introducing a radioiodine atom into a phenyl ring have been documented in the literature.[9] However, radioiodination via organometallics or direct electrophilic radioiodination that required oxidation conditions was found to be unsuitable for use with ascorbic acid susceptible to oxidation. Thus, a direct exchange of radioiodine for aromatic iodide was chosen in this work: A high specific activity was not required because L-ascorbic acid is normally present at high concentrations in mammalian cells.[10] 2-^{125}IBA and 3-^{125}IBA were prepared by simple heating with Na^{125}I in the absence of any solvent in a sealed tube. The exchange required 13 min at 150°C with 3-IBA and 9 min at 160°C with 2-IBA to give an isolated radiochemical yield of 27–60 and 12–45%, respectively, after HPLC purification. Similarly, the preparation of 6-^{125}IPA was achieved by isotope exchange of the MOM-protected (**12**) in a melt procedure (150°C, 10 min) and subsequent removal of the protecting group with HCl in an overall radiochemical yield of 20–48% after HPLC purification. The time required for both the radioiodination and purification was less than 2 h for all preparations and the radiochemical purity of the labeled materials was excellent. When the exchange reaction was performed with 1 mg of the iodinated compounds and 2.5–3.3 MBq of Na^{125}I, specific activities in the range of 200–350 MBq/mmol were produced, which were of the same level as that of commercially available ^{14}C-labeled ascorbic acid and were considered to be sufficient to permit preliminary biodistribution studies.

Animal studies

The distributions of 2-^{125}IBA, 3-^{125}IBA and 6-^{125}IPA after intravenous injections of these compounds in mice bearing 3-methylcholanthrene-induced fibrosarcoma are shown in Tables 3–5. There are marked differences in the distribution of these compounds. A fast disappearance of radioactivity from the blood was similar for 2-^{125}IBA and 3-^{125}IBA, but the level of radioactivity in the blood of 6-^{125}IPA remained much higher relative to the other two compounds throughout the during of the study. At an early time following injection, 3-^{125}IBA showed the highest uptake of radioactivity in the liver, whereas 2-^{125}IBA had the highest kidney uptake of radioactivity and the highest uptake of radioactivity of 6-^{125}IPA was found in the lungs. These organs showed

Table 3. Biodistribution of 2-^{125}IBA in mice bearing fibrosarcoma

	Uptake (% dose/g)[a]			
Tissue	1 min	10 min	30 min	60 min
Blood	15.66 + 0.83	6.15 + 4.82	1.30 ± 0.82	0.53 ± 0.32
Lung	7.66 ± 0.05	2.88 ± 2.20	0.75 ± 0.55	0.44 ± 0.32
Liver	7.18 ± 0.45	4.20 ± 2.15	1.04 ± 0.42	0.68 ± 0.31
Adrenals	3.18 ± 0.10	2.03 ± 2.17	0.67 ± 0.69	0.45 ± 0.38
Kidneys	46.38 ± 2.02	17.70 ± 5.09	4.47 ± 2.73	1.14 ± 0.67
Tumor	1.56 ± 0.20	1.43 ± 0.72	0.69 ± 0.53	0.41 ± 0.15
Muscle	1.61 ± 0.25	1.14 ± 0.73	0.37 ± 0.28	0.17 ± 0.0.8
Brain	0.74 ± 0.07	0.28 ± 0.34	0.05 ± 0.05	0.04 ± 0.04
Thyroid	6.02 ± 1.34	3.03 ± 1.73	2.00 ± 0.30	6.28 ± 1.58

[a]means ± SD (n = 3).

Table 4. Biodistribution of 3-^{125}IBA in mice bearing fibrosarcoma

	Uptake (% dose/g)[a]			
Tissue	1 min	10 min[b]	30 min	60 min[b]
Blood	12.03 + 0.55	3.39 + 0.35	0.65 ± 0.12	0.43 ± 0.23
Lung	10.17 ± 1.26	2.79 ± 0.45	0.50 ± 0.08	0.30 ± 0.13
Liver	21.15 ± 1.00	17.51 ± 5.58	2.20 ± 0.62	1.47 ± 0.74
Adrenals	6.65 ± 0.10	2.33 ± 0.46	0.63 ± 0.21	0.36 ± 0.16
Kidneys	16.70 ± 2.34	18.08 ± 2.71	3.22 ± 0.21	1.93 ± 0.99
Tumor	1.10 ± 0.28	1.86 ± 0.30	0.60 ± 0.17	0.41 ± 0.23
Muscle	1.92 ± 0.38	1.21 ± 0.12	0.24 ± 0.07	0.17 ± 0.12
Brain	0.55 ± 0.04	0.20 ± 0.03	0.06 ± 0.01	0.03 ± 0.01
Thyroid	4.55 ± 0.53	2.28 ± 0.71	1.47 ± 0.26	1.04 ± 0.33

[a]means ± SD (n = 3). [b]n = 4.

