Ascorbate Analogs for Use in Medical Imaging: Synthesis and Radical Scavenging Activity of 5-O-(4′-Iodobenzyl)-L-ascorbic Acid

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As part of our program to develop potential imaging agents for ascorbate bioactivity in the brain, 5-O-(4′-iodobenzyl)-L-ascorbic acid was prepared through a seven-step sequence which involved C5-O-alkylation with p-iodobenzyl bromide in the presence of Ag2O and CaSO4 as the key step, starting from L-ascorbic acid. The scavenging activity of the p-iodobenzylated analog against 2,2-diphenyl-1-picrylhyrazyl (DPPH) radical was almost the same as that of L-ascorbic acid itself.

Key words 5-O-(4′-iodobenzyl)-L-ascorbic acid; synthesis; radical scavenging

L-Ascorbic acid (AsA) is highly concentrated in the neurons in the brain, which likely indicates the essential roles in neuronal function and antioxidant protection.1–3 Recent research on the biochemistry of AsA has been focused on the transport and accumulation mechanism, by which the brain acquires AsA. It has been shown that AsA and its oxidized form, dehydroascorbic acid (DHA), have distinct transport mechanisms, mediated by the Na+-vitamin C transporter SVCT2, and the facilitative glucose transporter GLUT1, respectively.3–5

Considerable effort has been made toward synthesizing numerous derivatives of AsA substituted at various positions for their potential applications in biochemistry and medicine.6–9 The C6-hydroxyl group of AsA is not critical for transport. The hydroxyl groups of enediol lactone in C2 and C3 are at the focus of the reaction site in the redox process of AsA and are required for reducing properties of vitamin C. The 6-deoxy-6-haloascorbic acid analogs have proven to be useful as competition inhibitors for the study of AsA transport.10,11 In previous studies we reported the synthesis and characterization of 6-deoxy-6-[18F]fluoro-L-ascorbic acid and several 125I-labeled AsA analogs as radiotracer probes for imaging the bioactivity of AsA in vivo.12,13 In an attempt to improve the brain targeting of an AsA analog we considered that utilizing the DHA transport pathway, in which ascorbate accumulation in the brain is mediated by DHA, might be a potential approach to enhance the transfer of AsA analogs to the brain in vivo. Thus, we became interested in the preparation of C5-O-modified AsA analogs, the oxidized form of which, if stable, might be expected to have the hydrated bicyclic hemiketal structure in aqueous solution, in contrast to the known 6-deoxy-6-haloascorbic acid analogs.

Structural modifications of the C5 side chain of AsA have received limited attention from a synthetic standpoint as well as in terms of its biochemistry and, therefore, no monosubstituted C5-O-derivatives of AsA have been reported in the literature. In this paper we describe the synthesis of 5-O-(4′-iodobenzyl)-L-ascorbic acid and evaluate its reducing activity. The substituent iodobenzyl moiety was chosen as the unit for iodine radiolabeling in our work, taking account of the fact that an iodine atom on aromatic rings is usually regarded as chemically and biochemically more stable moiety relative to aliphatic iodides.14

Results and Discussion

It is known that O-alkylation of AsA is fairly sensitive to reaction conditions, the nature of the agents and the derivatives used.15–17 We envisioned that derivatization of the C5-oxygen of AsA could be accessed by suitable manipulation of the protection and deprotection processes of the four-hydroxyl functional groups of AsA. However, a general problem of protected AsA derivatives is that a secondary proton at C5, adjacent to the enediol framework, is often susceptible to abstraction by a base, followed by an E2 elimination process leading to unsaturated 4,5-didehydro compounds.18,19 At the beginning point for the present study, the 2,3-di-O-benzyl derivative (1) of AsA as a model compound, in order to obtain the preliminary information about C5-O-iodobenzylated, was prepared from easily available AsA according to the procedure given in the literature,20 although the benzyl group had been considered unsuitable as a protecting group. The primary alcohol of 1 was selectively silylated with t-butyldimethylsilyl (TBDMMS) chloride to give 3 in good yield. Generally O-benzylated of AsA has been done under strongly alkaline conditions. We sought an O-iodobenzylated method, under which undesirable C5-deprotonation is likely to be avoided. Thus, the secondary hydroxyl group of 3 was then O-alkylated by treatment with p-iodobenzyl bromide in the presence of Ag2O and CaSO4 in dry benzene, according to a procedure described by Marm-sater et al.,21 to give the desired iodobenzylated 4 requiring a long reaction time, albeit in low yield. Not unexpectedly, the lower acidity and steric inaccessibility of the C5-hydroxyl group necessitated a longer reaction time for O-alkylation. Compound 4 was further characterized as its desilylated compound 5.

