Flavor Contribution and Formation of the Intense Roast-Smelling Odorants 2-Propionyl-1-pyrroline and 2-Propionyltetrahydropyridine in Maillard-Type Reactions

Thomas Hofmann and Peter Schieberle*

Institut für Lebensmittelchemie and Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Application of aroma extract dilution analysis on two different model mixtures of proline and glucose, reacted under aqueous or dry-heating conditions, revealed 2-propionyl-1-pyrroline (PP) and 2-propionyltetrahydropyridine (PTHP; occurring in two tautomers), besides 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine, as important roast-smelling odorants in both mixtures. A comparison of the isotope distribution in PP and PTHP formed from either $^{13}C_6$-labeled or unlabeled glucose suggested a formation pathway for both odorants from the same intermediate, 1-pyrroline, when reacted with either 2-oxobutanal (yielding PP) or 1-hydroxy-2-butanone (yielding PTHP). 2-Oxobutanal, a possible precursor of 1-hydroxy-2-butanone, was shown to be formed in high yields (29 mol %) by reacting acetaldehyde and glycolaldehyde, two well-known degradation products of carbohydrates.

Keywords: *Maillard reaction; labeling experiments; 2-acetyl-1-pyrroline; 2-acetyltetrahydropyridine; 2-propionyl-1,4,5,6-tetrahydropyridine; 2-propionyl-3,4,5,6-tetrahydropyridine; 2-propionyl-1-pyrroline*

INTRODUCTION

The Maillard-type reaction between the amino acid proline and reducing carbohydrates is well-known to generate popcorn-like, roasty odors upon thermal treatment (Hunter et al., 1969; Lane and Nursten, 1983). 2-Acetyltetrahydropyridine [ATHP, occurring in an equilibrium of two tautomers; cf. Tressl et al. (1981) and Schieberle (1990a)] and 2-acetyl-1-pyrroline (AP) exhibit such odor notes at the very low odor thresholds of 0.2 and 0.02 ng/L (in air), respectively (Schieberle, 1995a).

Both odorants have earlier been identified in the volatile fraction of several Maillard-type model reactions containing proline (Hunter et al., 1969; Tressl et al., 1981), and it had, therefore, been proposed that ATHP and AP mainly contribute to the overall roasty odors of such processed flavors. The application of gas chromatography olfactometry (GCO) techniques, such as Charm analysis, or aroma extract dilution analysis (AEDA) [cf. review by Schieberle (1995b)] allows the evaluation of the relative odor potencies of a single volatile in complex extracts of volatile compounds by means of the "extract dilution to odor threshold in air" approach. Using Charm analysis, Roberts and Acree (1994) recently proved the two tautomers of ATHP to be the key odorants (74% of Charm) in a thermally treated (200 °C; 1 min; aqueous conditions) equimolar mixture of proline and glucose, followed by AP (18% of Charm). 2,3-Butanedione (4% of Charm) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4% of Charm) were found to contribute less to the overall odor. Similar results for ATHP and AP had earlier been reported for a heated proline/2-oxopropanal mixture (Schieberle, 1990a).

* Author to whom correspondence should be addressed (telephone +49-89-289 141 70; fax +49-89-289 141 83; e-mail LEBCHEM.Schieberle@lrz.tu-muenchen.de).

Besides AP and ATHP, 2-propionyl-1-pyrroline (PP) has been identified as a further key odorant in freshly popped corn (Schieberle, 1991) and, very recently, in the roasted skin of fried chicken (Kerscher and Grosch, personal communication). 2-Propionyltetrahydropyridines had been tentatively identified in thermally treated proline/glucose systems (Tressl et al., 1981). However, to date, these compounds have not been characterized in foods or reaction flavors based on sensory and analytical data.

To get insight into the precursors and formation of the propionyl homologues of AP and ATHP, the key odorants formed during thermal treatment of proline/glucose mixtures were reinvestigated by AEDA and their formation was elucidated using ^{13}C-labeled glucose.

EXPERIMENTAL PROCEDURES

Chemicals. 2,3-Butanedione, 1-hydroxy-2-butanone, acetaldehyde, glycolaldehyde, 1,2-diaminobenzene, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone were from Aldrich (Steinheim, Germany). [$^{13}C_6$]Glucose was from Sigma (Munich, Germany).