Table 5. Biodistribution of 6-^{125}IBA in mice bearing fibrosarcoma

	Uptake (% dose/g)[a]			
Tissue	1 min	10 min	30 min	60 min
Blood	54.18 ± 25.45	8.83 ± 2.91	5.28 ± 0.30	4.54 ± 0.885[c]
Lung	215.73 ± 136.24	17.32 ± 6.69	5.10 ± 1.56	26.07 ± 31.28[c]
Liver	36.72 ± 13.09	16.49 ± 3.98	6.13 ± 0.73	9.71 ± 5.59[c]
Adrenals	25.69 ± 9.12	4.72 ± 2.05	2.40 ± 0.46	4.58 ± 2.58[c]
Kidneys	26.20 ± 11.69[b]	22.24 ± 5.34	7.47 ± 0.58	9.21 ± 5.24[c]
Tumor	1.86 ± 0.64	2.38 ± 0.58	1.85 ± 0.18	2.21 ± 1.40[c]
Muscle	3.84 ± 1.45	1.58 ± 0.47	0.92 ± 0.15	1.20 ± 3.80
Brain	6.03 ± 3.05	0.63 ± 0.17	0.26 ± 0.01	0.26 ± 0.07[c]
Thyroid	17.82 ± 5.04	7.95 ± 2.65	17.56 ± 4.63	21.79 ± 8.32[c]

[a]means ± SD (n = 4). [b]n = 3. [c]n = 5.

subsequent fast washout of radioactivity with time. The thyroid showed relatively low radioactivity concentrations for 2-^{125}IBA and 3-^{125}IBA, suggesting the reasonable stability of the label in regard to metabolic

deiodination. On the other hand, a much higher thyroid accumulation was observed with 6-^{125}IPA. Very little concentration of radioactivity in the brain indicated very poor blood-brain permeability of all these compounds. Biodistribution studies indicated that the propensity for tumor uptake as well as the preferential uptake in the adrenal glands, characteristic of tissue distribution of exogenously administrated L-ascorbic acid,[4,5] were not mirrored by any of these radioiodinated analogs of ascorbic acid. The reasons for these different behaviors are not clear, but it appears likely that increased lipophilicities of the ^{125}I-analogs has a significant influence on *in vivo* distribution behavior. It is also conceivable that structural modification of ascorbic acid by substitution with iodobenzyl or iodophenyl groups may have led to a loss of the affinities for the transporters of ascorbic acid which play central roles in the absorption and accumulation of ascorbic acid in tissues.[11,12]

Conclusion

The three ^{125}I-labelled forms of ascorbic acid possessing a *m*-iodobenzyl or *m*-iodophenyl moiety on the hydroxy group were prepared by a melt exchange procedure. These radioiodinated analogs of ascorbic acid markedly differed in their biodistribution profiles from each other, and from ^{14}C-labeled ascorbic acid or ^{18}F-DFA. Therefore, none of these seems to be suitable radioiodinated analogs of L-ascorbic acid for imaging study of its *in vivo* biochemistry.

Experimental

All melting points were obtained on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL GX-270 spectrometer (270 MHz), and the chemical shifts are reported in parts per million downfield from tetramethylsilane. ^{13}C-NMR spectra were obtained on a Varian Unity 500 spectrometer (125 MHz), and the chemical shifts are reported in parts per million downfield from tetramethylsilane. Infrared (IR) spectra were recorded with a JASCO IR Report-100 spectrometer. Mass spectra and high resolution-FAB-mass spectra were obtained with a JEOL JMS DX-610 and JEOL SX-102 mass spectrometer, respectively. The elemental

