Identification of Two Degradation Products from Aqueous Dehydroascorbic Acid

Masayoshi Sawamura,* Kiyoshi Takemoto, Yoshihisa Matsuzaki, Hiroyuki Ukeda, and Hirozo Kusunose

Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku City, Kochi 783, Japan

The chemical structures of two compounds formed from an L-dehydroascorbic acid (DHA) solution were identified. They were 3,4-dihydroxy-5-methyl-2(5*H*)-furanone (MW 130), brown color, and 2-furancarboxylic acid (MW 112), colorless. The DHA solution produced a browner color under nonoxidative conditions than under oxidative conditions. The former increased under nonoxidative conditions during storage. The latter consists of explicit white crystals, and its aqueous solution never becomes colored during storage. It overlapped partly with the other brown pigment on a column chromatogram of Toyopearl HW-40F.

INTRODUCTION

The browning of processed citrus juices is one of the most important considerations in terms of quality, and a number of workers have studied it (Clegg, 1964; Kacem et al., 1987; Lee and Nagy, 1988; Handwerk and Coleman, 1988; Nagy et al., 1990). The authors (Li et al., 1989a,b) have pointed out that the browning is mainly caused by the degradation of L-ascorbic acid (AA) in citrus juices and that the behavior of browning of an AA model solution was similar to that of the citrus juices. It begins with the degradation of L-dehydroascorbic acid (DHA) after the oxidation of AA (Sawamura et al., 1991a). Moreover, by using [1-^{14}C]DHA it was found that the DHA skeleton was first decarboxylated (loss of C-1) (Sawamura et al., 1991b). This indicates that both brown pigments (B-I to B-IV) and products (C-I to C-III) formed in the degradation of DHA are essentially free from the C-1 of DHA. In addition, gel filtration chromatography suggests that the molecular weights of the pigments and the degradation products do not seem to be as large as that of DHA (174). Thus, further studies are required to determine the chemical structures of overall degraded compounds including brown pigments formed from DHA. The present paper deals with one pigment (B-III) and one colorless product (C-III) formed from DHA.

MATERIALS AND METHODS

Storage Conditions of Juice. To prepare the oxidative and nonoxidative samples, aliquots of aqueous DHA solution (10 mL) were poured into 13.3-mL vials and tightly sealed by fastening rubber stoppers with aluminum caps. The headspace was replaced by O_2 for oxidative samples or by N_2 for nonoxidative ones. The samples were stored at 37 °C in the dark. The difference of formation of B-III between oxidative and nonoxidative samples was compared by the percentage of the peak area on chromatograms monitored at 250 nm.

Chromatography of Degradation Products from DHA. An aqueous solution of 1% DHA, which is prepared from AA by using Br_2 (Rose and Nahrwold, 1981), was stored for 1 month at 37 °C to cause browning. The solution was then lyophilized and fractionated by a column chromatograph (3.5 cm i.d. × 100 cm) packed with Toyopearl HW-40F (Tosoh Co., Tokyo) at 4 °C in the dark. The eluent was distilled water, and the flow rate was 0.25 mL/min. The effluent was fractionated in 5-mL fractions with monitoring absorbance at 420, 290, and 250 nm (Sawamura et al., 1991b).

HPLC. The fraction of B-III and the mixed one of C-III and B-IV were lyophilized. Then, the latter fraction was subjected again to column chromatography and lyophilized. Further purification of the samples and confirmation of their purity after column chromatography were carried out on a Toyo Soda high-performance liquid chromatograph composed of a CCPE pump and a UV-8000 variable-wavelength UV–vis detector. An octadecyl silica gel column (Wakosil 5C_{18}, 4.6 mm i.d. × 150 mm, Wako Chemicals Co., Osaka) was used. The mobile phase was either H_2O for purification or 0.2% metaphosphoric acid for the purity confirmation run, which is isocratic at 1.0 mL/min at 40 °C. After monitoring at 250 nm for the purification run, the peak was lyophilized and dried over P_2O_5 and then used for instrumental analysis. The purities for B-III and C-III were confirmed with monitoring at 420 and 290 nm. Additionally, thin-layer chromatography was carried out by using the liquid phase (benzene–methanol–acetic acid 45:8:8) on Kieselgel 60 GF_{254} (Merck). The spot was detected visually or by an ultraviolet lamp.

Identification of Structures. Chemical structures of the materials from DHA were determined by the following instruments: visible and ultraviolet spectroscopy, Hitachi double-beam spectrophotometer 220; elemental analysis, Yanagimoto element analyzer Model MT2; MS, Shimadzu gas chromatograph–mass spectrometer LKB-9000 equipped with electron ionization; IR, Shimadzu Fourier transform infrared spectrometer FTIR-4300; NMR, Japan Electronics nuclear magnetic resonance JNM-GSX400 and JNM-FX60Q.

