^{14}C Studies on Browning of Dehydroascorbic Acid in an Aqueous Solution

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Aqueous solutions of L-dehydroascorbic acid (DHA) and a mixture of DHA and glycine including [1-^{14}C]-L-DHA were aged to yield browning pigments. The lyophilized browning pigments were separated into several fractions by gel filtration chromatography. Radioactivity was absent in any browning fraction. It is certain that C-1 of DHA has to first be decarboxylated to form browning pigments. All of the browning fractions were eluted after L-ascorbic acid (AA). It is suggested that the molecular weights of most browning pigments are not greater than that of AA. There were some colorless fractions detectable by UV, and one of them immediately turned into dark brownish materials in the atmosphere. This is a precursor of browning pigments.

INTRODUCTION

Nonenzymic browning is a major factor in quality deterioration of processed citrus juices and has been discussed for over half a century as reviewed recently by Handwerk and Coleman (1988). It is generally understood that the degradation products of L-ascorbic acid (AA) and/or sugars, e.g., furfural (FUR), 5-(hydroxymethyl)furfural (HMF), or other carbonyl compounds, would participate in juice browning and polymerize each other or react with amino acids to yield browning materials (Clegg, 1964; Robertson and Samaniego, 1986; Kacem et al., 1987; Lee and Nagy, 1988a,b; Kennedy et al., 1990). There have been numerous studies on the mechanism and intermediates of AA degradation. However, nothing is known about the browning compounds formed from AA.

A series of our works (Li et al., 1989a,b; Sawamura et al., 1991) on the browning of citrus juice has revealed that FUR or HMF is little related with browning and that browning materials were formed through oxidative degradation of AA via L-dehydroascorbic acid (DHA).

Hayashi et al. (1983a,b) proposed that a mixture of DHA and an amino acid in an nonaqueous solution gave rise to red or yellow pigment as an intermediate in the browning pathway. The pigment is an oxidative condensate from two or three molecules of L-scorbamic acid (SCA) produced from the reaction of DHA with α-amino acids (Kurata et al., 1973). Their result also implies that the browning material has a larger molecular weight than that of DHA.

Although isotopic AA was originally used for the study on its metabolism in vivo by Burns et al. (1958), it has not been applied yet to the study of nonenzymic browning. The present study was carried out to investigate the relationship between DHA and browning pigments by use of a model aqueous solution of labeled DHA.

MATERIALS AND METHODS

Chemicals. [1-^{14}C]-L-AA (0.37 GBq/mmol), CO_2 absorber (Oxisorb-CO_2), and scintillation cocktail (Aquasol 2) were purchased from NEN Research Products through Japan Radioisotope Association.

Model Systems. [1-^{14}C]-L-DHA was prepared from [1-^{14}C]-L-AA in the same manner as the oxidation of AA to DHA with Br_2 (Rose and Nahrwold, 1981). Two kinds of model solutions examined were as follows: one is a single DHA solution (100 mL) composed of 1.0% DHA with approximately 1.85 MBq of [1-^{14}C]-L-DHA; the other (100 mL) is composed of a mixture of 0.5% DHA and 0.5% glycine with approximately 1.85 MBq of [1-^{14}C]-L-DHA. Portions (10 mL) of these sample solutions were poured into vials and tightly covered with aluminum foil lined rubber stoppers. After the headspace was replaced by N_2, the samples were kept at 37 ± 0.1 °C for 4 weeks in the dark.

Table I. Radioactive Distribution[a] of Aged Aqueous Samples

model	total	[^{14}C]CO_2	[^{14}C]AA	[^{14}C]DHA	others
DHA	100[b]	78.2	8.0	13.8	0
DHA–Gly	100[c]	91.8	7.9	0.3	0

[a] Expressed as percentage of total radioactivity. Average of triplicates. [b] 1.423×10^8 dpm. [c] 1.445×10^8 dpm.

Chromatography of Browning Pigments and Intermediates. The 4-week-aged sample solutions were bubbled with N_2 for 15 min to absorb CO_2 in the headspace and then lyophilized. The concentrate was fractionated by column chromatography (3.5 cm i.d. × 100 cm) using Toyopearl HW-40F (Tosoh Co.) at 4 °C in the dark. The eluent was distilled water, and the flow rate was 0.25 mL/min. Each fraction (5 mL) was monitored by absorption at 420, 290, and 250 nm, respectively. The radioactivity was measured by a Beckman SC-1 scintillation counter.

RESULTS AND DISCUSSION

Experiment 1: DHA Model. As shown in Table I, about 80% of the total radioactivity occurred in CO_2 absorber. The result indicates that the DHA undergoes decarboxylation on C-1 in its chemical form during storage. Table I also indicates that about 14% of the initial DHA remained and 8% of that was converted to AA under the storage conditions described.