analyses were performed by the staff of the micro-analytical section of Kyushu University. Column chromatography was performed on Kieselgel 60 (70–230 mesh, Merck), and analytical TLC was carried out on Silica gel 60F 254 (Merck) or FL-60D (Fuji-gel). In the synthetic procedures, organic extracts were routinely dried over anhydrous Na_2SO_4 and evaporated with a rotary evaporator under reduced pressure. HPLC was done using a Waters Associate Model 440 HPLC system fitted with a Whatman Partisil 5PAC (10×100 mm) with monitoring of the radioactivity as well as UV absorption (at 254 nm). The radioactivity was also quantified with a Capintec radioisotope calibrator (CRC-5). The identity of radiolabeled compounds was supported by HPLC co-injection studies and specific activities were determined by HPLC using cold mass calibration lines. Radiochemical yields were expressed at the end-of-synthesis, relative to the amount of the $Na^{125}I$ as total activity present in the reaction vessel. All chemicals were purchased from Nacalai Tesque, Japan and $Na^{125}I$ was obtained from Amersham Pharmacia Biotech. All animal experiments were carried out in accordance with the regulations on animal experiments of the Faculty of Pharmaceutical Sciences, Kyushu University.

5,6-O-Isopropylidene-3-O-methoxymethyl-L-ascorbic acid (**2**). Under argon atmosphere, to a solution of 5,6-*O*-isopropylidene-L-ascorbic acid (**1**) (216 mg, 1.0 mmol) in DMF (2 ml) was added K_2CO_3 (276.4 mg, 2.0 mmol) and chloromethyl methyl ether (95.4 mg, 1.2 mmol). The mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with water (5 ml) and then extracted with ethyl acetate (30 ml). The organic layer was washed with a saturated NaCl aqueous solution, dried and evaporated to dryness. The residue was purified by chromatography on silica gel (chloroform and then chloroform:methanol = 10:1) to give compound (**2**) (77.2 mg, 30%) as a colorless powder, mp 66–69°C. ^{1}H-NMR ($CDCl_3$) δ(ppm): 5.32(2 H, s), 4.62 (1 H, d, $J - 2.97$ Hz), 4.33 (1 H, dt, $J = 2.97$, 6.77 Hz), 4.16 (1 H, dd, $J = 6.76$, 6.77 Hz), 4.06 (1 H, dd, $J = 6.60$, 8.58 Hz), 3.61 (3 H, s), 1.39 (3 H, s), 1.36 (3 H, s). IR(neat): 3350, 1740, 1680 cm^{-1}. FAB-MS *m*/*z*: 261(MH^+).

5,6-O-Isopropylidene-2-O-(m-iodobenzyl)-3-O-methoxymethyl-L-ascorbic acid (**3**). Under argon atmosphere, to a solution of (**2**) (680 mg, 2.61 mmol) in DMF (7 ml) was added K_2CO_3 (433 mg, 3.12 mmol) and *m*-iodobenzyl bromide (775 mg, 2.61 mmol). The mixture was stirred at

room temperature for 40 min.The reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate (150 ml). The organic layer was washed with saturated NaCl aqueous solution, dried and evaporated to remove the solvent. The residue was purified by chromatography on silica gel (hexane: ethyl acetate = 4:1) to give compound (**3**) (706 mg, 57%) as a colorless powder, mp 68–71°C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.80 (1 H, t, $J = 1.49$ Hz), 7.67 (1 H, dt, $J = 7.91$, 1.32 Hz), 7.39 (1 H, dt, $J = 8.24$, 1.32 Hz), 7.10 (1 H, t, $J = 7.75$ Hz), 5.36 (1 H, d, $J = 5.61$ Hz), 5.32 (1 H, d, $J = 5.28$ Hz), 5.12 (1 H, d, $J = 10.89$ Hz), 5.05 (1 H, d, $J = 11.22$ Hz), 4.58 (1 H, d, $J = 2.97$ Hz), 4.32 (1 H, dt, $J = 2.97$, 6.7 Hz), 4.15 (1 H, dd, $J = 6.77$, 8.42 Hz), 4.06 (1 H, dd, $J = 6.76$, 8.41 Hz), 3.50 (3 H, s), 1.39 (3 H, s), 1.37 (3 H,s). IR(neat): 1760, 1690 cm^{-1}. FAB-MS m/z: 477 (MH^+).

