2-Oxopropanal, Hydroxy-2-propanone, and 1-Pyrroline—Important Intermediates in the Generation of the Roast-Smelling Food Flavor Compounds 2-Acetyl-1-pyrroline and 2-Acetyltetrahydropyridine

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On the basis of labeling experiments with $[^{13}C]_6$-glucose/unlabeled proline as well as quantitative data obtained in model studies using stable isotope dilution assays, 1-pyrroline and hydroxy-2-propanone were identified as effective intermediates in generating the roast-smelling food odorant 2-acetyltetrahydropyridine (ATHP; two tautomers). Synthesis of the key precursor compound, 2-(1-hydroxy-2-oxo-propyl)pyrrolidine, and studies on its degradation confirmed the important role of this intermediate in ATHP formation. Boiling of the intermediate for 30 min in aqueous solution generated >30% of ATHP on a molar basis. 1-Pyrroline and 2-oxopropanal were confirmed as important intermediates in the generation of the further roast food odorant, 2-acetyl-1-pyrroline (AP). On the basis of results of labeling experiments with $[^{13}C]_6$-glucose/unlabeled proline, two different mechanisms could be proposed. One leads to AP via 2-(1,2-dioxopropyl)pyrrolidine as the precursor with elimination of the aldehyde group in 2-oxopropanal as carbon dioxide. The other one suggests elimination of carbon-2 of the pyrroline ring. The latter mechanism was further established by a result showing that from the reaction of 2-methyl-1-pyrroline with 2-oxopropanal AP was also generated.

Keywords: *1-Pyrroline; 2-oxopropanal; hydroxy-2-propanone; 2-acetyl-1-pyrroline; 2-acetyltetrahydropyridine; 2-acetyl-3-methyl-3,4,5,6-tetrahydropyridine; 2-(1-hydroxy-2-oxo-1-propyl)pyrrolidine*

INTRODUCTION

On the basis of high odor activity values (ratio of concentration to odor threshold), the intense roast odorants 2-acetyl-1-pyrroline (AP) and 2-acetyltetrahydropyridine (ATHP) have been established as key contributors to the roasty, popcorn-like odor of several processed foods (Table 1). It has long been suggested that the Maillard reaction between amino acids and carbohydrates, or degradation products thereof, generates both odorants in foods, and several investigations have been performed to clarify the intermediates and reaction pathways leading to AP and ATHP during food processing.

Hodge et al. (1972) were the first to postulate the amino acid proline and the carbohydrate degradation product 2-oxopropanal as key intermediates in the formation of ATHP. They speculated that a hypothetical intermediate, *N*-acetonyl-4-aminobutanal (**I** in Figure 1), should give the ATHP simply by elimination of water. However, recent results by de Kimpe et al. (1994) questioned this mechanism, because they were unable to generate the ATHP from the synthesized intermediate.

Tressl et al. (1985) found trace amounts of AP and ATHP when proline was reacted in the presence of several carbohydrates. Quantitative investigations performed by using stable isotope dilution assays (Schieberle, 1989, 1990) established the important role of the amino acid proline as precursor of both odorants and indicated that the AP is preferentially formed when proline is reacted with trioses such as glycerine aldehyde, 1,3-dihydroxyacetone, or their common dehydration product 2-oxopropanal. In contrast, the ATHP was shown to be formed in higher amounts when proline was reacted with hexoses. Further findings (Schieberle, 1990) indicated the amino acid ornithine as another, but more effective, precursor of AP. However, ATHP was not generated from this amino acid.

1-Pyrroline, which may be formed either from proline via a Strecker degradation initiated by α-dicarbonyls, such as 2-oxopropanal (Figure 1), or, alternatively, from ornithine via 4-aminobutanal (Schieberle, 1990) is

Table 1. Concentrations and Odor Activity Values (OAVs) of AP and ATHP in Processed Foods[a]

	AP		ATHP	
food	concn (μg/kg)	OAV[b]	concn (μg/kg)	OAV[b]
wheat bread crust	19	2602	53	981
popcorn	24	3288	437	8092
toasted wheat bread	8.8	1205	1.5	28
roasted sesame	30	4110	nd[c]	
basmati rice	610[d]	83516	nd[c]	
cooked sweet corn	4[d]	6027	nd[c]	

[a] Based on results reported by Schieberle and Grosch (1987), Schieberle (1991), and Rychlik and Grosch (1996). [b] OAVs were calculated by dividing the concentrations by the odor thresholds in wheat starch: AP, 0.0073 μg/kg; ATHP, 0.054 μg/kg (Rychlik and Grosch, 1996). [c] nd, not determined. [d] Data from Buttery et al. (1994).

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S0021-8561(97)00990-4 CCC: $15.00
Published on Web 05/13/1998

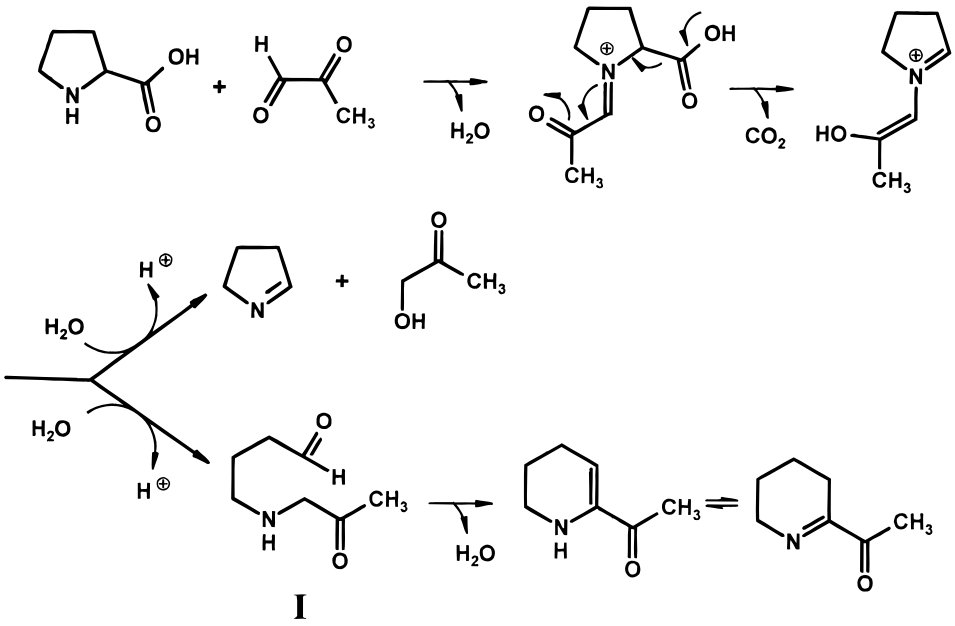

Figure 1. Formation of ATHP, 1-pyrroline, and hydroxy-2-propanone from proline and 2-oxopropanal [according to Hodge et al. (1972)].