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As the introduction of the iodobenzyl group into the protected AsA derivative became possible, in our second set of experiments the synthetic route was modified by using either the tert-butyldiphenylsilyl (TBDPS) group or 2-methoxyethoxymethyl (MEM) group as the 2,3-di-hydroxy protecting group, instead of the 2,3-O-di-benzyl ethers. These protecting groups were selected in view of their known stability under a wide variety of reaction conditions and the fact that they can be selectively cleaved in the presence of benzyl ethers. 2,3-Di-O-silylation of 5,6-O-isopropylidene-L-ascorbic acid (2) was carried out with tert-butylidiphenylsilyl chloride in the presence of imidazole in DMF at 60 °C to afford the desired silylated 6 in good yield. The isopropylidene group was then removed from 6 with stannous chloride dihydrate in methanol using the conditions reported by Baer et al. to give the desired compound 7. On the other hand, as expected, treatment of 6 with 50% acetic acid resulted in the cleavage of the isopropylidene group as well as the TBDPS group (data not shown). The primary alcohol of 7 was protected with a TBDMS group to quantitatively give 8, and the secondary alcohol of 8 was subjected to O-alkylation with p-iodobenzyl bromide using the conditions developed for the model compound. However, in this case only the starting material was recovered with no trace of the desired compound. We speculated that the bulky silyl ether groups are likely sterically encumbered to undergo a C5-O-iodobenzylation reaction. In seeking an alternative protecting group, we replaced the TBDPS group with a 2-methoxyethoxymethyl (MEM) group.

The two enolic hydroxyls in 2 were protected by the MEM groups using MEM chloride and N,N-disopropylethylamine to give 9 in quantitative yield. Deprotection of the isopropylidene of 9 with 80% acetic acid, followed by protection of the resulting primary alcohol with TBDMS bromide provided the TBDMS ether (11). Compound 11 was then iodobenzylated in the same way, affording the desired compound 12, although again requiring a long reaction time and in low yield (20%). The reaction was still clean and gave only the unreacted starting material 11 without appreciable side reactions besides the desired product. Removal of the protecting groups with 1% HCl–EtOH provided the final target AsA derivative (13). The chemical shifts and coupling patterns observed in the 1H-NMR spectrum of 13, indicating a p-iodobenzyl group attached at C5, are consistent with those expected. Also, inspection of the proton-decoupled 13C-NMR spectrum of 13 showed eleven assignable peaks for the thirteen carbons of the compound, as expected.

The reducing ability of 13 in a mixture of Tris–HCl buffer (pH 7.4) and ethanol (2:3, v/v) was determined by the calibration method.
orimetric method using a stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), which accepts an electron or hydrogen radical to become a stable diamagnetic molecule.25,26 The change of absorbance at 517 nm due to the scavenging of the DPPH radical was continuously measured with a spectrophotometer. The rate constant for the disappearance of the DPPH color, which is referred to as the radical scavenging activity, was calculated from the slope of the initial period. Compound 13, as characterized by a rate constant of 0.525 ± 0.038 s⁻¹, exhibited a very strong scavenging activity against the free radical DPPH, almost equal to that of AsA itself having a rate constant of 0.391 ± 0.037 s⁻¹.

