Note

Formation of 2,3-Dihydro-3,4-dihydroxy-5-acetylfuran in the Reaction between D-Fructose and β -Alanine

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Received October 8, 1990

In our previous paper, the effects of dough materials on flavor formation in baked cookies were investigated.^{1,2)} Ten peaks were detected from the methanolic extracts of baked cookies by HPLC, and among them, peaks 3, 5, and 6 were the most remarkable peaks. Peaks 5 and 6 were identified as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and 5-hydroxymethylfurfural (HMF), respectively. Furthermore, DDMP seemed to be one of the main substances enhancing the good qualities of cookies with its favorable aroma. On the other hand, it was suggested that peak 3 is also another substance with a sweet aroma. Therefore, this paper deals with the formation of P3 in the reaction between D-fructose and β -alanine as a model system of cookies. In a preliminary experiment of the baking flavor from sugars and amino acids, the products from the reaction of fructose and β -alanine had a strong sweet aroma similar to the baked cookie and showed the comparable peak pattern of HPLC.

Fig. 1. HPLC Profile of Products (A) from the Reaction Mixture of D-Fructose and β -Alanine, and Maltol (B).

P3 was identified as 2,3-dihydro-3,4-dihydroxy-5-acetylfuran (DDAF). P5 was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP). Chromatographic conditions: column, Develosil ODS-5, 250 mm $\times$ 4.6 mm i.d.; mobile phase, H₂O–MeOH (6:1, v/v) 0.8 ml/min; UV detection, 283 nm.

Then, we have used the fructose- β -alanine system as a model of the baked cookie.

One millimole each of D-fructose and β -alanine placed in test tubes were heated in a block-bath at 150°C for 2 min and then extracted with 1 ml of methanol–water (3:1, v/v). The extracts were clarified by filtration and analyzed by HPLC. As shown in Fig. 1, HPLC of products from this reaction mixture gave only two main peaks, peak 3 (P3) and 5 (P5). P3 was isolated and purified repeatedly from the preparative HPLC. The P3 isolated from this reaction mixture gave the same retention time of HPLC with peak 3²⁾ from baked cookie extracts. Peak 3 from baked cookie extracts was regarded as containing at least one or two other compounds, other than P3 from this reaction mixture. However, the P3 isolated from this reaction mixture had the same sweet aroma as peak 3 from baked cookie extracts. From the data of UV, NMR, FAB-MS, EI-MS, and HR-MS, P3 was proposed to be 2,3-dihydro-3,4-dihydroxy-5-acetylfuran (DDAF). Then, the isolated P3 was acetylated with acetic anhydride and anhydrous pyridine. The acetylated P3 (Ac.P3) was purified by HPLC and analyzed by the same method as P3. The presence of two additional acetyl groups in Ac.P3 was confirmed, and it was identified as 2,3-dihydro-3,4-diacetoxy-5-acetylfuran. From the results described above, P3 from this reaction mixture was identified as DDAF.

Severin and Seilmeier³⁾ isolated a product, C₆H₈O₄, from the reaction mixture of D-glucose and a primary amine salt and identified it as DDAF. It was readily converted to the O-methyl isomaltol in methanol–hydrogen chloride. Furthermore, the same product, C₆H₈O₄, was isolated by Tatum *et al.*,⁴⁾ and they judged it to be practically odorless.⁵⁾ However, the compound, C₆H₈O₄, was actually revised to 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP).^{5–7)}

Fisher *et al.*⁸⁾ isolated piperidino-hexose reductone, DDMP, and 4-hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone (HHMF) from the reaction mixture of aqueous solutions of 1-deoxy-D-erythro-2,3-hexodiulose and ethanolic solutions of piperidine acetate. The NMR data on their HHMF and our DDAF were very similar,

Fig. 2. The Structures of DDAF, DDMP, and HHMF. DDAF, 2,3-dihydro-3,4-dihydroxy-5-acetylfuran; DDMP, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; HHMF, 4-hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone.

but there were large differences in the fragmentation of their MS spectra. They reported that the MS fragmentation of HHMF was m/z : 126 (12), 111 (20), 98 (35), 85 (33), 84 (73), 57 (52), 56 (44), 55 (22), 43 (100), 42 (34), and the molecular ion M^+ (m/z 144) showed only a minimal peak, because of the rapid dehydration of water from HHMF to form the fragment of m/z 126. Hiebl *et al.*⁹⁾ reported that the UV-absorbing band of HHMF was 254 nm. Comparing these facts, DDAF isolated in our laboratory was ascertained to be different from HHMF. Figure 2 shows the structures of DDAF, DDMP, and HHMF.