RESULTS AND DISCUSSION

Ascorbic acid is known to be an antioxidant. It is often effective for suppressing enzymatic browning in processing fruit juice products such as apple and peach. It has been thought that polyphenols are oxidized to form *o*-quinones, being subsequently polymerized to brown pigments. AA plays a role in turning it back to the starting polyphenols. As long as AA remains in the juice, a slight amount of enzymatic browning occurs. While the browning of citrus juice is caused by a nonenzymatic process, and the substantial starting material is DHA (Sawamura et al., 1991a), it degrades to form brown pigment even if oxygen is present or not in the solution. As various osones are formed by DHA degradation, amino acids would accelerate the browning involving an amino–carbonyl reaction (Li et al., 1989c), though it is not dealt with here relative to this problem. Thus, it may be important to prevent the degradation of DHA in juice processing. We have been investigating the degradation products of DHA using a model system as a first step.

A schematic chromatogram (Figure 1) may be helpful in reviewing the products that were formed by the

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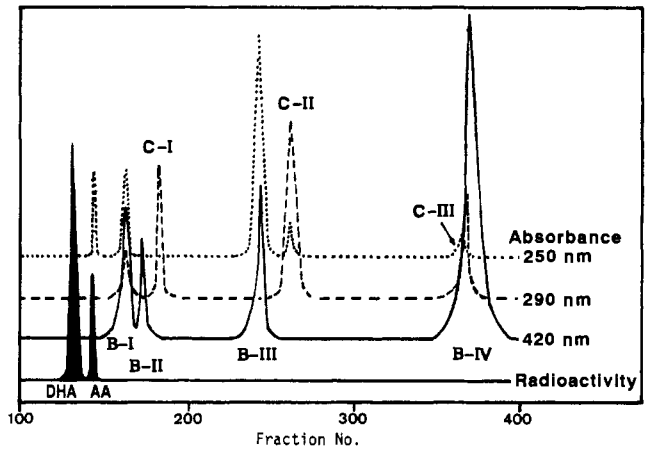

Figure 1. Schematic chromatogram of brown pigments and intermediates from a DHA aqueous solution by gel chromatography.

Table 1. Analytical Data for Brown Pigment (B-III)

IR ν_{max} (KBr), cm^{-1}: 3355, 3065 (OH); 2945, 1458 (C—CH_3); 1738 (O—C=O); 1641 (C=O); 1342, 1300 (C—O); 1163, 1080 (C—O—C)

EIMS, *m/z*: 130 (M^+), 85 ($M - C_2H_5O^+$), 57 ($C_2HO_2^+$)

elemental analysis: found, C, 45.84; H, 4.55; O; 49.61
Calcd for $C_5H_6O_4$, C, 46.15; H, 4.62; O, 49.23%

NMR (DMSO-d_6), δ_H: 1.32 (3H, doublet, J = 6.7 Hz, CH—C*H*$_3$), 4.70 (1H, quartet, J = 6.5 Hz, C*H*—Me), 8.22 (1H, C—O*H*), 10.95 (1H, C—O*H*)

degradation of DHA as demonstrated previously (Sawamura et al., 1991b). The radioactive peaks of AA and DHA from [1-^{14}C]DHA were also inserted in the scheme. It is demonstrated that the degradation products of DHA have no carbon-1 of AA at least, because no radioactive peak can be seen after the peaks of AA and DHA. This may be useful for knowledge of their structures. There are four brown products (B-I to B-IV) detected at 420 nm and three colorless products (C-I to C-III) detected at 290 and 250 nm. The purified B-III and C-III could be obtained by means of repetition of chromatography. They gave a single peak on the liquid chromatogram and a single spot on the thin-layer chromatogram. It was difficult to separate clearly B-I, B-II, and C-I in this condition as their retention times were very close. Though C-III and B-IV almost overlapped at the first separation, C-III could be successfully isolated by combined methods of the repetition of column chromatography and HPLC. B-IV has not been isolated yet because of the tailing of C-III into peak B-IV. We are now trying to remove C-III from B-IV using some procedures such as ion-exchange resin chromatography. Our results will be reported later. As mentioned previously (Sawamura et al., 1991b), the colorless fraction C-II could be isolated by chromatography, but it turned into the brown pigment B-IV as soon as it was placed in an atmospheric condition after completion of lyophilization. Special handling will be necessary for instrumental measurement. Thus, we first dealt with a brown pigment (B-III) and a colorless product (C-III).