Syntheses: Preparation of Roast Odorants. AP and ATHP were prepared using the new synthetic approaches described recently (Hofmann and Schieberle, 1998a). PP was prepared from 1-pyrroline and 2-oxobutanal as previously reported (Schieberle, 1991). 1-Pyrroline was generated by oxidation of L-proline as described recently (Schieberle, 1991). [$^{13}C_4$]-2,3-Butanedione was synthesized as recently described (Schieberle and Hofmann, 1997a).

2-Propionyltetrahydropyridine (PTHP). On the basis of a reaction route verified recently for the homologous ATHP (Hofmann and Schieberle, 1998b), 1-pyrroline (0.1 mmol) and 1-hydroxy-2-butanone (0.1 mmol) were reacted at 100 °C in phosphate buffer (50 mL; 0.1 mol/L; pH 7.0) for 30 min. The volatiles formed were isolated by extraction with diethyl ether followed by sublimation in vacuo (Sen et al., 1991). ^{1}H NMR

S0021-8561(97)01101-1 CCC: $15.00
Published on Web 06/06/1998

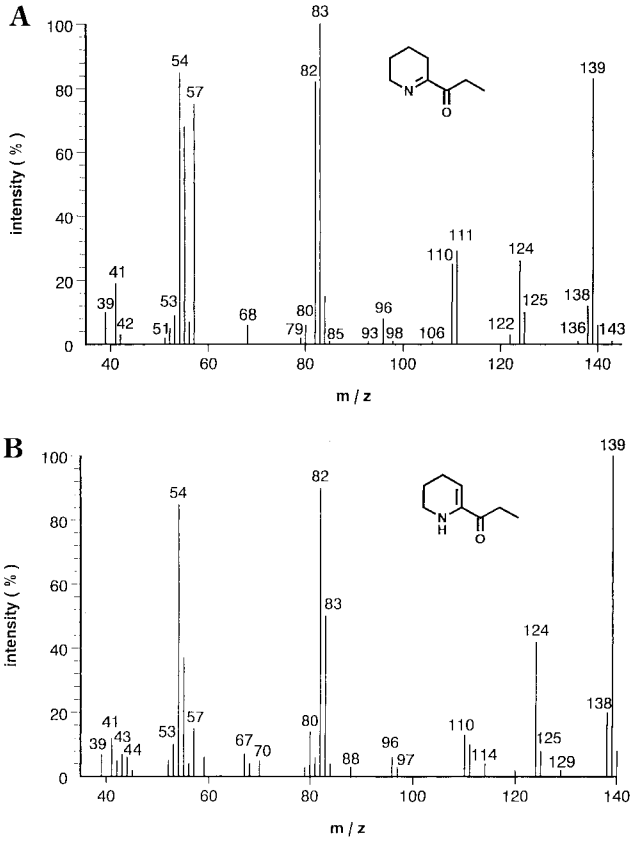

Figure 1. Mass spectra (MS/EI) of 2-propionyl-3,4,5,6-tetrahydropyridine (**I**; A) and 2-propionyl-1,4,5,6-tetrahydropyridine (**II**; B).