On the other hand, for about thirty years, maltol and isomaltol have been known to be trace aroma constituents of bread, in which they may contribute to the fragrant aroma of freshly baked bread and to the final flavor of bread.^{10,11)} Furthermore, maltol and isomaltol were isolated from various foods by other investigators.^{12,13)} However, in our model system of baked cookies, only DDMP and DDAF were detected as the baked flavors, but not maltol and isomaltol. Hodge *et al.*⁷⁾ reported that DDMP was odorless in a pure state at room temperature; nevertheless, the isolated and purified DDMP and DDAF by HPLC in our laboratory had a strong sweet aroma. From the results described above, DDAF similarly to DDMP, seemed to be an important substance having a fragment property in baked cookies.

Experimental

High performance liquid chromatography (HPLC). HPLC analyses were done using a Toyo Soda Model HLC-803D pump. Test parameters were as follows: column, Develosil ODS column (250 × 4.6 mm and 250 × 20 mm); mobile phase, water-methanol (6:1, v/v); flow rate, 0.8 ml/min and 5 ml/min; detection, UV 283 nm.

Isolation. Mixtures of D-fructose (180 mg) and β -alanine (89 mg) were placed in test tubes and heated in a block-bath at 150°C for 2 min. The reaction mixtures were extracted with 1 ml of methanol-water (3:1, v/v). The extracts were clarified by filtration, and P3 was isolated and purified repeatedly by preparative HPLC to give a pure material having a strong sweet aroma. The yield of P3 was 0.5 mg.

Acetylation. Mixtures of the isolated P3 (3 mg), acetic anhydride (0.5 ml), and anhydrous pyridine (0.5 ml) were kept at room temperature for 24 hr. Excess acetic anhydride was decomposed with methanol and the mixed solution was concentrated with a small portion of toluene under reduced pressure. Ac.P3 was purified by HPLC fitted with Develosil SI 60-10, 250 × 20 mm as follows: mobile phase, hexane-ethyl acetate (7:3, v/v), flow rate, 6.0 ml/min, detection, UV 298 nm. The yield was 2.1 mg (70%).

Spectrometric analyses. UV spectra were recorded with a Hitachi Model 200-10 spectrophotometer. NMR spectra were recorded with a JEOL GX-200 (¹H, 200 MHz and ¹³C, 50 MHz). ¹H-NMR and ¹³C-NMR were measured in D₂O, CD₃OD and CHCl₃ as solvents, respectively. Fast atom bombardment-(FAB-MS) and electron impact-mass spectrometry (EI-MS) were done on a JEOL DX-303. Glycerol was used as a matrix for FAB-MS analyses.

Spectroscopic characterization of the isolated compounds (P3).

1) *2,3-Dihydro-3,4-dihydroxy-5-acetylfuran (DDAF).* UV spectrum: $\lambda_{\max}$ in water, 288 nm. ¹H-NMR (D₂O): 2.11 (3H, s), 3.71 (1H, q), 3.76 (1H, q), 3.88 (1H, q). ¹³C-NMR (CDCl₃): 13.0, 60.4, 83.9, 135.6, 174.0, 196.5 ppm. MS m/z : 144 (M^+ , 100), 126 (30), 113 (10), 101 (35), 84 (13), 73 (10), 55 (47), 43 (75), 31 (12), 18 (15). HR-MS: found, m/z 145.0571 [$M+H$]⁺; calcd. for C₆H₉O₄, 144.0562.

2) *2,3-Dihydro-3,4-diacetoxy-5-acetylfuran.* UV spectrum: $\lambda_{\max}$ in MeOH, 274 nm. ¹H-NMR (D₂O): 2.03 (3H, s), 2.16 (3H, s), 2.26 (3H, s), 4.43 (1H, q), 4.52 (1H, q), 5.43 (1H, q). MS m/z : 228 (M^+), 186 ($M^+ - COCH_3$), 144 ($M^+ - COCH_3 \times 2$), 126, 115, 101, 84, 55, 43.

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