generally accepted as a key intermediate in the generation of AP (Schieberle, 1989, 1990; Tressl et al., 1993a,b; Rewicki et al., 1993).

To gain more detailed insight into AP formation, model studies using labeled glucose have been performed in the literature. In AP formed from U-^{13}C-labeled glucose in the presence of proline, the main isotopomer (80%) contained two labeled carbon atoms (Schieberle, 1989). Depending on this result, an "acylation" of 1-pyrroline by 2-oxopropanal with elimination of formaldehyde was suggested as the key step in AP formation (Schieberle, 1990, 1995). Tressl et al. (1993a,b) and Rewicki et al. (1993) detected a 1:1 mixture of either unlabeled or $^{13}C_1$-labeled AP when proline was reacted with 1-^{13}C-labeled glucose. They proposed an acylation of 1-pyrroline by acetylformoine leading to 2-acetylpyrrolidine, which should subsequently be oxidized into the AP. However, this key reaction step has not yet been proven by systematic quantitative studies.

The literature survey indicates that, first, the intermediates and reaction pathways leading to the formation of ATHP are still unclear and, second, it is not known in detail how the AP is formed from 1-pyrroline. The following investigations were, therefore, undertaken to further the understanding of the mechanisms governing the formation of these two very important food odorants during thermal processing of foods.

EXPERIMENTAL PROCEDURES

Chemicals. L-Proline, pipecolinic acid, 4-aminobutanal (as diethyl acetal), 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, 2-methyl-1-pyrroline, hydroxy-2-propanone, 1-hydroxy-2-butanone, 2-oxopropanal (40% solution in water), and 2-(hydroxymethyl)pyrrolidine were from Aldrich (Steinheim, Germany). 2-[(*tert*-Butoxycarbonyl)oximino]-2-phenylacetonitrile was supplied by Lancaster (Mühlheim, Germany). [^{13}C]$_6$-Glucose was from Sigma (Munich, Germany).

Syntheses. AP, 2-acetylpyrrolidine, ATHP, and 2-acetylpiperidine were prepared as described recently (Hofmann and Schieberle, 1998). 1-Pyrroline was generated by an oxidative decarboxylation of L-proline (Yoshikawa et al., 1965; Schieberle, 1990). [^{2}H]-2-Acetyl-1-pyrroline and [^{2}H]-2-acetyltetrahydropyridine were synthesized as described by Schieberle and Grosch (1987) and Schieberle (1995).

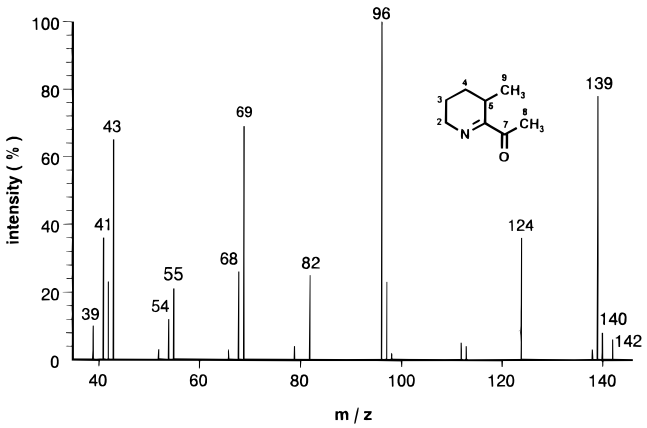

Figure 2. Mass spectrum (MS/EI) of AMTHP.

2-Acetyl-3-methyl-3,4,5,6-tetrahydropyridine. 2-Methyl-1-pyrroline (5 mmol) and hydroxy-2-propanone (50 mmol) were dissolved in phosphate buffer (500 mL, 0.5 mol/L; pH 7.0) and the solution was refluxed for 2 h. The aqueous mixture was extracted eight times with diethyl ether (total volume = 250 mL); the etheral solution was treated with a sodium bicarbonate solution (50 mL, 0.5 mol/L) and finally dried over Na_2SO_4. After concentration to 2 mL, the target compound was isolated by flash chromatography on a Diol-phase (column, 20 cm × 1.9 cm; J. T. Baker BV, Deventen, The Netherlands) using an *n*-pentane/diethyl ether gradient. HRGC separation on a Silicone SE-54 column afforded two peaks showing nearly identical mass spectra (Figure 2). One major peak (~95%) was eluted at a retention index of 1068 (relative to *n*-alkanes) and a minor one (5%) at a retention index of 1166. The major compound was isolated by preparative GLC using the equipment described previously (Schieberle, 1991). Its ^{1}H NMR data (Table 2) confirmed the structure of the target compound as 2-acetyl-3-methyl-3,4,5,6-tetrahydropyridine. The minor compound was assigned as 2-acetyl-3-methyl-1,4,5,6-tetrahydropyridine.