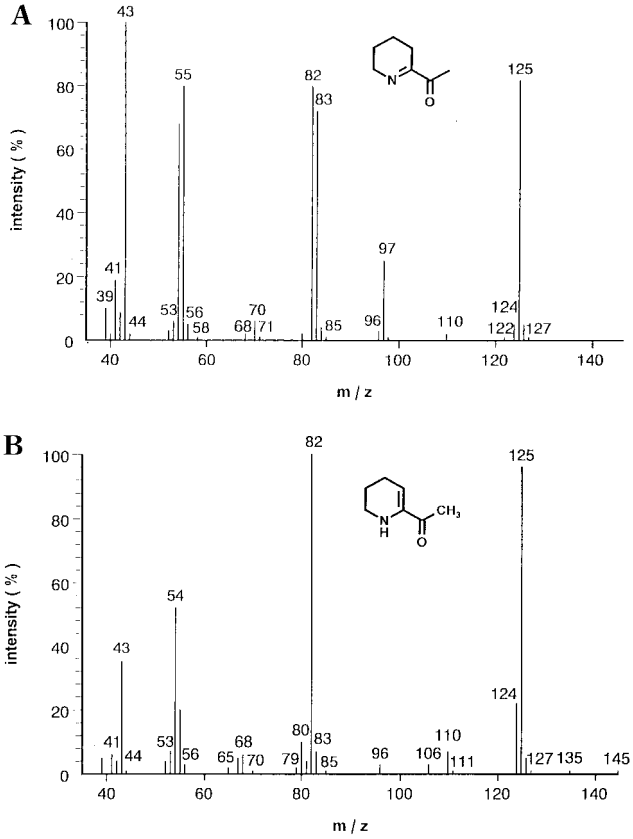

Figure 2. Mass spectra of 2-acetyl-3,4,5,6-tetrahydropyridine (A) and 2-acetyl-1,4,5,6-tetrahydropyridine (B).

and ^{13}C measurements in an aliquot of the distillate gave signals for 2-propionyl-1,4,5,6- and 2-propionyl-3,4,5,6-tetrahydropyridine, which were in agreement with those published recently by De Kimpe and Keppens (1996). High-resolution gas chromatography on an SE-54 column− showed two main products eluting at retention indices (relative to *n*-alkanes) of 1148 (**I**) and 1239 (**II**). Their mass spectra are displayed in Figure 1 [A (**I**) and B (**II**)]. By MS/CI, for both a molecular mass of 139 was established. To correlate the GC peaks with the structure of the tautomers, compound **I** was isolated by preparative gas chromatography using the method described recently (Schieberle, 1991). The signals obtained by ^1H NMR were identical with the data for the synthetic 2-propionyl-3,4,5,6-tetrahydropyridine (De Kimpe and Keppens, 1996). On the basis of this result, compound **II** was assigned as 2-propionyl-1,4,5,6-tetrahydropyridine. This order of elution is further corroborated by the following results: It was recently shown (Hofmann and Schieberle, 1998b) that on an SE-54 column the imine tautomer of 2-acetyl-3-methyltetrahydropyridine is eluted before the enamine tautomer. Furthermore, the higher intensity of the fragment *m/z* 57 (CH_3-$CH_2C{\equiv}O^+$) found for **I** (Figure 1A) is more reasonable for the imine tautomer (cf. parts A and B of Figure 1). Similar differences were also found in the mass spectra of the homologous 2-acetyltetrahydropyridines, also showing a more intense fragment of the acetyl group *m/z* 43 ($CH_3C{\equiv}O^+$) in the imine tautomer (cf. parts A and B of Figure 2). Both propionyltetrahydropyridines showed the same low odor threshold of 0.2 ng/L in air.

Model Reactions: Isolation of the Volatiles. Two different model systems were studied. In mixture I, a diluted aqueous mixture of L-proline (2 mmol) and D-glucose (1 mmol) was boiled in phosphate buffer (200 mL; pH 7.0; 0.1 mol/L) for 2 h and the volatiles formed were simultaneously steam-distilled and extracted using the apparatus described by Nickerson and Likens (1966). In mixture II, L-proline (2 mmol) and D-glucose (1 mmol) were mixed with silica gel (2.7 g containing 300 μL of the same phosphate buffer) and then heated for 10 min at 160 °C in closed glass vials (1 cm i.d.; total volume = 10 mL). Mixture II was extracted with diethyl ether (total volume = 300 mL), and the volatiles were isolated by sublimation in vacuo (Sen et al., 1991).

The distillates obtained from both mixtures were concentrated to 0.2 mL by distilling off the solvent at 35 °C using a Vigreux column (60 cm × 1 cm i.d.) followed by microdistillation (Schieberle, 1991). The most odor-active volatiles were then evaluated by AEDA as described previously (Schieberle, 1991).

Labeling Experiments. In mixtures I and II, D-glucose was substituted by [$^{13}C_6$]-D-glucose and, after thermal treatment, the volatile fractions were isolated as described above. The carbon isotope ratios in the labeled odorants generated were determined by mass chromatography of the cluster of molecular ions generated in the electron impact mode. The relative concentration of each isotopomer was calculated by using the computer of the mass spectrometer. The data were compared with results obtained for the respective unlabeled aroma compounds.