Figure 1 shows the distribution of the radioactivity and absorbance in the concentrate from the DHA model sample on the chromatogram. We can see two radioactive peaks: A-I is assigned to [^{14}C]DHA and A-II to [^{14}C]AA. The radioactivity was not detected after A-II and did not remain in the residue.

Monitoring at 420 nm gives us the distribution of browning pigments. Each fraction was yellow or reddish yellow. It is suggested that the browning pigments produced by the degradation of DHA would consist of at least four compounds. The fraction of B-IV seems to contribute greatly to the browning due to strong absorption at 420 nm.

The peaks detected at 290 and 250 nm are the degradation products of DHA. There were two main peaks

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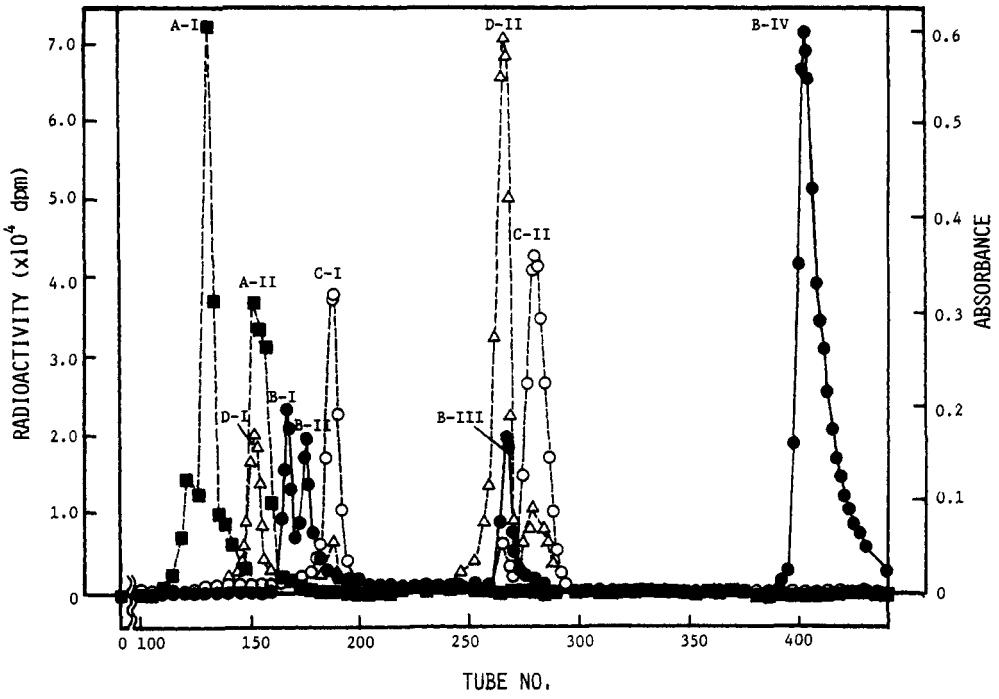

Figure 1. Separation of browning pigments and intermediates from a DHA aqueous solution by gel filtration column chromatography: (■) radioactivity; (●) OD_{420}; (○) OD_{290}; (△) OD_{250}.

