Reaction of Ascorbic Acid with Aliphatic Amines

Monika Pischetsrieder,* Bernd Larisch, Ursula Müller, and Theodor Severin*

Institut für Pharmazie und Lebensmittelchemie der Universität München, Sophienstrasse 10, D-80333 München, Germany

Ascorbic acid (**1**) reacts with propylamine or α-*N*-acetyllysine to afford *N*-substituted 3-deoxy-3-aminoascorbic acid of general structure **3**.

Keywords: *Ascorbic acid; aliphatic amines; aminoreductones*

INTRODUCTION

It is well documented that ascorbic acid (AA) can react with proteins (Bensch et al., 1985; Meucci et al., 1987), a process which is of considerable interest in food chemistry. Degradation of AA can lead to discoloration of fruit juices (Nagy et al., 1990) and other foods (Liao and Seib, 1987), and the formation of browning products and melanoidines has been studied in detail (Löschner et al., 1990, 1991; Rogacheva et al., 1995). The importance of AA interaction with proteins has been recognized in medical biochemistry as well. In the human body, predominantly long-living proteins are supposed to react with AA. The proteins of eye lenses have been thoroughly investigated, and it has been demonstrated that the lysine side chains react with AA, dehydroascorbic acid (DHA), or degradation products of these compounds (Ortwerth and Olesen, 1988; Ortwerth et al., 1988). During prolonged interaction, cross-linking of proteins is observed as well as the formation of colored compounds, but the structures of the products remained unknown.

Previously it has been shown that degradation of DHA (**7**) in the presence of α-amino acids leads to the formation of scorbamic acid (**9**) and 2,2′-nitrilodi-2(2′)-deoxyascorbic acid (**11**), a red pigment (Koppanyi et al., 1945; Kurata et al., 1973). It might be expected that AA and DHA react with primary amines in different ways. Here we report our results that verify this assumption.

MATERIALS AND METHODS

Apparatus. ^{1}H NMR (400 MHz) and ^{13}C NMR (400 MHz) spectra were recorded with a Jeol 400 GSX spectrometer with $(CH_3)_4Si$ as internal standard. Chemical shifts are reported in ppm. MS analysis was performed with a HP 5989 A MS Engine. Analytical HPLC was performed with a Merck L-6200 gradient pump, and a Merck D-6500 photodiode array detector that included Merck DAD-Manager software and a NEC pinwriter P60. UV spectra were directly taken from this system and are given in nm. For preparative chromatography, a Merck L-6250 pump, a Merck L-4000 UV detector, and a Merck D-2500 chromatointegrator were used.

Reagents. The water used for HPLC was distilled and filtered through a nylon membrane of 0.45 μm. HPLC grade methanol was used without further purification. All solvents were degassed with helium.

HPLC. Analytical HPLC was performed on a column packed with LiChroSorb (RP 18, 280 × 4 mm i.d., 5-μm particle size). The column was protected with a guard cartridge (25 × 4 mm) packed with the same material as the column. The eluents used were water (10 mmol of KH_2PO_4, pH 3.0 adjusted with H_3PO_4; solvent A) and methanol (solvent B) with a gradient of 0−75% B in 25 min, then continuing with 100% B from 25 to 40 min. The substances were detected with a diode array detector from 225 to 400 nm. Preparative HPLC was performed on a HiBar Column packed with LiChroSorb (RP 18, 250 × 20 mm i.d., 7-μm particle size).

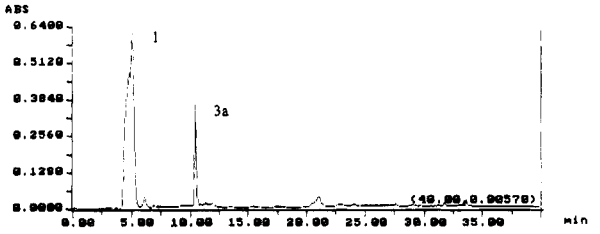

Figure 1. High-pressure liquid chromatogram of the reaction mixture of **1** and propylamine in methanol heated for 2 h at 100 °C (UV detection at 278 nm). Numbers on top of peaks refer to structures in Scheme 1.