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 helium carrier gas was 2.5 mL/min. At the end of the capillary, the eluate was split 1:1 (by volume) into an FID and a sniffing port using deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). The FID and the sniffing port were held at 180 °C. Linear retention indices (RI) of the compounds were calculated from the retention times

Table 1. Key Odorants (FD ≥ 16) Generated in Thermally Treated Proline/Glucose Reaction Mixtures[a]

no.	odorant[b]	odor quality[c]	RI on SE-54	FD factor[d] in mixture I	FD factor[d] in mixture II
1	2,3-butanedione	buttery	592	16	16
2	2-acetyl-1-pyrroline	popcorn-like	922	64	16384
3	2-propionyl-1-pyrroline	popcorn-like	1024	16	512
4	2-acetyl-3,4,5,6-tetrahydropyridine	popcorn-like	1049	4096	2048
5	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	1068	<1	8192
6	2-acetyl-1,4,5,6-tetrahydropyridine	popcorn-like	1145	4096	2048
7	2-propionyl-3,4,5,6-tetrahydropyridine	popcorn-like	1148	128	128
8	2-propionyl-1,4,5,6-tetrahydropyridine	popcorn-like	1239	128	128
9	unknown	roasty	1352	<1	16

[a] Mixtures of L-proline (2 mmol) and D-glucose (1 mmol) were reacted under different conditions. Mixture I, simultaneous steam distillation/extraction for 2 h; mixture II, dry heating for 10 min at 160 °C. [b] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on two capillary columns given in the table, mass spectra obtained by MS(EI) and MS (CI), and odor quality and odor threshold perceived at the sniffing port. [c] Odor quality perceived at the sniffing port. [d] Flavor dilution (FD) factor determined in distillates containing the complete set of the volatiles. Analyses were performed by two assessors in duplicates. The data differed by not more than two FD factors. RI: Linear retention index.

of *n*-alkanes by using a computer program. MS analysis was performed with an 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.

Quantification of 2-Oxobutanal and 2,3-Butanedione. Glycolaldehyde (1 mmol) and acetaldehyde (1 mmol) were reacted for 20 min at 145 °C in phosphate buffer (50 mL; 0.5 mol/L; pH 7.0). After cooling, the solution was made up to a defined volume (100 mL) and 1 mL of this mixture was spiked with [$^{13}C_4$]-2,3-butanedione (20 μg) as the internal standard. The aqueous solution was three times extracted with diethyl ether (total volume = 10 mL), 1,2-diaminobenzene (2 mg) was added, and the mixture was stored overnight at 8 °C. The quinoxaline derivatives formed were separated by HRGC on the SE-54 column, and the amount of 2,3-butanedione and 2-oxobutanal was calculated by using the $^{13}C_4$-labeled quinoxaline formed from the labeled 2,3-butanedione as the internal standard (Schieberle and Hofmann, 1997a).

RESULTS AND DISCUSSION

AEDA. A distillate, obtained by boiling and simultaneously extracting an aqueous mixture of L-proline and D-glucose, was judged to elicit an intense roasty, popcorn-like odor. Application of AEDA to the distillate revealed seven odor-active volatiles in the flavor dilution (FD) factor range of 16−4096. Among them, six odorants elicited a popcorn-like, roasty odor. The identification experiments, which were based on a comparison of two sensory criteria (odor quality and odor threshold) and at least two of four analytical criteria (RI on SE-54; RI on FFAP; MS/CI; MS/EI) with the reference odorants revealed that 2-acetyl-3,4,5,6-tetrahydro- (**4** in mixture I, Table 1) and 2-acetyl-1,4,5,6-tetrahydropyridine (**6**, Table 1) showed by far the highest FD factors. This result is in good agreement with data published by Roberts and Acree (1994) using similar and, also, aqueous reaction conditions in the thermal treatment of proline/glucose. Next in rank to the two 2-acetyltetrahydropyridines, we identified 2-propionyl-3,4,5,6-tetrahydro- (**7**, Table 1) and 2-propionyl-1,4,5,6-tetrahydropyridine (**8**), both PTHPs showing the same popcorn-like odor note and the same low odor threshold as the ATHP tautomers. The structures of these propionyl homologues were elucidated for the first time in a reaction flavor or a food, respectively, based on mass spectrometric data and synthetic experiments. On the basis of the FD factors, their contribution to the overall odor of the mixture was, however, lower than the contribution of the ATHP isomers. In agreement with the findings of Roberts and Acree, AP (**2**, Table 1) showed a low odor contribution to the overall roasted odor of mixture I. The sixth popcorn-like-smelling odorant present in the distillate was identified as PP (**3** in Table 1). Although PP is an established contributor to food flavors [cf. Schieberle (1991) and Buttery et al. (1997)], it has, however, not yet been reported as a flavor constituent in processed flavors.