The objective of this work was to synthesize the C₅-O-monosubstituted analog of AsA, aimed at introducing radical stabilization and introducing variety of functional groups. Compound 13 was therefore prepared as a prototype model for further structural modifications at C₅ on the ascorbic acid side chain. In addition, the present route is acceptable for producing the target compound in the quantities needed for our further biological studies. We are currently examining radiolabeling with radioactive iodine for the assessment of its radiosensitizing efficacy.

Experimental

Chemical reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. 2,3-Di-O-benzyl-L-ascorbic acid (1) and 5,6-O-isopropylidene-L-ascorbic acid (2) were prepared from AsA according to the procedure given in the literature.20 All melting points are uncorrected. ¹H-NMR spectra were obtained on a Varian Unity 400 (400 MHz), and the chemical shifts are reported in parts per million downfield from tetramethylsilane. ¹³C-NMR spectra were recorded with a Shima duzu FTIR-8400 spectrometer and mass spectra were obtained with a JEOL JMS DX-610 (FAB-MS), or an Applied Biosystems Mariner System 5299 spectrometer (EI Mass). UV–VIS spectra were obtained on a Hitachi U-2810 spectrophotometer. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. Column chromatography was performed on Kieselgel 60 (70—230 mesh, Merck), the progress of the reaction was monitored by TLC on Silica gel 60F254 plates (Merck), and the spots were visualized with UV light or by spraying with 5% alcoholic molybdophosphoric acid. In the synthetic procedures, the organic extracts were routinely dried over anhydrous Na₂SO₄ and evaporated with a rotary evaporator under reduced pressure. All reactions involving air- or moisture-sensitive compounds were carried out under a positive argon atmosphere.

Synthesis

2,3-Di-O-benzyl-6-O-tert-butyldimethylsilyl-5-O-(4’-iodobenzyl)-L-ascorbic acid (3) tert-Butylidimethylsilyl chloride (1.35 g, 8.08 mmol) was added to a solution of 2,3-di-O-benzyl-L-ascorbic acid (1) (2.62 g, 7.35 mmol) in dry benzene (5 ml) at room temperature, and Ag₂O (286 mg, 1.22 mmol), CaSO₄ (296 mg, 2.17 mmol) and p-iodobenzene bromide (603 mg, 2.03 mmol) were then added sequentially. The mixture was covered in aluminum foil and stirred for 4 d at room temperature. The reaction mixture was filtered through a paper filter, and the solid residue was washed with EtOAc and the combined organic layers were concentrated to dryness. The residue was purified by silica gel chromatography (EtOAc:hexane=3:40) to give iodobenzylated (4) (249 mg, 36%) as a viscous oil.¹H-NMR (CDCl₃) δ: 0.02—3.86 (m, 1H), 0.04 (3H, 9H), 3.65—3.68 (m, 1H), 3.74—3.77 (m, 1H), 4.34 (d, 1H, J = 12.0 Hz), 4.43 (d, 1H, J = 12.4 Hz), 4.81 (s, 1H), 5.07—5.17 (m, 4H), 6.91 (d, 2H, J = 8.1 Hz), 7.15—7.17 (m, 2H), 7.32—7.60 (m, 8H), 7.69 (d, 2H, J = 8.4 Hz); IR (neat) cm⁻¹: 1763, 1676.

A solution of compound (4) (237 mg, 0.34 mmol) in 1% HCl–ethanol (6 ml), prepared from 99% ethanol and 37% hydrochloric acid, was stirred for 3.5 h at room temperature. The mixture was then evaporated to dryness and the residue was chromatographed on silica gel (EtOAc: hexane=1:2) to give the desilylated product (5) (172 mg, 88%) as a viscous oil.¹H-NMR (CDCl₃) δ: 3.70—3.72 (m, 1H), 3.76—3.77 (m, 2H), 4.44 (s, 2H), 4.76 (d, 1H, J = 2.4 Hz), 5.10—5.19 (m, 4H), 6.93 (d, 2H, J = 8.4 Hz), 7.15—7.18 (m, 2H), 7.33—7.36 (m, 8H), 7.60 (d, 2H, J = 8.3 Hz); IR (neat) cm⁻¹: 3400, 1759, 1674.