2-O-(m-Iodobenzyl)-L-ascorbic acid (**4**). To a solution of (**3**) (706 mg, 1.48 mmol) in methanol (50 ml) was added 10% HCl aqueous solution (9.9 ml). The reaction mixture was stirred for 15.5 hrs at room temperature followed by the evaporation of the solvent. The residue was dissolved in water (10 ml) and extracted with ethyl acetate (30 ml). The organic layer was washed with saturated NaCl aqueous solution, dried and evaporated to dryness. The residue was recrystallized from ethyl acetate to give compound (**4**)(558 mg, 96%) as a colorless powder, mp 148–150°C. ^{1}H-NMR (acetone-d_6) δ (ppm): 7.84 (1 H, d, $J = 1.65$ Hz), 7.67 (1 H, d, $J = 7.92$ Hz), 7.46 (1 H, dd, $J = 7.59$, 1.65 Hz), 7.16 (1 H, t, $J = 7.76$ Hz), 4.99 (2 H, s), 4.83 (1 H, d, $J = 1.98$ Hz), 3.94 (1 H, ddd, $J = 1.98$, 6.27, 9.57 Hz), 3.69 (1 H, dd, $J = 6.27$, 10.56 Hz), 3.64 (1 H, dd, $J = 7.42$, 10.72 Hz). ^{13}C-NMR (CD_3OD) δ (ppm): 172.61, 161.86, 140.94, 138.44, 138.28, 131.19, 128.68, 121.52, 94.64, 76.94, 73.34, 70.63, 63.42. IR(neat): 3300, 1730, 1640 cm^{-1}. FAB-MS m/z: 393 (MH^+). Analytically Calculated for $C_{13}H_{13}IO_6$: C, 39.82; H, 3.34. Found: C, 40.02; H, 3.32.

2-O-(m-[^{125}I]Iodobenzyl)-L-ascorbic acid (**5**). To a glass vessel containing a solution of 2-*O*-(*m*-iodobenzyl)-L-ascorbic acid (**4**) (1.3 mg, 0.0033 mmol) in acetonitrile (130 μl) was added an aqueous NaOH solution of $Na^{125}I$ (carrier free, 4–5 μl, 0.3–5.9 MBq). The mixture was evaporated to dryness. The vessel was closed tightly and heated at 160°C in an oil bath for 9 min, and then cooled to room temperature. The labeled (**5**) was purified by HPLC (Whatman Partisil 5PAC, 0.003 M potassium phosphate buffer (pH = 6): methanol = 1:1, 3 ml/min). The

radioactive fraction eluting at $t_R = 16$ min, corresponding to authentic 2-IBA, was collected. The radiochemical yield was 27–60% and the specific activity was 200–360 MBq/mmol. Chemical purity and radiochemical purity were higher than 98%, respectively, as determined by HPLC.

5,6-O-Isopropylidene-3-O-(m-iodobenzyl)-L-ascorbic acid (**6**). Under argon atmosphere, to a solution of 5,6-*O*-isopropylidene-L-ascorbic acid (**1**) (1.0 g, 4.63 mmol) in DMF (15 ml) was added K_2CO_3 (830 mg, 6.00 mmol) and *m*-iodobenzyl bromide (1.79 g, 6.00 mmol). The reaction mixture was stirred for 3.5 h at room temperature, then diluted with water (50 ml) and extracted with ethyl acetate (180 ml). The organic layer was washed with a saturated NaCl aqueous solution, dried and evaporated to dryness. The residue was purified by chromatography on silica gel (hexane:ethyl acetate = 8:2 → 6:4) to give compound (**6**) (984 mg, 49%) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ(ppm): 7.76 (1 H, d, $J = 1.65$ Hz), 7.70 (1 H, dd, $J = 7.92$, 1.65 Hz), 7.36 (1 H, m), 7.13 (1 H, m), 5.43 (2 H, s), 4.58 (1 H, d, $J = 2.97$ Hz), 4.30 (1 H, dt, $J = 3.30$, 6.60 Hz), 4.13 (1 H, dd, $J = 6.93$, 8.25 Hz), 4.04 (1 H, dd, $J = 6.60$, 8.57 Hz), 1.39 (3 H, s), 1.37 (3 H,s). IR(neat): 3300, 1760, 1680 cm^{-1}. FAB-MS *m/z*: 433 (MH^+).