The analytical data for B-III are summarized in Table 1. The IR spectrum of the pigment suggests the existence of hydroxyl and methyl groups. A lactone ring at 1738 cm^{-1}, a carbonyl group at 1641 cm^{-1}, and a five-membered ring at 1342 and 1300 cm^{-1} were also observed in the IR spectrum. The maximum peak in the MS spectrum was 130. The data for elemental analysis coincided exactly with the calculated data for $C_5H_6O_4$. The measurement

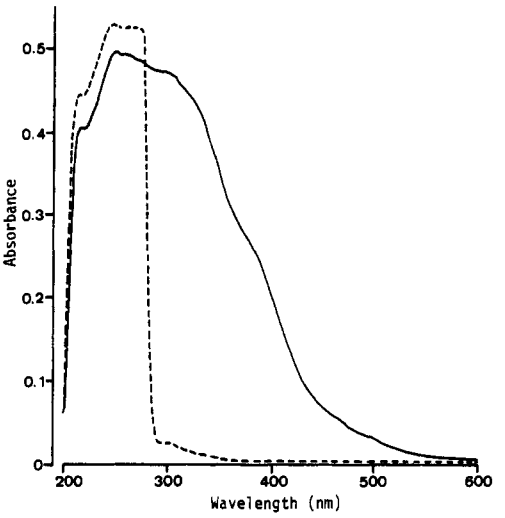

Figure 2. Visible and ultraviolet absorption spectra of brown pigment B-III (—) and degradation product (- - -).

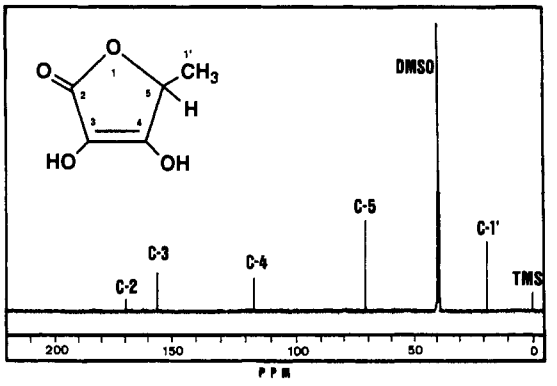

Figure 3. Proton-decoupled ^{13}C spectrum of brown pigment B-III and its chemical structure: 3,4-dihydroxy-5-methyl-2(5*H*)-furanone.

for ^{1}H and ^{13}C NMR was carried out in $(CD_3)_2SO$. The maximum in visible, and the ultraviolet absorption spectrum drawn by a continued line was 250 nm, as shown in Figure 2. There was no peak of visual absorption which coincided with the spectra of brown pigment in orange juice (Meydav et al., 1977). The ^{13}C NMR spectrum is given in Figure 3. The complete decoupling method showed that B-III was constructed of five carbons, while the off-resonance measurement gave three singlets, one doublet, and one quartet for each peak. The peaks were assigned as follows: 18.2 ppm (C-1'), 71.3 ppm (C-5), 116.6 ppm (C-4), 156.1 ppm (C-3), and 169.7 ppm (C-2). In conclusion, we defined the chemical structure of the brown pigment B-III as 3,4-dihydroxy-5-methyl-2(5*H*)-furanone (MW 130), as shown in Figure 3.

It has been generally recognized that the molecular weight of a brown pigment would be greater than that of the starting material, since browning involves polymerization (Kennedy et al., 1990). There is little knowledge concerning the chemical structure of browning because of the complexity due to the polymerization. However, the molecular weight of B-III is 130 and actually smaller than that of DHA.

We reported that the browning of an aqueous DHA solution proceeded under nonoxidative conditions more readily than under oxidative ones (Li et al., 1989b). It had been, however, unknown what kind of browning material is responsible for that phenomenon. Figure 4 shows that a nonoxidative solution of DHA favors the formation of B-III. There is an assumption on the

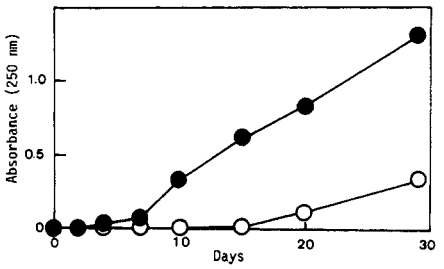

Figure 4. Effect of oxygen on the formation of B-III in a DHA solution during storage at 37 °C: (○) oxidative condition; (●) nonoxidative condition.

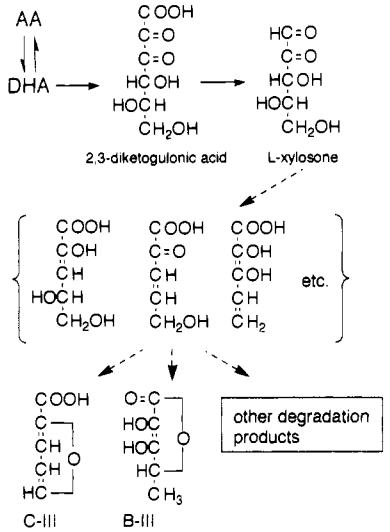

Figure 5. Tentative scheme for the degradation of DHA.