2,3-Di-O-tert-butyldimethylsilyl-5,6-O-isopropylidene-L-ascorbic Acid (6) tert-Butyldimethylsilyl chloride (4.80 g, 18.45 mmol) was added to a solution of 5,6-O-isopropylidene-L-ascorbic acid (2) (3.32 g, 6.15 mmol) in dry benzene (5 ml) containing imidazole (1.67 g, 24.60 mmol). The mixture was stirred at room temperature for 12 h under argon atmosphere. The mixture was concentrated and the residue was chromatographed on silica gel (EtOAc: hexane=1:2) to give the desilylated product (7) (1.27 g, 88%) as a colorless solid, mp 146—147 °C. ¹H-NMR (CDCl₃) δ: 0.98 (s, 9H), 1.02 (s, 9H), 1.13 (s, 3H), 1.29 (s, 3H), 3.63 (dd, 2H, J = 2.4, 7.3 Hz), 3.83 (d, 1H, J = 1.0 Hz), 3.90 (td, 1H, J = 7.1, 0.9 Hz), 7.30—7.50 (m, 12H), 7.66—7.74 (m, 8H); IR (CHCl₃) cm⁻¹: 1780, 1670, 1625 (M+M⁺). FAB-MS (m/z): 715 (M+Na⁺)⁻.

2,3-Di-O-tert-butyldimethylsilyl-6-O-isopropylidene-L-ascorbic Acid (8) tert-Butyldimethylsilyl chloride (90 mg, 0.60 mmol) was added to a solution of 7 (130 mg, 0.20 mmol) in CH₂Cl₂ (6 ml) containing imidazole (40 mg, 0.60 mmol) and the mixture was stirred at room temperature for 2 h. The reaction mixture was filtered off and the solid residue was washed with CH₂Cl₂. The combined organic layers were washed with water, saturated aqueous sodium chloride, and dried. Infrared (IR) spectra were recorded with a Shimadzu FTIR-8400 spectrometer and mass spectra were obtained with a JEOL JMS DX-610 (FAB-MS), or an Applied Biosystems Mariner System 5299 spectrometer (EI Mass). UV–VIS spectra were obtained on a Hitachi U-2810 spectrophotometer. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. Column chromatography was performed on Kieselgel 60 (70—230 mesh, Merck), the progress of the reaction was monitored by TLC on Silica gel 60F254 plates (Merck), and the spots were visualized with UV light or by spraying with 5% alcoholic molybdophosphoric acid. In the synthetic procedures, the organic extracts were routinely dried over anhydrous Na₂SO₄ and evaporated with a rotary evaporator under reduced pressure. All reactions involving air- or moisture-sensitive compounds were carried out under a positive argon atmosphere.

Synthesis

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iodobenzyl)-L-ascorbic Acid (10) (2.00 g, 53%) as a viscous oil. ^1H-NMR (CDCl3, 400 MHz) δ: 3.38 (s, 6H), 3.55—3.59 (m, 4H), 3.70—3.79 (m, 3H), 4.35 (d, 1H, J=11.8 Hz), 4.58 (d, 1H, J=12.0 Hz), 4.85 (d, 1H, J=1.3 Hz), 7.07 (d, 2H, J=8.1 Hz), 7.64 (d, 2H, J=8.2 Hz); ^13C-NMR (CDCl3, 100 MHz) δ: 62.2, 74.0, 76.4, 78.5, 93.6, 120.2, 130.8, 138.5, 139.5, 154.2, 173.2; IR (KBr) cm^-1: 3373, 3209, 1762, 1701; ESI-MS (m/z): 390.9664 Calcd for C_{11}H_{10}O_{2}(M): 390.9684. ^109C NMR (CDCl3, 100 MHz) δ: 133.7, 133.8, 133.9. Anal. C_{11}H_{10}O_{2} (427.7) C, 47.98; H, 3.78; O, 48.29. Found: C, 47.9; H, 3.8; O, 48.1.

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References