3-O-(m-Iodobenzyl)-L-ascorbic acid (**7**). To a solution of 5,6-*O*-isopropylidene-3-*O*-(*m*-iodobenzyl)-L-ascorbic acid (**6**) (150 mg, 0.35 mmol) in methanol–water (10:1, 4.4 ml) was added *p*-toluenesulfonic acid monohydrate (66 mg, 0.35 mmol). The mixture was stirred for 12 h at room temperature and evaporated to dryness. The residue was dissolved in water (10 ml) and extracted with ethyl acetate (30 ml). The organic layer was washed with a saturated NaCl aqueous solution, dried and evaporated to dryness. Recrystallization from ethyl acetate gave compound (**7**) as a colorless powder (112 mg, 89%), mp 137–140°C. ^{1}H-NMR (acetone-d_6) δ(ppm): 7.87 (1 H, m), 7.72 (1 H, m), 7.52 (1 H, m), 7.20 (1 H, t, $J = 7.76$ Hz), 5.54 (1 H, d, $J = 12.2$ Hz), 5.43 (1 H, d, $J = 12.2$ Hz), 4.85 (1 H, d, $J = 1.65$ Hz), 4.20 (1 H, bs), 3.92 (1 H, t, $J = 6.43$ Hz), 3.70 (2 H,m). ^{13}C-NMR (CD_3OD) δ(ppm): 172.72, 151.17, 140.30, 138.50, 137.86, 131.35, 128.19, 121.36, 94.73, 76.72, 72.85, 70.51, 63.42. IR(neat): 3350, 1740, 1680 cm^{-1}. FAB-MS *m/z*: 393 (MH^+). Analytically Calculated for $C_{13}H_{13}IO_6$: C, 39.82; H, 3.34. Found: C, 39.72; H, 3.33.

3-O-(m-[^{125}I]Iodobenzyl)-L-ascorbic acid (**8**). To a glass vessel containing a solution of 3-*O*-(*m*-iodobenzyl)-L-ascorbic acid (**7**) (1.0 mg,

0.0026 mmol) in acetonitrile (100 µl) was added an aqueous NaOH solution of $Na^{125}I$ (carrier free, 10 µl, 2.9–4.7 MBq). The mixture was evaporated to dryness under a stream of argon. The vessel was closed tightly and heated at 150°C in an oil bath for 13 min, and then cooled to room temperature. The labeled (**8**) was purified by HPLC (Whatman Partisil 5PAC, H_2O:methanol = 7:3, 3 ml/min). The radioactive fraction eluting at $t_R = 8$ min, corresponding to authentic 3-IBA, was collected. The radiochemical yield was 12–45% and the specific activity was 200–340 MBq/mmol. Chemical purity and radiochemical purity were higher than 98%, respectively, as determined by HPLC.

6-O-(m-Iodophenyl)-L-ascorbic acid (**11**). Under argon atmosphere, to a solution of potassium *m*-iodophenoxide (1.173 g, 4.55 mmol) in acetonitrile (10 ml) was added methyl 2,3-*O*-isopropylidene-2-keto-L-glonate 4,6-cyclic sulfate[3] (**9**) (403 mg, 1.3 mmol). The mixture was refluxed for 1 h and evaporated to dryness. To the residue was added 35% HCl (6.5 ml, 5 ml/mmol), and the mixture was refluxed for 2 hrs. After cooling to room temperature, the reaction mixture was extracted with ethyl acetate (60 ml). The organic layer was washed with a saturated NaCl aqueous solution, dried and evaporated to dryness. The residue was purified by chromatography on silica gel (ethyl acetate and then ethyl acetate: methanol = 9:1 → 8:2 → methanol) to give compound (**11**) (370.8 mg, 76%) as a yellow powder. Further purification by HPLC (nacalai tesque COSMOSIL 5C18MS-AR300, H_2O:methanol = 45:55, flow rate = 3 ml/min) afforded an analytical sample, mp 155–157°C. ^{1}H-NMR (CD_3OD) δ(ppm): 7.31–7.26 (2 H, m), 7.05–6.94 (2 H, m), 4.53 (1 H, s), 4.25 (1 H, bt, $J = 5.2$ Hz), 4.14 (1 H, dd, $J = 5.5$, 9.57 Hz), 4.07 (1 H, dd, $J = 7.3$, 9.3 Hz). IR(neat): 3300, 1710, 1590 cm^{-1}. FAB-MS m/z: 401 ($M + Na^+$), 379 (MH^+). HR FAB-MS m/z calculated for $C_{12}H_{12}INaO_6$: 401.9576, found: 401.9577.