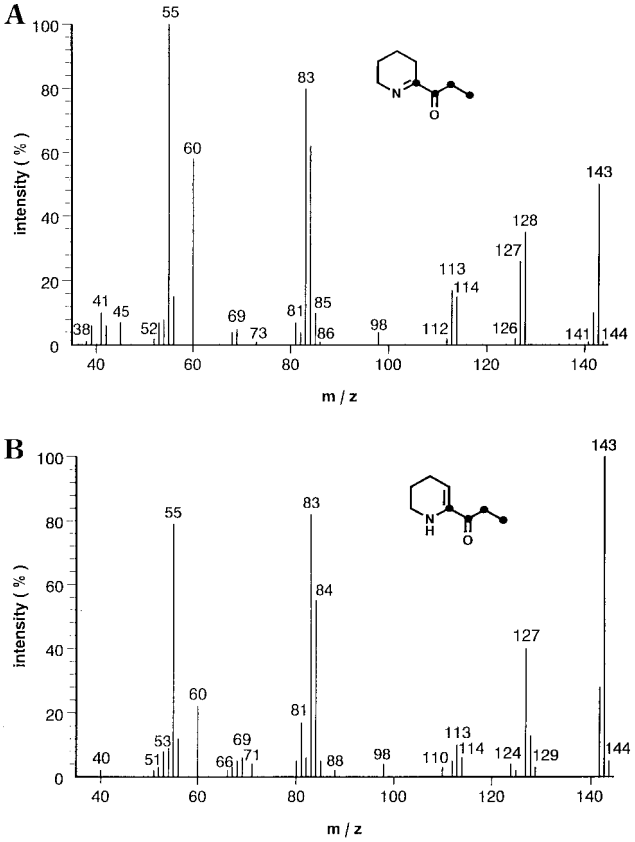

Figure 3. Mass spectra of [$^{13}C_4$]-2-propionyl-3,4,5,6-tetrahydropyridine (A) and [$^{13}C_4$]-2-propionyl-1,4,5,6-tetrahydropyridine (B): ●, carbon-13 label from U-^{13}C-labeled glucose.

It is well-known that in the production of Maillard-type processed flavors, the reaction parameters significantly influence the overall odors (Lane and Nursten, 1983; Schieberle and Hofmann, 1997b). To gain insight into the influence of the reaction conditions on the formation of the popcorn-like-smelling odorants, another proline/glucose mixture was thermally treated at 160 °C under dry-heating conditions (mixture II). The

Table 2. Carbon Isotope Ratio in PTHP Generated from Proline and Glucose or [$^{13}C_6$]Glucose[a]

	rel distribution (%) in PTHP generated in	
m/z	(A) glucose	(B) [$^{13}C_6$]glucose
137	0.1	<0.1
138	20.1	<0.1
139	73.7	<0.1
140	5.8	<0.1
141	0.3	1.4
142	<0.1	26.1
143	<0.1	68.5
144	<0.1	3.8
145	<0.1	0.2

[a] L-Proline (2 mmol) and either glucose (1 mmol) or [$^{13}C_6$]glucose (1 mmol) were dissolved in phosphate buffer (200 mL; 0.1 mol/L: pH 7.0) and simultaneously distilled and extracted for 2 h.