*2-(1-Hydroxy-2-oxo-1-propyl)pyrrolidine (**IV** in Figure 3).* The target compound was synthesized using the four-step

Table 2. ^{1}H NMR Data Obtained for AMTHP

hydrogen at carbon[a]	δ (ppm)	multiplicity	J (Hz)	TOCSY[b]
9	1.04	d, 3H	7.63	H at C-9/C-5
4	1.48−1.63	m, 2H		H at C-4/C-5
				H at C-4/C-3
3	1.61−1.75	m, 2H		H at C-3/C-4
				H at C-3/C-2
8	2.03	s, 3H		
5	2.8−2.9	m, 1H		H at C-5/C-9
				H′ at C-5/C-4
2	3.6−3.7	m, 1H		H at C-2/C-3
	3.82−3.93	m, 1H		H at C-2/C-3

[a] Numbering refers to the structure displayed in Figure 2. [b] Total correlated (^{1}H, ^{1}H) shift spectroscopy.

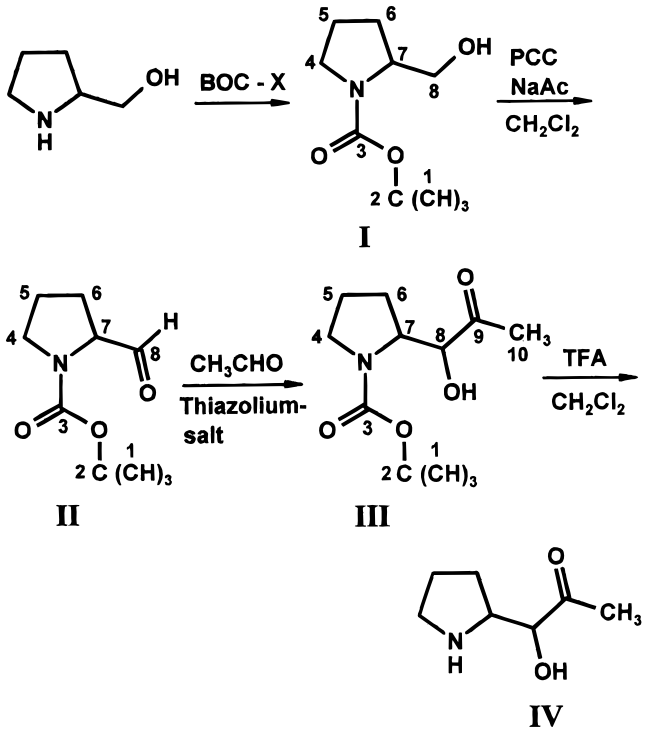

Figure 3. Synthetic route used for the preparation of HOP.

synthesis detailed in Figure 3 starting from 2-(hydroxymethyl)-pyrrolidine.

*N-(tert-Butoxycarbonyl)-2-(hydroxymethyl)pyrrolidine (**I** in Figure 3).* 2-Hydroxymethyl pyrrolidine (15 mmol), triethylamine (22.5 mmol), and 2-[(*tert*-butyoxycarbonyl)oximino]-2-phenylacetonitrile (16.5 mmol) were stirred for 2 h at room temperature in dioxane/water (20 mL; 1+1 by vol). The excess of solvent was then distilled off at 20 °C in vacuo, and, after addition of water (50 mL) and cooling, the oily phase obtained was purified by flash chromatography on a Diol-phase suspended in *n*-pentane (column, 15 × 1.9 cm; J. T. Baker BV). After flushing with *n*-pentane (150 mL), the target compound was eluted with *n*-pentane/diethyl ether (150 mL; 9+1 by vol) followed by *n*-pentane/diethyl ether (150 mL; 8+2 by vol). A colorless oil (2.3 g, =75% of theory) was obtained after distilling off the solvent. The target compound was characterized by HRGC/mass spectrometry: MS/EI [*m*/*z* (%)] 114 (100), 41 (95), 170 (84), 70 (82), 57 (78), 128 (77); MS/CI [*m*/*z* (%)] 146 (100), 102 (83), 128 (49), 114 (31), 130 (15), 170 (13), 202 (12), 84 (9).

By ^{1}H NMR measurements the following signals were obtained (δ; multiplicity): δ 1.47 (s, 9H; C-1 in Figure 3), 1.70−2.12 (m; 4H; C-5 and C-6), 3.29−3.72 (m; 2H; C-4), 3.65−3.83 (m; 1H; C-7), 3.95−4.05 (m; 1H; C-8), 4.12−4.21 (m; 1H; C-8).

*N-(tert-Butoxycarbonyl)-2-formylpyrrolidine (**II** in Figure 3).* A solution of **I** (11.3 mmol) and pyridinium chlorochromate (17 mmol) in dichloromethane (25 mL) was stirred for 150 min at room temperature. After addition of diethyl ether (40 mL), the organic phase was separated and dried over Na_2SO_4, and the solvent was distilled off. The remaining material was purified by flash chromatography as described above. The target compound (1.1 g, =49% of theory) was obtained and characterized by mass spectral and ^{1}H NMR measurements: MS/EI [*m*/*z* (%)] 114 (100), 70 (96), 41 (92), 57 (82), 126 (77), 170 (77), 82 (52); MS/CI [*m*/*z* (%)] 144 (100), 100 (95), 114 (49), 126 (45), 128 (26), 170 (12), 172 (9), 200 (7), 184 (6).

The ^{1}H NMR signals of **II** were very similar to those of the corresponding alcohol (**I** in Figure 3) with the exception of the signal at δ 9.5 (d; 1H; C-8) for the aldehyde hydrogen.

*N-(tert-Butoxycarbonyl)-2-(1-hydroxy-2-oxo-1-propyl)pyrrolidine (**III** in Figure 3).* To a mixture of **II** (5.5 mmol), freshly distilled acetaldehyde (17.3 mmol), and 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (1.5 mmol) was added dropwise dry triethylamine (9 mmol), and the mixture was heated for 90 min at 80 °C under pure argon with vigorous stirring. After cooling, water (50 mL) was added and the pH adjusted to 5.0 by using hydrochloric acid (1 mol/L). Extraction with diethyl ether, drying over Na_2SO_4, and distilling off the solvent afforded a crude mixture from which **III** was purified by flash chromatography (yield = 0.28 g, =21% of theory). **III** was purified from the coeluting isomer N-(*tert*-butoxycarbonyl)-2-(2-hydroxy-1-propanoyl)pyrrolidine by preparative HPLC on reversed phase material (C_{18}; Shandon Hypersil, 5 μ; Eastmore, U.K.) and by using an acetonitrile/water gradient from 20+80 to 80+20 by vol within 30 min. HRGC/mass spectral measurements of the purified compound gave the following signals: MS/EI [*m*/*z* (%)] 43 (100), 170 (95), 114 (92), 57 (83), 74 (72), 70 (71), 100 (70), 144 (63), 126 (36); MS/CI [*m*/*z* (%)] 114 (100), 188 (51), 70 (34), 144 (31), 170 (21), 142 (13), 126 (8), 186 (5), 244 (2).