Table 2. Analytical Data for DHA Degradation Product (C-III)

IR ν_{max} (KBr), cm^{-1}: 2584–3142 (OH), 1691 (C=O), 1585 (C=C), 1481 (C—C), 1306 (C—O), 1196 (C—O—C), 766 (C—H)

EIMS, m/z: 112 (M^+), 95 (M^+ – OH), 39 ($C_3H_3^+$)

elemental analysis: found, C, 53.47; H, 3.90; O; 42.54
Calcd for $C_5H_4O_3$, C, 53.57; H, 3.57; O, 42.86%

NMR (DMSO-d_6), δ_H: 6.64 (1H, quartet, J = 1.8 Hz, CH=C*H*—CH), 7.19 (1H, doublet, J = 0.9 Hz, CH—C*H*=C), 7.88 (1H, doublet, J = 0.9 Hz, CH=C*H*—O), 11.89 (1H, C—O*H*)

degradation pathway of DHA postulated by Kurata and Sakurai (1967). A tentative scheme for the DHA degradation is outlined in Figure 5. L-Xylosone is formed by decarboxylation of 2,3-diketogulonic acid, and then several intermediates including reductones will be produced. Some of them will be responsible for the precursors of brown pigments. If these intermediates undergo oxidative degradation, the development of brown pigments would be suppressed. This may be one reason for the decline of formation of B-III under oxidative conditions.

Further purification of the fraction containing B-IV by HPLC gave two different peaks. One was a brown compound B-IV, detectable at 420 nm, while the other, which was eluted just before the peak of B-IV, was colorless (C-III) and showed absorbance at 250 nm but not at 420 nm.

The analytical data for C-III is summarized in Table 2. The IR spectrum indicates a carbonyl group at 1691 cm^{-1} and a furan ring at 1585 and 1196 cm^{-1}. The λ_{max} was 245 nm in the spectrum drawn by a broken line as shown in Figure 2, and the melting point was 130 °C. The maximum peak in the MS spectrum was 112, followed by two small

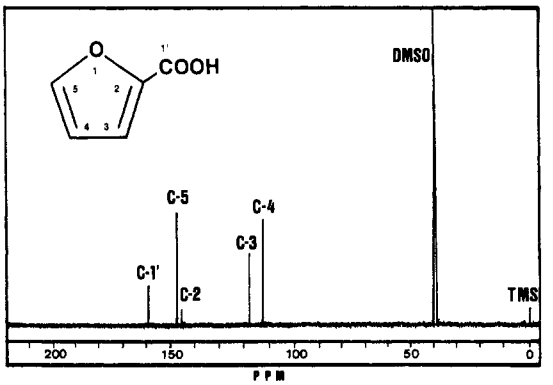

Figure 6. Proton-decoupled ^{13}C spectrum of degradation product C-III and its chemical structure: 2-furancarboxylic acid.

peaks of 113 and 114 that resulted from the isotope. The relative intensities were 5.6% and 0.8% in the masses of 113 and 114, respectively. This knowledge is useful for the assumption of the molecular formula. The result of the elemental analysis coincided with the calculated data for $C_5H_4O_3$. The ^{13}C NMR spectrum is given in Figure 6. C-III is composed of five carbons, and they were assigned as follows: 112.0 ppm (C-4), 117.6 ppm (C-3), 144.9 ppm (C-2), 146.9 ppm (C-5), 159.2 ppm (C-1′). From these overall data we have now determined the chemical structure of the colorless product (C-III) of DHA as 2-furancarboxylic acid or 2-furoic acid ($C_5H_4O_3$), as shown in Figure 6. All of these data were in accordance with those of its authentic chemical (Sigma Chemical Co., St. Louis, MO).

It was reported that 2-furoic acid would come out in the degradation mechanism of AA in an aqueous solution (Kurata and Sakurai, 1967; Tatum et al., 1969). Our result proves the occurrence of 2-furoic acid as one of the degradation products of an aqueous solution of DHA. C-III is sublime, and it never colors even if stored at 37 °C for 1 month. Therefore, 2-furoic acid is not related to browning of DHA, although it is one of the degradation products of DHA. It is suggested that the molecular weight of B-IV might be similar to that of 2-furoic acid, because the peak of C-III was very close to the biggest one of the brown pigment (B-IV) in column chromatography (Figure 1). It is also important, however, to consider the adsorption property involved in Toyopearl HW-40F (Derdelinckx and Jerumanis, 1984). In any case, other products formed by the degradation of DHA will be further studied.

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