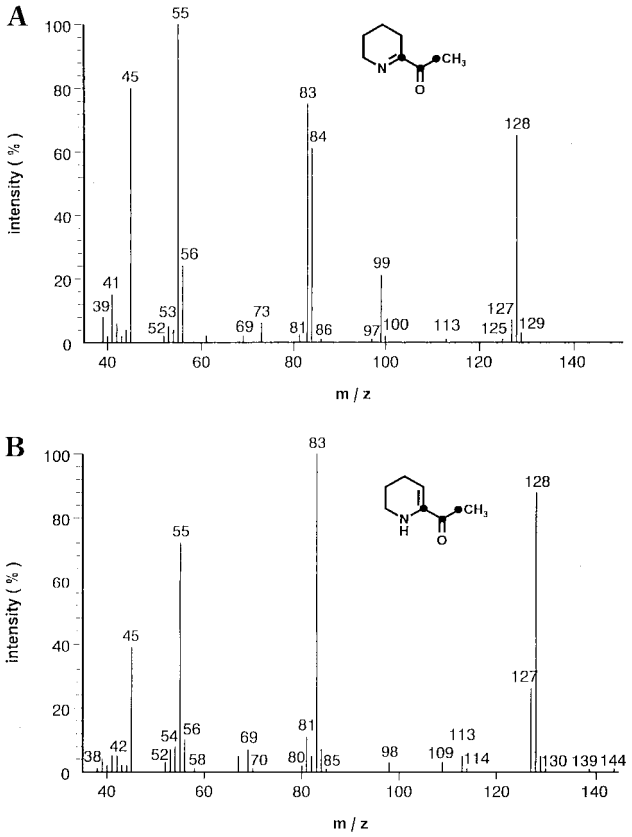

Figure 4. Mass spectra of [$^{13}C_3$]-2-acetyl-3,4,5,6-tetrahydropyridine (A) and [$^{13}C_3$]-2-acetyl-1,4,5,6-tetrahydropyridine (B): ●, carbon-13 label from U-^{13}C-labeled glucose.

results revealed the same key odorants as in mixture I. However, as displayed by their FD factors (mixture II in Table 1), the different reaction parameters significantly changed the odor contributions of the respective odorants. Compared to mixture I (aqueous conditions), especially, AP was drastically increased under the dry-heating conditions to become the most important odorant in this mixture (**2**, Table 1). Besides AP, also the homologous PP was significantly enhanced. In contrast to the two pyrrolines, the four tetrahydropyridine derivatives remained constant in their odor activities (cf. compounds **4** and **5** and **7** and **8** in mixtures I and II).

The caramel-like-smelling 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) was detected among the key odorants only under dry-heating conditions. However, HDMF is not steam volatile and, therefore, the data do

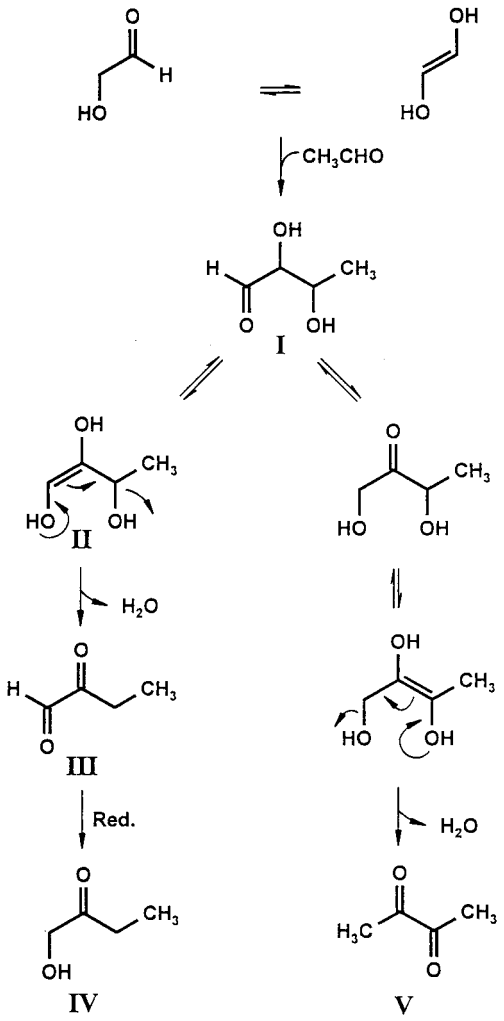

Figure 5. Formation of 2-oxobutanal (**III**), 2,3-butanedione (**V**), 1-hydroxy-2-butanone (**IV**), and 2,3-dihydroxybutanal (**I**) from glycolaldehyde and acetaldehyde.

not allow the conclusion that this odorant had not been formed in mixture I.