The structure of **III** was confirmed by ^{1}H NMR measurements (360 MHz; $CDCl_3$): δ 1.43 (s, 9H; C-1; cf. Figure 3), 1.62−1.88 (m; 3H; C5/C6), 1.90−2.06 (m; 1H; C-5), 2.26 (s, 3H; C-10), 3.28−3.37 (m; 1H; C-4), 3.38−3.57 (m, 1H; C-4), 4.00−4.4 (m, 2H; C-7/C-8).

*2-(1-Hydroxy-2-oxo-1-propyl)pyrrolidine (**IV** in Figure 3).* **III** (1 mmol) was dissolved in dichloromethane (2 mL) and, after addition of trifluoroacetic acid (60 mg), stirred for 3 h at room temperature. The mixture was dried at 20 °C in a stream of pure nitrogen, the residue was taken up in water (100 mL), and aliquots (1 mL) of this solution were used in the model experiments without further purification.

Labeling Experiments. L-Proline (2 mmol) and [^{13}C]$_6$-D-glucose (1 mmol) were intimately mixed with silica gel (2.7 g containing 300 μL of a phosphate buffer (0.1 mol/L; pH 7.0) and heated for 10 min at 160 °C in closed glass vials (total volume = 10 mL). The mixture was extracted three times with diethyl ether (total volume = 50 mL) and concentrated to 200 μL by distilling off the solvent at 35 °C using a Vigreux column (60 cm × 1 cm) followed by microdistillation (Schieberle, 1991).

Model Reactions. The composition of the model systems and the reaction parameters applied are detailed in the respective tables. The amounts of AP and ATHP formed were determined from the volatile fractions obtained by sublimation in vacuo by means of stable isotope dilution assays and by using [^{2}H]-AP and [^{2}H]-ATHP as internal standards (Schieberle, 1995).

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC was performed with a type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) by using the following capillaries: FFAP (30 m × 0.32 mm fused silica capillary, free fatty acid phase, 0.25 μm; J&W Scientific, Fisons Instruments, Mainz, Germany) and SE-54 (30 m × 0.32 mm fused silica capillary DB-5; 0.25 μm; J&W Scientific, Fisons Instruments). The samples were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was quickly raised at 40 °C/min to 50 °C (SE-54) or 60 °C (FFAP), respectively, held for 5 min isothermally, then raised at 6 °C/min to 230 °C, and held for 15 min. The flow of the carrier gas helium was 2.5 mL/min. Linear retention indices (RI) of the compounds were calculated from the retention times of *n*-alkanes by using a computer program [cf. Schieberle (1991)]. MS analysis was performed with an

Table 3. Carbon Isotope Ratio (Percent) in ATHP Generated from Proline in the Presence of Glucose (Glc) or [^{13}C]$_6$-Glucose ([^{13}C]$_6$-Glc), Respectively[a]

ATHP (*m*/*z*)	Glc	[^{13}C]$_6$-Glc	ATHP (*m*/*z*)	Glc	[^{13}C]$_6$-Glc
123	0.1	<0.1	127	0.3	**5.2**
124	**4.9**	<0.1	128	<0.1	**89.2**
125	**88.3**	<0.1	129	<0.1	**4.7**
126	**6.4**	0.7	130	<0.1	0.2

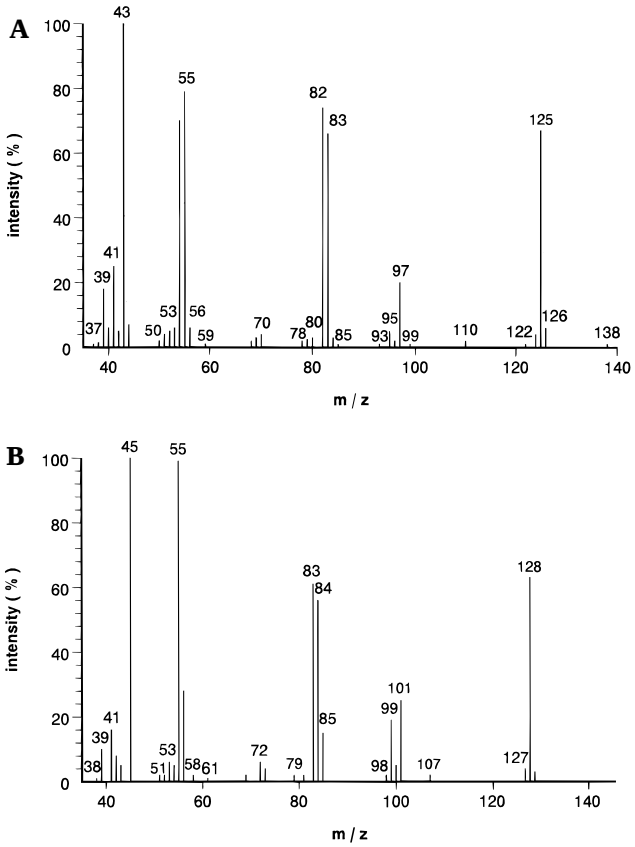

Figure 4. Mass spectra of ATHP (A) and [^{13}C]$_3$-ATHP (B).

MS 95 S (Finnigan, Bremen, Germany) in tandem with the capillaries described above. Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with isobutane as reactant gas.