3-Deoxy-3-(propylamino)-5,6-*O*-isopropylideneascorbic Acid (4a). 5,6-*O*-Isopropylideneascorbic acid (**2**; Micheel and Hasse, 1936; 800 mg, 3.7 mmol) and propylamine (240 mg, 4 mmol) were heated in a closed vessel in tetrahydrofuran (THF) (8 mL) at 100 °C for 4 h. The solvent was removed under reduced pressure, and the residue was dissolved in water:methanol (6:4). Isolation of **4a** was achieved by preparative HPLC; the eluent was water:methanol (6:4), the flow rate was 6 mL/min, and UV detection was at 278 nm. The fraction between 24 and 26 min was collected, and the methanol was removed under reduced pressure. The aqueous solution was lyophilized and **4a** was obtained as a colorless solid (yield after purification, 20%). Anal. Calcd for $C_{12}H_{19}O_5N$: C, 56.02; H, 7.44; N, 5.44. Found: C, 55.66; H, 7.52; N, 5.73. ^{1}H NMR (CD_3OD, COSY) δ 0.95 (m, 3H, CH_2CH_3), 1.3 (2s, 6H, $(CH_3)_2CO_2$), 1.6 (m, 2H, CH_2CH_3), 3.3 (m, 2H, NCH_2), 4.0 (m, 1H, CH_aH_bO), 4.1 (m, 1H, CH_aH_bO), 4.5 (m, 1H, CH_2OCHO), 4.6 (m, 1H, CHOC=); ^{13}C NMR (CD_3OD, COSY, DEPT) δ 11.4 (CH_2CH_3), 25.3 (CH_2CH_3), 25.7 and 26.1 ($(CH_3)_2CO_2$), 46.3 (NCH_2), 66.4 (CH_2O) 75.3 (CHOC=), 75.7 (CH_2OCHO), 111.2 ($CO_2(CH_3)_2$), 115.0 (HOC=), 148.0 (NC=), 174.3 (C=O); MS (*m*/*z*, CI) 258 (M + 1), 200; UV λ_{max} = 278 nm (UV spectra were directly taken from the analytical HPLC system).

3-Deoxy-3-(propylamino)ascorbic Acid (3a). 3-Deoxy-3-(propylamino)-5,6-*O*-isopropylideneascorbic acid (**4a**; 10 mg, 0.05 mmol) was dissolved in methanol (2 mL) and allowed to stand over Amberlite IR 120 for 12 h. After decanting the solution, the solvent was removed under reduced pressure, and a light yellow solid was obtained: ^{1}H NMR (CD_3OD) δ 0.96 (t, 3H, CH_2CH_3), 1.6 (m, 2H, CH_2CH_3), 3.4 (m, 2H, NCH_2), 3.6 (dd, 2H, CH_2O), 3.9 (m, 1H, CH_2OCHO), 4.8 (d, 1H, CHOC=); MS (*m*/*z*, EI) 217 (M), 186; UV λ_{max} = 278 nm (UV spectra were directly taken from the analytical HPLC system).

Reaction of AA with Propylamine or α-*N*-Acetyllysine in Methanol. AA (100 mg, 0.57 mmol) and propylamine (33 mg, 0.56 mmol) or α-*N*-acetyllysine (108 mg, 0.57 mmol) were dissolved in methanol (1 mL) and kept for 2 h at 100 °C in a closed vessel. A diluted sample of each reaction mixture was analyzed by HPLC (Figures 1 and 2).