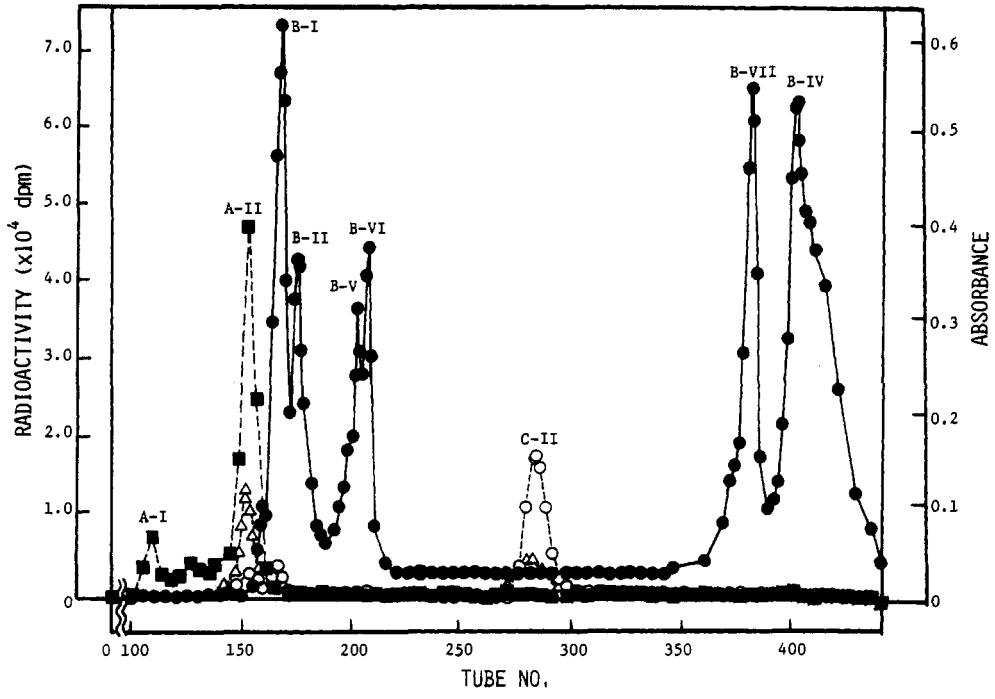

Figure 2. Separation of browning pigments and intermediates from a mixture of DHA and glycine in an aqueous solution by gel filtration column chromatography: (■) radioactivity; (●) OD_{420}; (○) OD_{290}; (△) OD_{250}.

detectable at 290 nm (C-I and C-II). Although the C-II fraction was colorless during lyophilization under vacuum, it dramatically turned into dark brown as soon as the internal pressure was released to atmosphere. It was proved by HPLC that this browned material was just the same as B-IV. Therefore, C-II is a colorless precursor of the browning pigment B-IV, and the conversion of C-II to B-IV occurs easily under oxidative conditions. The λ_{max} of C-II was 293 nm.

The chromatogram monitored at 250 nm gave two main peaks: D-I was assigned to AA, and D-II overlapped with B-III. A fraction equivalent to DHA was not observed, because DHA has no absorption at 250 nm but does at 210 nm. The λ_{max} of D-II was 249 nm.

The Toyopearl HW-40F is in principle a type of molecular exclusion (100–7000 for dextrans) and is often used for gel filtration chromatography. Thus, Figure 1 also gives us information on the molecular weight of the browning fractions, B-I–IV, that is, equal to or less than that of AA (176). Moreover, the absence of radioactivity in these browning fractions indicates that they ought to be decarboxylates and have lower molecular weights than DHA. It has been imagined that the molecular weight of browning pigments probably would be much higher than that of their substrate due to polymerization. Our result suggests the molecular weight of the browning compounds would be as low as 130. It is known that Toyopearl HW-40F does not always work on the principle of gel filtration

but also on that of absorption chromatography (Ozawa, 1982; Derdelinckx and Jerumanis, 1984). There might be a slight disorder on the molecular weights of the materials eluted in this chromatography. The mass spectral datum regarding B-IV was, however, 112. The chemical structures of browning materials are being studied in detail.

Kurata and Sakurai (1967a,b) reported that DHA changed into 2,3-diketo-L-gulonic acid in an aqueous solution and was subsequently decarboxylated to form 2-furoic acid, 3-hydroxy-2-pyrone, and 3-keto-4-deoxypentosulose, which are assumed to have some relationship with browning. We support their result that the decarboxylation of DHA is necessary to reach browning compounds.

Experiment 2: DHA–Glycine Model. The radioactivity of about 91% of initial administration occurred in CO_2 and others did in DHA and AA, as given in Table I. Three browning fractions (B-V–VII) were obtained in addition to B-I, B-II, and B-IV (Figure 2). All of these fractions were free from radioactivity as well, but fractions B-V–VII probably include amino or nitrogenous groups in their chemical formula because they did not appear in the single DHA model. It may be concluded again that none of the browning compounds shown in Figure 2 has a molecular weight greater than that of DHA.

Two radioactive peaks could be found due to DHA and AA as shown in Figure 2, but the radioactivity of DHA was much less than that in the single DHA model as shown in Figure 1. The reactivity of DHA seems to increase in the presence of amino acid more than in its absence. Since the fraction C-II is labile and tends to change into B-IV also detected in this model, it has no nitrogenous group. The color of the fractions B-V–VII was yellow, while B-IV was rather reddish brown.

SCA is known to be one of the initial products of the reaction of DHA with α-amino acids. It reacts with dehydro-SCA in nonaqueous solvent to provide 2,2′-nitrilodi-2(2′)-deoxy-L-ascorbic acid monoammonium salt, which is a red pigment (Kurata et al., 1973). Subsequently, Hayashi et al. (1983a,b) reported that the reaction of the red pigment with SCA was allowed to form a yellow product in an ethanolic solution and was followed by browning. Consequently, even that yellow product prior to browning consists of three molecules of SCA (MW 175) and possesses a molecular weight of more than 500.

Our conclusion, however, presents a different view. From the ^{14}C tracer experiment it was ascertained that C-1 of DHA was first decarboxylated and then the degradation products reacted with α-amino acids but that the resulting yellow or browning compounds had a molecular weight below that of DHA. There might be a difference between the reactivities in an aqueous solution and in a nonaqueous solution. We believe a similar browning reaction occurs in citrus juice as well as in the DHA aqueous model systems. Further qualitative studies on browning pigments and their precursors are necessary.

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Registry No. DHA, 490-83-5; AA, 50-81-7; glycine, 56-40-6.