RESULTS AND DISCUSSION

Formation of ATHP. To gain a first insight into the formation of ATHP from glucose and L-proline, U-^{13}C-labeled glucose was reacted with the amino acid. On the basis of their molecular ions monitored by MS/EI, the relative amounts of the isotopomers in ATHP formed were determined. The main isomer (*m*/*z* 128; Table 3) formed from [^{13}C]$_6$-glucose showed a clear upshift of 3 mass units compared to the ATHP generated in the same experiment with unlabeled glucose (*m*/*z* 125). This result indicates the presence of three carbon-13 atoms in the labeled ATHP. The positions of the labels were derived from a comparison of the mass spectra of [^{13}C]$_3$-ATHP and ATHP (Figure 4). A shift of 2 mass units in the fragment representing the acetyl group (*m*/*z* 45 vs *m*/*z* 43) indicates the presence of two carbon-13 labels (Figure 4B). In addition, the fragment *m*/*z* 83 of the tetrahydropyridine ring (Figure 4B) indicated one labeled carbon atom in the ring (*m*/*z* 83 vs *m*/*z* 82).

Table 4. Influence of the pH Value on the Formation of ATHP from 1-Pyrroline and Hydroxy-2-propanone[a]

pH	ATHP μg	ATHP %	pH	ATHP μg	ATHP %
3.0	<0.1		7.0	10.8	0.9
5.0	0.9	0.1	9.0	38.4	3.1

[a] 1-Pyrroline (10 μmol) and hydroxy-2-propanone (10 μmol) were reacted for 30 min at 100 °C in phosphate buffer (5 mL, 0.5 mol/L).

The data would be in good agreement with the reaction mechanism proposed by Hodge et al. (1972) suggesting 2-oxopropanal (a carbon-3 compound) as the key intermediate in ATHP formation (Figure 1). However, as mentioned in the Introduction, recent studies had shown that the intermediate *N*-acetonyl-4-aminobutanal (Figure 1) did not yield ATHP (de Kimpe et al., 1994).

A Strecker degradation is most probable if an amino acid is reacted with an α-dicarbonyl compound, such as 2-oxopropanal. From proline, the reaction products are 1-pyrroline and hydroxy-2-propanone (Figure 1). Both compounds are reactive intermediates and, therefore, further reactions can be proposed. To study their efficiency as precursors of ATHP, 1-pyrroline and hydroxy-2-propanone were reacted by boiling for 30 min in an aqueous buffer. Under weak acidic conditions (pH 3.0; Table 4), no ATHP was formed. However, increasing the pH to 9.0 led to a significant formation of the odorant in a 3.1% molar yield.

In Figure 5, a reaction pathway leading from 1-pyrroline and hydroxy-2-propanone to ATHP is proposed. From the result that higher pH values favor the formation of the odorant (Table 4), it can be assumed that the key step in the reaction is the attack of carbon-1 in the enolized hydroxy-2-propanone at carbon-2 of 1-pyrroline. Once formed, the 2-(1-hydroxy-2-oxopropyl)pyrrolidine (HOP) intermediate may undergo a ring opening leading to 5,6-dioxoheptylamine, which should finally give ATHP by a Schiff reaction.

To prove that a ring enlargement takes place in the reaction, 2-methyl-1-pyrroline instead of 1-pyrroline was reacted with hydroxy-2-propanone. 2-Acetyl-3-methyl-3,4,5,6-tetrahydropyridine (AMTHP; Figure 2) was formed as the main volatile reaction product (3 mol %; data not shown). Because the methyl group at carbon-2 of the 1-pyrroline was shifted into position 3 in the AMTHP, this result unambiguously confirms a ring enlargement reaction during the formation of ATHP (Figure 5).

It is interesting to note that, compared to ATHP, the AMTHP mainly occurred in one tautomeric form during HRGC, namely the 3,4,5,6-tetrahydro compound. The corresponding 1,4,5,6-tautomer was present only in a more than 30-fold lower amount (data not shown). Obviously the electron-donating property of the methyl group stabilizes the imine tautomer. As recently shown (Hofmann et al., 1995), the reverse was true for the thio homologue 5-acetyl-2,3-dihydro-1,4-thiazine identified in a thermally treated cysteine/ribose mixture. In the latter molecule, obviously the electron-withdrawing sulfur atom stabilized predominantly the enamine structure.

AMTHP showed the same popcorn-like odor at the same low odor threshold (0.06 ng/L in air) as ATHP (Schieberle, 1991).

As a final proof for the mechanism suggested in Figure 5, the proposed intermediate HOP was synthe-

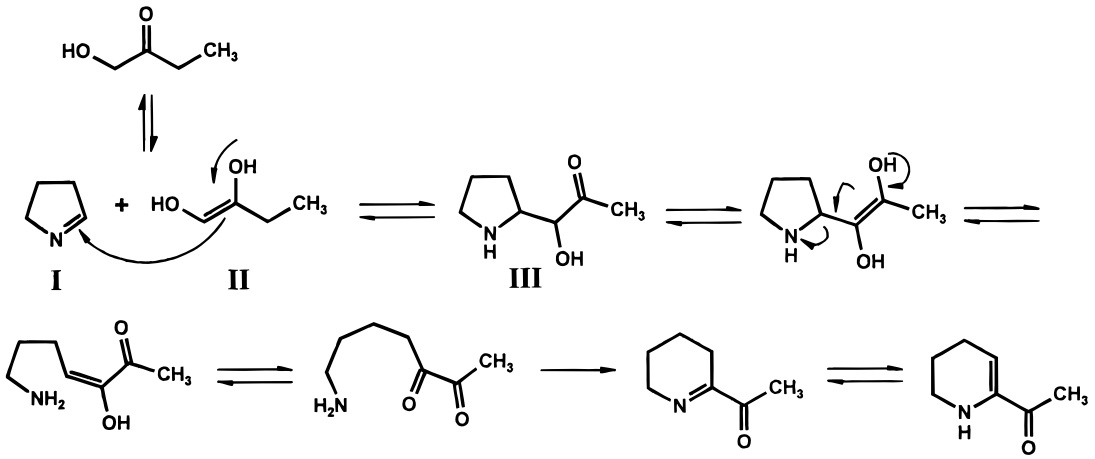

Figure 5. Hypothetical pathway leading from 1-pyrroline and hydroxy-2-propanone to ATHP.