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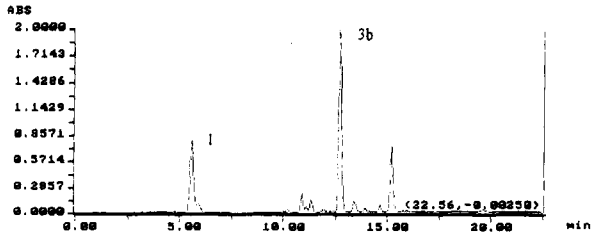


Figure 2. High-pressure liquid chromatogram of the reaction mixture of 1 and α-*N*-acetyllysine heated in methanol for 24 h (UV detection: 0–10 min, 245 nm; then 278 nm). Numbers on top of peaks refer to structures in Scheme 1.

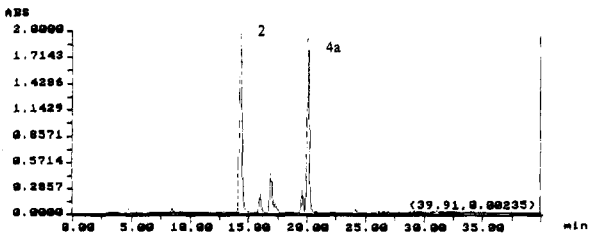


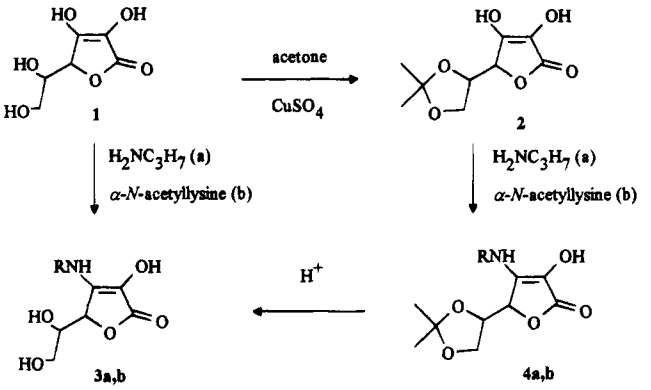
Figure 3. High-pressure liquid chromatogram of the reaction mixture of **2** and propylamine heated in THF for 4 h at 100 °C (UV detection: 0–18 min, 245 nm; 18–40 min, 278 nm). Numbers on top of peaks refer to structures in Scheme 1.

Reaction of AA with Propylamine in Water. AA (100 mg, 0.57 mmol) and propylamine (33 mg, 0.56 mmol) were dissolved in water (1 mL), and the solution was neutralized with phosphoric acid. The mixture was heated for 2 h at 100 °C. A diluted sample was analyzed by HPLC. Chromatographic and spectroscopic data revealed the identity of the product with **3**.

Preparation of 3-Deoxy-3-propylamino-5,6-*O*-isopropylidene-2-*O*-methylascorbic Acid (6) from 5,6-*O*-Isopropylidene-2-*O*-methylascorbic Acid (5). A mixture of 5,6-*O*-isopropylidene-2-*O*-methylascorbic acid (**5**; 800 mg, 3.7 mmol), which can be obtained by the method of Lu et al. (1984), and propylamine (218 mg, 3.7 mmol) were heated in 8 mL of THF in a closed vessel for 4 h at 100 °C. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate. This solution was extracted with an aqueous solution of sodium bicarbonate (1 M, 3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification was achieved by preparative HPLC. The residue was dissolved in methanol, filtered through a Durapore poly(vinylidene fluoride) membrane (0.45 μm), and injected into the system (see Apparatus); the eluent was water:methanol (5:5), the flow rate was 10 mL/min, and UV detection was at 273 nm. The fraction between 20 and 23.5 min was collected, and the methanol was removed under reduced pressure. The aqueous solution was lyophilized, and **6** was obtained as a slightly yellow solid: ^{1}H NMR ($CDCl_3$, COSY) δ 0.97 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.36 and 1.45 (2s, $(CH_3)_2CO_2$), 1.60 (m, 2H, CH_2-CH_3), 3.40 (m, 2H, NCH_2), 3.75 (dd, J = 6.8 and 8.6 Hz, 1H, CH_aH_bO), 3.77 (s, 3H, OCH_3), 4.04 (dd, J = 6.8 and 8.6 Hz, 1H, CH_aH_bO), 4.55 (m, 1H, CH_2OCHO), 4.74 (NH), 4.82 (d, J = 3.8 Hz, 1H, CHOC=); ^{13}C NMR ($CDCl_3$, COSY, DEPT) δ 11.1 (CH_2CH_3), 23.8 (CH_2CH_3), 24.1 and 25.8 ($(CH_3)_2CO_2$), 45.6 (NCH_2), 60.5 (OCH_3), 64.1 (CH_2O), 72.1 (CHOC=), 74.2 (CH_2OCHO), 110.0 ($CO_2(CH_3)_2$), 118.9 (HOC=), 148.7 (NC=), 169.2 (C=O); MS (m/z, CI) 272 (M + 1); UV λ_{max} = 273 nm (UV spectra were directly taken from the analytical HPLC system).