6-O-(m-Iodophenyl)-2,3,5-tri-O-methoxymethyl-L-ascorbic acid (**12**). Under argon atmosphere, to an ice-cooled solution of dimethoxymethane (1 ml, 11.5 mmol) and P_2O_5 (ca.100 mg) in $CHCl_3$ (1 ml) was added a solution of 6-*O*-(*m*-iodophenyl)-L-ascorbic acid (**11**) (35.0 mg, 0.0926 mmol) in $CHCl_3$ (1 ml). The mixture was stirred for 18 h at room temperature. The reaction mixture was cooled at 0°C and a saturated $NaHCO_3$ aqueous solution (10 ml) was added. The mixture was

extracted with ethyl acetate (50 ml). The organic layer was dried and evaporated to dryness. The residue was purified by chromatography on silica gel with ethyl acetate and then ethyl acetate: methanol = 2:1 to give compound (**12**) (30 mg, 64%) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ(ppm): 7.34–7.26 (2 H, m), 7.04–6.86 (2 H,m), 5.56 (1 H, d, $J = 5.27$ Hz), 5.42 (1 H, d, $J = 5.61$ Hz), 5.20 (2 H, dd, $J = 14.52$, 5.94 Hz), 4.96 (1 H, d, $J = 1.65$ Hz), 4.68 (2 H, dd, $J = 10.23$, 6.93 Hz), 4.19 (1 H, ddd, $J = 5.60$, 5.60, 1.65 Hz), 4.18 (2 H, s), 3.55 (3 H, s), 3.54 (3 H, s), 3.38 (3 H, s). IR(KBr): 2500, 1760, 1670, 1580 cm^{-1}. FAB-MS *m/z*: 549 ($M + K^+$), 533 ($M + Na^+$), 511 (MH^+).

6-O-(m-[^{125}I]Iodophenyl)-L-ascorbic acid (**13**). To a glass vessel containing 6-*O*-(*m*-iodophenyl)-2,3,5-tri-*O*-methoxymethyl-L-ascorbic acid (**12**) (1.0 mg, 0.0020 mmol) was added an aqueous NaOH solution of Na^{125}I (carrier free, 5 μl, 1.9–2.7 MBq). The mixture was evaporated to dryness under a stream of argon. The vessel was closed tightly and heated at 150°C for 10 min in an oil bath, and then cooled to room temperature. To the reaction mixture was added 35% HCl (1 gtt) and the mixture was heated at 60°C for 30 min.The labeled compound (**13**) was purified by HPLC (Whatman Partisil 5PAC, 0.003M potassium phosphate buffer (pH = 6):methanol = 1:2, 2.5 ml/min). The radioactive fraction eluting at $t_R = 9.3$ min, corresponding to authentic 6-IPA, was collected. The radiochemical yield was 20–48%, and the specific activity was 200–350 MBq/mmol. Chemical purity and radiochemical purity were higher than 98%, respectively, as determined by HPLC.

Partition coefficients. The log *P* values were measured using a standard shake flask method. The non-radioactive samples were well shaken with a mixture of 1-octanol (2.5 ml) and 0.05M phosphate buffer (2.5 ml, pH7.4) for 20 min at 25°C, after which aliquots of both phases were taken for analysis by HPLC quantitation.

Reducing activity. According to the literature procedure[3], 2-IBA, 3-IBA, 6-IPA or L-ascorbic acid was added to a solution of α,α-diphenyl-β-picrylhydrazyl (DPPH) (10^{-4} M) in ethanol. After 20 min, the absorbance at 517 nm was measured. The percentage of the absorbance change to that of the control was taken as the reducing activity.

Biodistribution in tumor-bearing mice. 3-Methylcholanthrene-induced fibrosarcoma (FSa) was inoculated s.c. into the right hind leg muscle of female C3H/He mice (5 weeks old, 16–18 g). Tumors which developed with a diameter of about 1 cm at 9–14 days after inoculation were used.[3] The animals were allowed free access to water and food at all times.

Aliquots of 2-^{125}IBA, 3-^{125}IBA or 6-^{125}IPA in about 500 μl of saline solution, with activities ranging from 40–460 kBq, were injected through the tail vein of unanesthetized mice. At 1-, 10-, 30- and 60-min postinjection intervals, the animals were killed by cervical dislocation while under ether anesthesia. Samples of blood and the tissues of interest, blotted free of blood, were taken, weighed, and assayed for radioactivity in an Aloka Auto Well Gamma System ARC-300. The results were expressed as percent injected dose per gram of tissue weight (%dose/g).

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