Table 5. Formation of ATHP from HOP at Different pH Values[a]

	ATHP			ATHP	
pH	μg	mol %	pH	μg	mol %
3.0	2.1	1.7	7.0	29.3	23.4
5.0	14.3	11.4	9.0	43.7	35.0

[a] The N-shielded HOP (1 μmol) was treated with trifluoroacetic acid (5 μmol in 100 μL of dichloromethane) and, after addition of phosphate buffer (5 mL, 0.5 mol/L), heated for 30 min at 100 °C in a closed vessel.

Table 6. Carbon Isotope Ratio in AP Generated from Proline and D-Glucose (Glc) or [$^{13}C]_6$-D-Glucose ([$^{13}C]_6$-Glc), Respectively[a]

AP (*m/z*)	Glc	[$^{13}C]_6$-Glc	AP (*m/z*)	Glc	[$^{13}C]_6$-Glc
111	**93.9**	0.2	114	<0.1	**19.1**
112	**5.9**	3.1	115	<0.1	0.7
113	0.2	**76.8**	116	<0.1	<0.1

[a] L-Proline (2 mmol) and glucose (1 mmol) or [$^{13}C]_6$-glucose, respectively, were mixed with silica gel (3 g) and reacted for 10 min at 160 °C.

sized (cf. Figure 3). To elucidate its effectiveness in generating ATHP, aqueous solutions of HOP were boiled for 30 min at different pH values and the ATHP formed was quantified. The odorant was very effectively generated with increasing pH values increasing the amounts of the ATHP (Table 5). At pH 9.0, 35 mol % was formed, thereby confirming the reaction mechanism displayed in Figure 5.

Formation of AP. In a previous investigation (Schieberle, 1989), one of us had found that in AP generated from proline and [$^{13}C]_6$-glucose, the main isotopomer was the [$^{13}C]_2$-AP. Reinvestigation of the isotope distribution established our previous data (Table 6) and indicated a main isotopomer having two labeled carbon atoms (*m/z* 113 vs *m/z* 111). Further results had established the reaction of 1-pyrroline with 2-oxopropanal as an effective system to generate AP (Schieberle, 1995). Therefore, in the present study, the experiments on 1-pyrroline and 2-oxopropanal were extended.

Heating of 1-pyrroline and 2-oxopropanal gave high yields of AP, especially when the concentrations of 2-oxopropanal much exceeded those of 1-pyrroline (expt 1; Table 7). However, changing the ratio of 2-oxopropanal to 1-pyrroline from 5:1 to 1:5 (cf. expt 1 and 3) led to a drastic decrease in the formation of AP. Furthermore, under dry-heating conditions and at higher temperatures, a 50-fold lower amount of AP was formed compared to that formed in aqueous conditions (cf. expt 2 and 4; Table 7).

Table 7. Influence of the Concentration of 2-Oxopropanal on the Formation of AP from 1-Pyrroline[a]

	2-oxopropanal	AP	
expt	(μmol)	μg	%
1	50	319.1	28.7
2	10	58.5	5.3
3	10[b]	3.7	0.3
4	10[c]	1.1	0.1

[a] 1-Pyrroline (10 μmol, 690 μg) and 2-oxopropanal were reacted for 30 min at 100 °C in phosphate buffer (5 mL, 0.5 mol/L; pH 7.0). [b] 50 μmol of 1-pyrroline was reacted. [c] 1-Pyrroline (10 μmol) and 2-oxopropanal (10 μmol) were mixed with silica gel (2.7 g containing 300 μL of phosphate buffer (0.1 mol/L; pH 7.0) and reacted for 5 min at 180 °C.

On the basis of the results of the labeling experiments (Table 6) and assuming 1-pyrroline and 2-oxopropanal as the intermediates in AP formation from glucose and proline, one carbon atom has to be eliminated. We had recently proposed a mechanism of this reaction (Schieberle, 1995) suggesting formaldehyde as the leaving group. However, this was based on the assumption that AP is formed during the reaction without any oxidation step. In a recent publication (Hofmann and Schieberle, 1998) we could, however, show that 2-acetylpyrrolidine is easily oxidized with high yields into AP. On the basis of this result, we assume that the reaction sequence leading from 1-pyrroline and 2-oxopropanal to AP goes via 2-acetylpyrrolidine as the key intermediate.

Because higher amounts of AP are formed under aqueous conditions (Table 7), the hydrated 2-oxopropanal, which is easily formed in the presence of water (Buettner et al., 1996), might be the reactive species. Therefore, the reaction pathway might be suggested as follows (Figure 6): hydrated 2-oxopropanal, as the nucleophile, attacks 1-pyrroline at carbon-2. Due to its N-analogous reductone structure, the 2-(1,2-dioxopropyl)pyrrolidine formed is very susceptible to air oxidation and will easily be oxidized into 2-(1,2-dioxopropyl)-pyrroline. As found for triketones (Dao et al., 1974), this N-analogous triketone will then be hydrated and should undergo a rearrangement leading to 2-acetyl-2-carboxypyrrolidine. This β-keto acid easily loses water to yield 2-acetylpyrrolidine. The latter is in turn oxidized into AP (Hofmann and Schieberle, 1998).