Preparation of 3-Deoxy-3-(propylamino)-5,6-*O*-isopropylidene-2-*O*-methylascorbic Acid (6) from 3-Deoxy-3-(propylamino)-5,6-*O*-isopropylideneascorbic Acid (4a). 3-Deoxy-3-(propylamino)-5,6-*O*-isopropylideneascorbic acid (**4a**; 80 mg, 0.31 mmol) was dissolved in water (0.8 mL). While bubbling nitrogen through the solution, it was adjusted to pH 10.5 with aqueous sodium hydroxide (2 M), and dimethyl sulfate (0.032 mL, 0.33 mmol) was added. The reaction mixture was kept under nitrogen for 10 min at pH 10.5 and room temperature, adjusted to pH 4 with cold sulfuric acid, and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were reextracted with aqueous sodium carbonate solution (1 M; 3 × 10 mL) to remove educt and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was dissolved in 0.3 mL of methanol and filtered through a Durapore poly(vinylidene fluoride) membrane (0.45 μm). For purification, this solution was injected into the preparative HPLC system (see Apparatus); the eluent was water:methanol (6:4), the flow rate was 12 mL/min, and UV detection was at 273 nm. The fraction between 30 and 35 min was collected, and the methanol removed under reduced pressure. The aqueous solution was lyophilized, and a slightly yellow solid was obtained. The spectral data were identical with those of **6** just described.

Scheme 1



RESULTS AND DISCUSSION

When AA and propylamine are heated in neutral, alkaline, or acidic aqueous solution or are allowed to stand at 40 °C, a reaction product can be detected by HPLC that is characterized by an absorption maximum at 278 nm. The yield is increased when methanol is applied as solvent (Figure 1). Isolation and purification of the new compound proved to be difficult, but the same substance was more easy obtained by a slightly different route. When 5,6-*O*-isopropylideneascorbic acid (**2**) is heated with propylamine in THF or methanol, the aminoreductone **4a** is formed as main product in high yield (Figure 3). Acid hydrolysis of **4a** leads to the substitution product **3a** obtained directly from AA and propylamine (Scheme 1).

The structures of **4a** and **3a** are established by spectral data. In the ^{13}C NMR, the aminoreductone structure is represented by signals at 174.3, 114.5, and 148.2 ppm for C-1, C-2, and C-3, respectively. The substitution pattern (Scheme 1) is evidenced by a COLOC correlation between NCH_2 and C-3. Products **4** and **3** show a λ_{max} at 278 nm, whereas a λ_{max} for **9** is observed at 246.5 nm (Kurata et al., 1945).

