Model Reactions on Roast Aroma Formation. 14. Formation of 2-Acetylpyrido[3,4-*d*]imidazole by Heating of Glucose with Histidine

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2-Acetylpyrido[3,4-*d*]imidazole was formed by heating glucose with histidine to 120, 150, or 180 °C in an aqueous buffer system and also to 220 °C in a roast system. The structure of this new compound is described, and a formation pathway is proposed.

INTRODUCTION

Thermal aromas are well-known to consist of a great many aroma compounds. To obtain an overview of their structures and formation pathways, we have carried out some model reactions where sugars or sugar degradation compounds were reacted with selected amino acids under the conditions of food preparation [e.g., Baltes and Bochmann (1987); Baltes and Knoch (1993)]. To reach higher temperatures, we have also treated the reaction mixtures in a laboratory autoclave. In the volatiles obtained after reaction of D-glucose with histidine (Lingert and Waller, 1983), we identified, in addition to numerous pyrazines, pyrroles, furanes, pyridines, and some imidazoles, a compound of molecular mass 161, the mass spectrum of which indicated it to be an acetylpyrido[3,4-*d*]imidazole. This compound, as well as its basic ring structure, is to our knowledge unknown to date.

EXPERIMENTAL PROCEDURES

High-grade commercial L-histidine, sugar, and pyruvic aldehyde were used. Solvents for extraction and column chromatography were freshly distilled before use.

Sample Preparation. (*1*) *Glucose (0.04 mol) was added to 0.04 mol of histidine* in 60 mL of 1 M phosphate buffer, pH 5.8

(*2*) *Four milliliters of pyruvic aldehyde, freshly distilled, was added to 0.02 mol of histamine dihydrochloride* in 60 mL of 1 M phosphate buffer, pH 5.8

Model Reactions. Mixture 1 or 2 was heated and stirred at 120, 150, or 180 °C, respectively, in a laboratory autoclave fitted with a PTFE insert for 1 h. After cooling to room temperature, the mixture was extracted with 5 × 40 mL of ether. The combined ether fractions were treated with aqueous sodium bicarbonate, and the water dissolved in the ether was frozen out at −20 °C. After filtration on cotton wool, the ether extract was carefully concentrated to 2 mL with a Vigreux column.

(*3*) *Reaction of Tuna Meat with D-Glucose.* Fresh tuna meat (18 g) was ground and mixed with 1 g of D-glucose. The mixture was heated in 60 mL of 1 M phosphate buffer and worked up as described before. To separate fat, the concentrated ether extract (2 mL) was treated by means of gel chromatography (column SR 45, 450 × 25 mm, filled with Bio-Beads SX 3, 200–400 mesh, constant for solvent; extrusion limit 2000 D, Bio-Rad, Richmond, CA, which was filled to the column with dichloromethane). The elution of fat was carried out with 100 mL of dichloromethane. Subsequently it was eluted with 100 mL of dichloromethane, which was dried and carefully concentrated to 2 mL with a Vigreux column. The composition of this fraction was investigated by GC/MS.

(*4*) *Roast System (0.004 mol of Glucose plus 0.004 mol of Histidine).* The mixture was ground in a mortar with 10% 1 M phosphate buffer and 6 g of sand and was layered in the reaction tube together with quartz wool. After the tube had been covered with a heating tape and a contact thermometer, it was connected with the cooling traps (20, 0, and −196 °C). Roasting was carried out by heating the tube at 20 °C/min to a final temperature of 220 °C, which was maintained for 10 min. The volatiles were carried over to the cooling traps by means of a nitrogen stream of 50 mL/min. The condensates were extracted with ether, and the combined ether extract was treated as described before.

Gas Chromatography (GC)/Mass Spectrometry (MS). *Column 1*: fused silica DB-Wax (J&W, Folsom, CA) 60 m; 0.25-μm film; i.d. 0.32 mm; split, 1:5; temperature program, 40 °C, 5 min, 2 °C/min to 230 °C, 60 min.