As suggested in Figure 7, finally the aldehyde group of 2-oxopropanal is lost by oxidation into carbon dioxide. However, this has to be verified by an experiment using

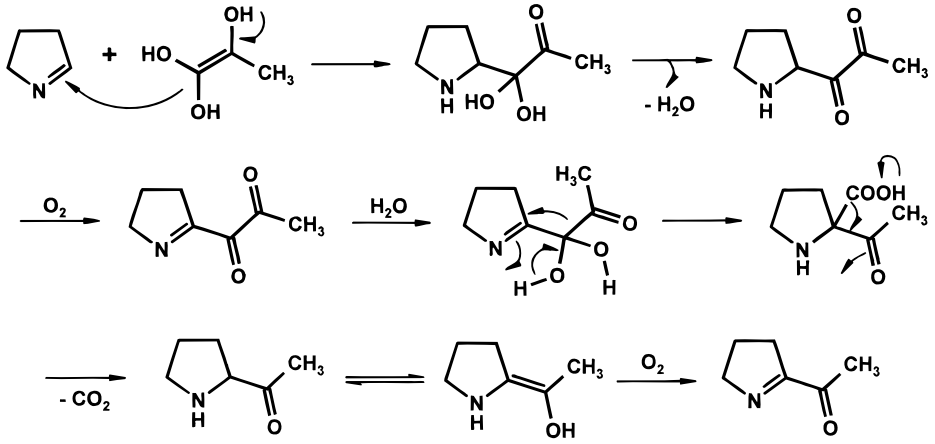

Figure 6. Formation of AP from 1-pyrroline and 2-oxopropanal hydrate.

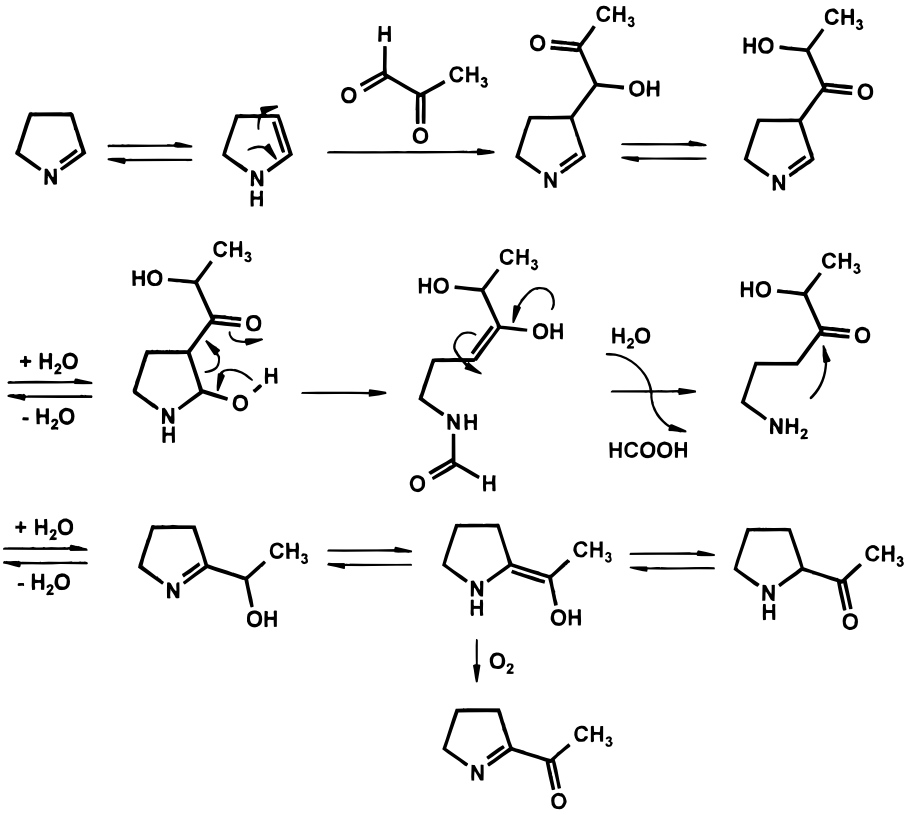

Figure 7. Alternative reaction pathway leading from 1-pyrroline and 2-oxopropanal to AP.

$[^{13}C]_1$-2-oxopropanal and by monitoring the carbon-13 labeling in the carbon dioxide eliminated.

Referring to Table 6, a minor amount of AP formed from labeled glucose had incorporated three labeled carbon atoms (*m/z* 114 vs *m/z* 111). Assuming also 1-pyrroline and 2-oxopropanal as the key intermediates in the formation of $[^{13}C]_3$-AP, it has to be concluded that one carbon of the 1-pyrroline (which stems from the unlabeled proline) is lost in the course of the reaction. To gain an insight into this alternative pathway, 2-methyl-1-pyrroline was reacted with 2-oxopropanal. As indicated in Table 8, somewhat lower, but significant, amounts of AP were formed from the homologous 2-methyl-1-pyrroline. The data establish that carbon-2 of both pyrrolines is lost during the reaction with 2-oxopropanal. In Figure 7, a reaction pathway is given explaining these results. It is assumed that the tautomeric 2-pyrroline, as a nucleophile, attacks carbon-1 in nonhydrated 2-oxopropanal. Hydration of the imine formed, followed by a ring opening and subsequent hydrolysis of the formamide generated, yields 4-oxo-5-hydroxyhexylamine. This intermediate will then cyclize into 2-acetylpyrrolidine, the precursor of AP (Hofmann and Schieberle, 1998).

Table 8. Amounts of AP Generated from 1-Pyrroline or 2-Methyl-1-pyrroline, Respectively, in the Presence of 2-Oxopropanal[a]

reactant	AP (μg)	yield (%)
1-pyrroline	53.5	6.5
2-methyl-1-pyrroline	39.5	4.8

[a] The reactants (10 μmol; 690 μg or 830 μg, respectively) were dissolved in phosphate buffer (5 mL, 0.5 mol/L; pH 7.0) and reacted for 30 min at 100 °C.