To further establish the structure of the aminoreductone (**4**) reactions of an *O*-methyl derivative of AA has been investigated. 2-*O*-Methylascorbic acid and 3-*O*-methylascorbic acid are described in the literature (Lu et al., 1984). The 2-*O*-methyl derivative is more acidic, and its dissociation in dilute aqueous solution leads to a shift of the UV maximum from 237 to 260 nm. When the 5,6-*O*-isopropylidene derivative of 2-*O*-methylascorbic acid **5** is heated with propylamine in THF solution, the substitution product **6** is obtained (Figure 4). The structure of this *O*-methylaminoreductone is derived from the NMR and MS spectral data (see Materials and Methods). The UV absorption of **6** is similar to that of **4**. The same *O*-methylaminoreductone **6** is obtained when the 5,6-*O*-isopropylideneascorbic acid/propylamine reaction product **4a** is treated with dimethyl sulfate in

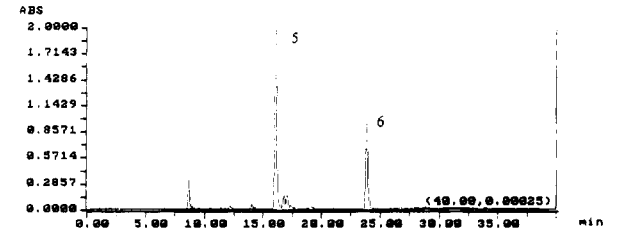
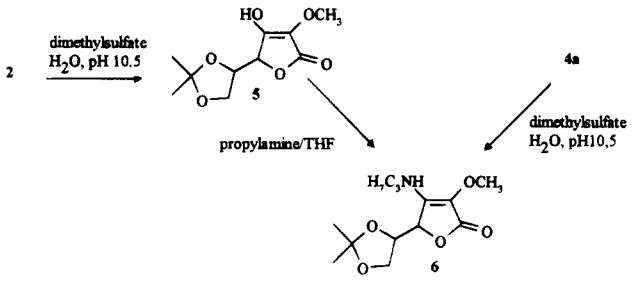
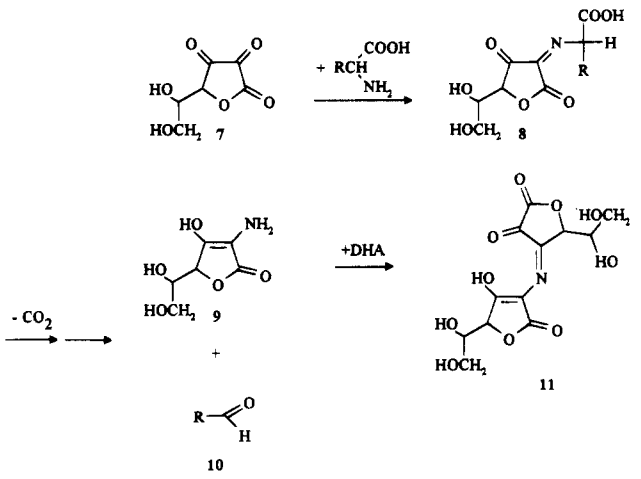


Figure 4. High-pressure liquid chromatogram of the reaction mixture of **5** and propylamine heated in THF for 4 h at 100 °C (UV detection: 0–20 min, 245 nm; 20–40 min, 273 nm). Numbers on top of peaks refer to structures in Scheme 2.

Scheme 2



Scheme 3



alkaline solution. Purification can be performed by HPLC. The identity of **6** obtained by different routes as just described above (Scheme 2) is shown by a comparison of the NMR, MS, and UV spectral data. Thus, the structure of the AA/propylamine reaction product is confirmed.

Reaction of AA or the isopropylidene derivative **2** with α-*N*-acetyllysine proceeds in an analogous manner (Figure 2). The formation of aminoreductones of structures **4b** and **3b** is derived from the characteristic UV absorption of the products. So, it seems justified to assume that aminoreductones of general structure **3** can be formed as well by interaction of AA with lysine side chains of proteins. But, it must be emphasized that aminoreductones of type **3** are not very stable. The mechanism of degradation is at present under investigation. The previously investigated reaction of DHA with amino acids follows a different reaction mechanism that must be considered as a Strecker degradation. The Schiff base **8** formed of DHA (**7**) and amino acid as an intermediate affords scorbamic acid (**9**) besides the aldehyde **10** by an intramolecular redox reaction and subsequent hydrolysis (Scheme 3).

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