Column 2: fused silica DB-1701 (J&W) 60 m; 025-μm film; i.d. 0.32; split, 1:5; temperature program, 40 °C, 5 min, 3 °C/min to 260 °C, 50 min.

Gas Chromatography: Carlo Erba 4130 with FID; retention indices calculated according to the method of van den Dool and Kratz (1963) via *n*-alkane (C_8–C_{30}) standards.

GC/MS System: Finnigan MAT 4500 with interface box 2010 and data system INCOS 2100. The technical data are as follows: closed coupling; temperature of ion source, 120 °C; ionization energy, 70 eV; cyclic scan, electron impact; mass range, 35–350 within 0.8 s; chemical ionization, methane as reactant gas; mass range, 80–350 within 0.8 s.

H/D isotope exchange was achieved in the DB-Wax column by previous injection of CD_3OD.

^{1}H Nuclear Magnetic Resonance: Bruker WM 400 NMR spectrometer; solvent, $CDCl_3$; tetramethylsilane as internal standard.

Infrared Spectroscopy: Pye Unicam SP 1100, KBr, 4000–200 cm^{-1}.

Preparation of Compound III. Mixture 2 was treated as described above. The concentrated ether solution was fractionated on a column (30 cm × 2 cm) with cooling jacket filled with 20 g of Al_2O_3 acidic (ICN Biochemicals, deactivated with 10% water) by successive application of pentane plus ether (mL): (1) 90 + 10; (2) 80 + 20; (3) 70 + 30; (4) 60 + 40; (5) 50 + 50. Fraction 5 yielded the pure compound, about 1 mg of which was obtained as colorless crystals.

RESULTS AND DISCUSSION

Among the various compounds formed by the heating of glucose with histidine at 120, 150, 180, and 220 °C, we identified one compound whose mass spectrum suggested that its formula might be 2-acetylpyrido[3,4-*d*]imidazole (III, Figure 2). The same peak was also obtained when we reacted histamine with pyruvic aldehyde under the same conditions. Another indication of the structure of III was obtained by exact mass determination (Wittkowski et al., 1984), resulting in the molecular formula $C_8H_7N_3O$. By treatment of the compound with CD_3OD, two to three hydrogen atoms were exchanged, because the acetyl group can possess active hydrogen atoms by means of keto–enol tautomerism. Another H/D exchange should be possible via the imidazole ring. Besides III, we identified in the gas chromatogram a compound with the molecular mass 165, the mass spectrum of which was very similar to that of III. We suggest that this compound represents the

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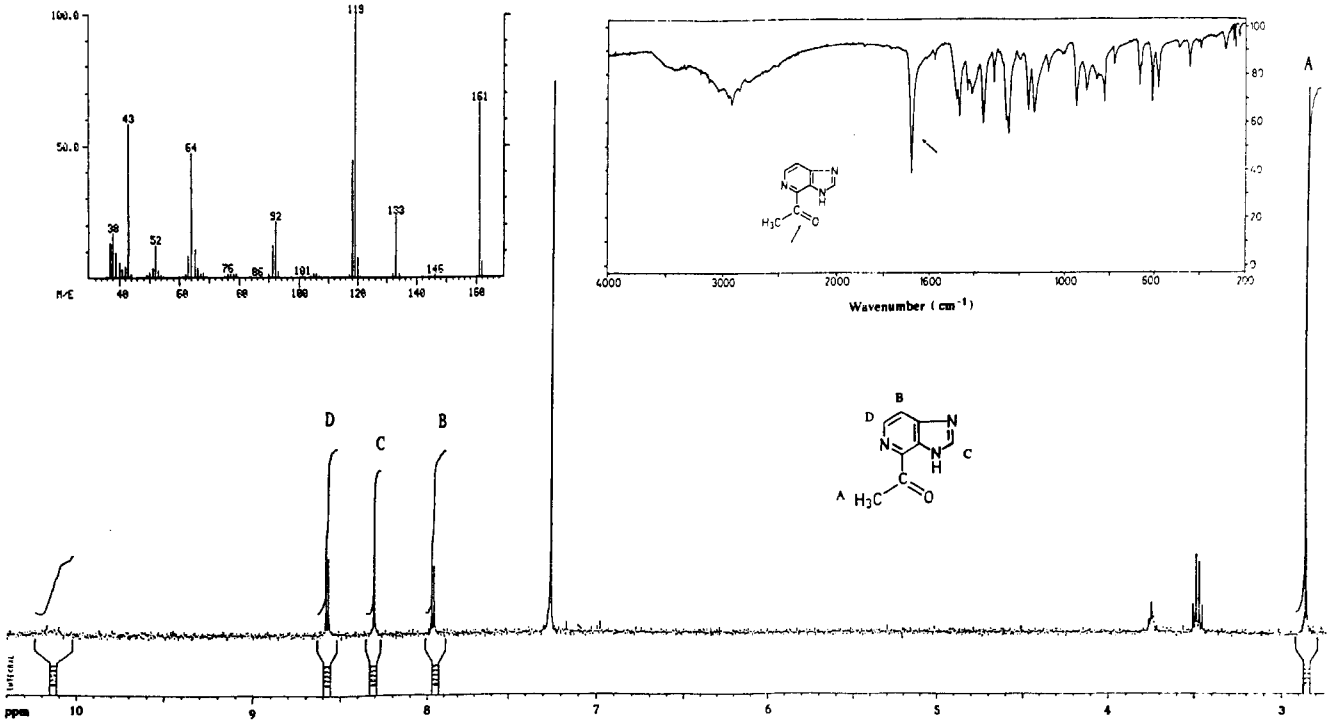