General Considerations. During food processing, the amino acid proline should react first with, for example, a deoxyosone generated from a carbohydrate. As shown in Figure 8, the Maillard reaction of the

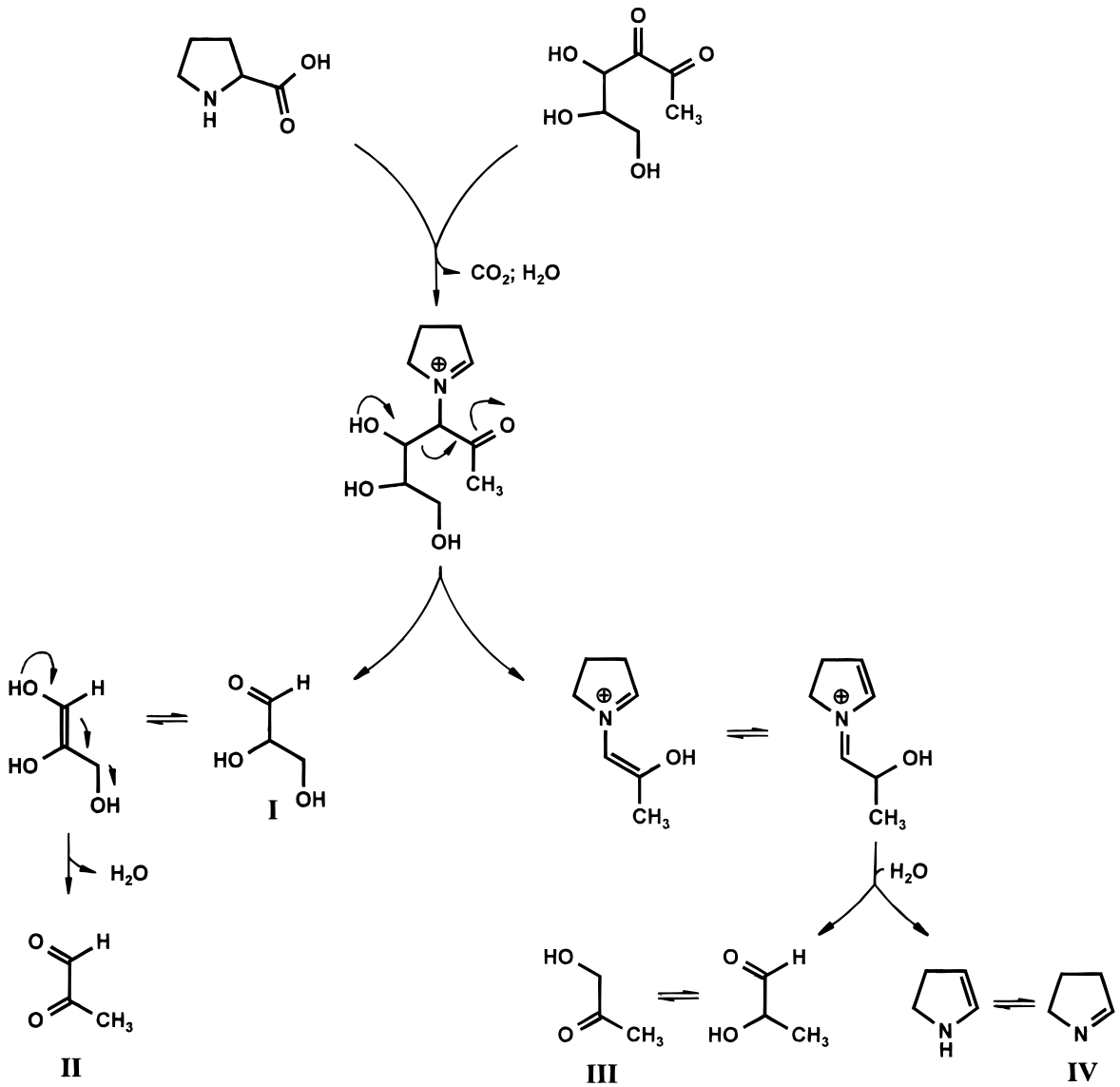

Figure 8. Generation of 1-pyrroline, glycerine aldehyde, 2-oxopropanal and hydroxy-2-propanone from proline and 1-deoxyglucosone.

1-deoxyglucosone with proline will yield 1-pyrroline, hydroxy-2-propanone, and glycerinaldehyde as the first reaction products. The latter aldehyde might subsequently lose water to yield 2-oxopropanal. Because the results presented here suggest 1-pyrroline as the key intermediate in the formation of both AP and ATHP, the relative concentrations of these odorants formed, for example, during thermal processing of foods, should be dependent on the relative amounts of the three carbohydrate cleavage product present in a food. Reacting the amino acid proline with increasing amounts of 2-oxopropanal resulted in the preferential formation of AP, whereas ATHP was preferentially formed from proline in the presence of lower amounts of 2-oxopropanal (Table 9). This result implies that a lower proline/2-oxopropanal ratio will preferentially generate the ATHP precursors 1-pyrroline and hydroxy-2-propanone by Strecker degradation (Figure 1). However, at higher ratios of proline/2-oxopropanal, the excess of 2-oxopropanal will immediately react with the 1-pyrroline formed, yielding preferentially AP.

Conclusions. The results suggest 1-pyrroline as the key intermediate in the formation of both food odorants AP and ATHP from the amino acid proline. Whether ATHP is predominantly formed, as in popcorn (Schieberle, 1991), or AP is preferentially generated, as in bread crust (Schieberle and Grosch, 1987), depends significantly on the carbohydrate cleavage products present. If high amounts of 2-oxopropanal are present, AP will be formed, whereas in the presence of its reduction product hydroxy-2-propanone, formation of ATHP is favored. This is because in the presence of high amounts of free amino acids, the Strecker reaction of 2-oxopropanal will be favored, hydroxy-2-propanone will predominate, and, consequently, generation of ATHP should be favored.

Table 9. Influence of the Concentration of 2-Oxopropanal on the Formation of AP and ATHP from Proline[a]

2-oxopropanal (mmol)	AP (μg)	ATHP (μg)
0.02	12.8	89.4
0.2	27.8	61.6
2.0	39.8	3.5

[a] Proline (2 mmol) was reacted in the presence of increasing concentrations of 2-oxopropanal for 30 min at 100 °C in phosphate buffer (5 mL; pH 7.0; 0.5 mol/L).

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Received for review November 21, 1997. Revised manuscript received March 18, 1998. Accepted March 23, 1998.

JF970990G