Figure 1. ^{1}H NMR spectrum (in $CDCl_3$), IR spectrum (in KBr), and EI mass spectrum of 2-acetylpyrido[3,4-*d*]imidazole.

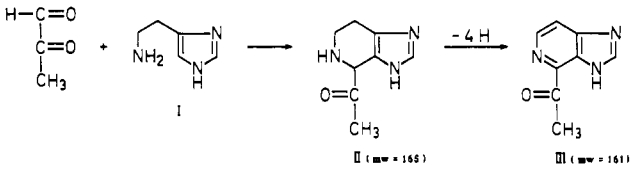

Figure 2. Formation of 2-acetylpyrido[3,4-*d*]imidazole (III) by reaction of pyruvic aldehyde with histamine (I) via 2-acetyltetrahydropyrido[3,4-*d*]imidazole (II).

tetrahydro derivative of III. As a byproduct, we identified 1,2-propanediol and hydroxyacetone under the reaction conditions described. To confirm the structure of III via infrared and nuclear magnetic resonance spectroscopy, we have reacted a mixture consisting of histamine with pyruvic aldehyde to obtain crystalline material. We have fractionated the numerous products by column chromatography with aluminum oxide acidic as an adsorbant. By treatment with pentane–ether mixtures of different compositions, we excluded the nonpolar byproducts and finally isolated about 1 mg of compound III in the crystalline state. Its structure was additionally confirmed by ^{1}H NMR and IR spectra (Figure 1).

We suggest that in the last reaction the aldehyde group of pyruvic aldehyde reacted with the amino residue of histamine (I), yielding the Schiff base, which formed II (Figure 2) by cyclization in analogy to the isoquinoline formation according to Pictet and Games (1909, 1910). By subsequent dehydrogenation III would have been formed.

2-Acetylpyrido[3,4-*d*]imidazole is a new compound in the field of Maillard reaction products. Compound III or

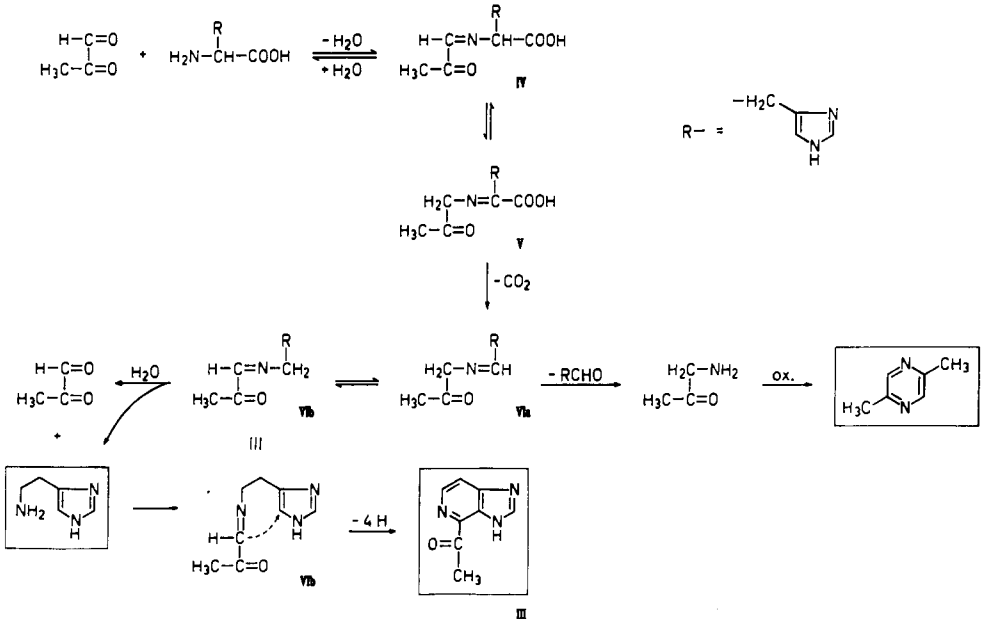

Figure 3. Mechanism proposed for the formation of 2-acetylpyrido[3,4-*d*]imidazole via Strecker degradation of histidine with pyruvic aldehyde.

its basic structure (without the acetyl residue) has not been previously described in the literature. Previously known isomers bear the nitrogen atom in other positions of the pyridine ring or the hydrogen atom is bound to the other imidazole nitrogen of the pyrido[3,4-*d*]imidazole ring. In a patent specification (Jonas, 1986) some pyrido[3,4-*d*]-imidazoles were described that possess different substituted phenyl rings at the position 2 of the imidazole ring.

In Figure 3 our suggestion of the reaction mechanism for the formation of III via the Maillard reaction is illustrated. We assume that the first step is the condensation of pyruvic aldehyde with histidine. In the course of the Strecker degradation, CO_2 is split off, forming compound VIa, which, by scission of the Strecker aldehyde, should form the corresponding pyrazine. In our case, this reaction takes place but seems not to be as prominent as in the case of most amino acids. Instead, structure VIb seems to be formed by tautomerism, which offers two alternatives: (1) hydrolysis to yield histamine, which can react once more with pyruvic aldehyde; (2) direct cyclization of VIb with subsequent dehydrogenation to III.

The first pathway should require the presence of histamine in the reaction mixture. This was not the case. Therefore, we suggest that the second pathway is more likely.

As has been pointed out elsewhere, the Schiff bases of tryptamine are rather stable and, on heating, form β-carbolines (Knoch and Baltes, 1992). Also, the structure VIb seems to be stable enough to make this cyclization possible. On the other hand, similar experiments with phenylalanine were not successful, which means that the scission of phenylacetaldehyde from the molecule corresponding to VIa is favored, which we have found in large amounts (Kunert-Kirchhoff and Baltes, 1990).

We have also carried out some experiments on the dehydrogenation of these molecules which seems to be extremely difficult. Under our conditions, pyruvic aldehyde seems to be a very active dehydrogenation agent, forming 1,2-propanediol and hydroxyacetone, which we have identified.

To test the relevance of 2-acetylpyrido[3,4-*d*]imidazole in natural systems, we have reacted fresh tuna meat with glucose under the same conditions. Tuna meat is well-known to contain relatively high amounts of histidine (about 1%). As expected, 2-acetylpyrido[3,4-*d*]imidazole was identified. In contrast, commercial samples of canned tuna products (three different samples) did not contain III, the recovery of which at a label of about 3.0 mg/kg was 60%. Toxicological experiments of III have not been carried out because the synthesis of large amounts of this compound was not successful until now.

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Received for review December 7, 1992. Accepted January 25, 1993.