Labeling Experiments. *Formation of 2-Propionyltetrahydropyridines.* To get some insight in the formation of the 2-propionyl compounds, the carbon isotope ratio in the 2-propionyltetrahydropyridines formed by heating proline and $^{13}C_6$-labeled glucose was determined from the cluster of their molecular ions. As indicated in Table 2, the main molecular ion was *m/z* 143 (B in Table 2). Compared with the main molecular ion *m/z* 139 in the 2-propionyltetrahydropyridines generated from unlabeled glucose (A in Table 2), this corresponds to an upward shift of 4 mass units, indicating the incorporation of four labeled carbons in the PTHP formed from [$^{13}C_6$]glucose. The mass spectra of the two labeled tautomers are displayed in Figure 3. A comparison with the spectra of the respective unlabeled PTHPs (Figure 1) revealed that in both tautomers, three labeled carbons are present in the propionyl group (*m/z* 60 versus 57), whereas one labeled carbon is incorporated in the piperidine ring (*m/z* 83/84 versus 82/83). A similar result was found for the two labeled ATHP tautomers (Figure 4). Compared with the spectra of the respective unlabeled ATHPs (Figure 2), two labeled carbon atoms were detected in the acetyl group (*m/z* 45 versus 43) and one labeled carbon in the piperidine ring (*m/z* 83/84 versus 82/83).

Table 3. Amounts of 2-Oxobutanal and 2,3-Butanedione Generated from Acetaldehyde and Glycolaldehyde[a]

reaction product	amount (μg)	yield (%)
2-oxobutanal	25100	29.2
2,3-butanedione	755	0.9

[a] Glycolaldehyde (1 mmol) and acetaldehyde (1 mmol) were reacted for 20 min at 145 °C in phosphate buffer (50 mL; 0.5 mol/L; pH 7.0).

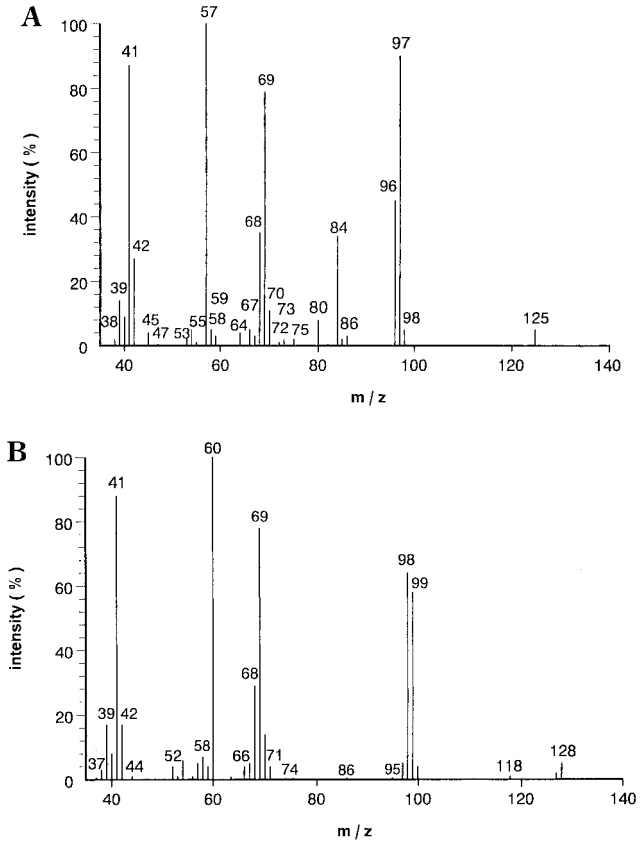

Figure 6. Mass spectra (MS/EI) of PP generated from proline/glucose (A) and proline/U-^{13}C-labeled glucose (B).

In a very recent investigation we could show (Hofmann and Schieberle, 1998b) that the homologous ATHP is formed in relatively high yields from a reaction of 1-pyrroline with hydroxy-2-propanone and have proposed a new reaction pathway involving a ring enlargement of the 1-pyrroline. Application of this pathway to the homologous reaction of 1-hydroxy-2-butanone with 1-pyrroline should yield PTHP. On the basis of a reaction of $^{13}C_4$-labeled 1-hydroxy-2-butanone (which might be formed from labeled glucose) and 1-pyrroline, a PTHP isotopomer would arise having three labeled carbons in the propionyl group and one in the ring. This assumption is in very good agreement with the analytical data described above.

To confirm both compounds as intermediates in PTHP formation, 1-pyrroline and 1-hydroxy-2-butanone (HB) were reacted in an aqueous buffer and the amounts of PTHP formed were determined by a stable isotope dilution analysis using deuterium-labeled ATHP as the internal standard (Schieberle, 1995a). Heating for 1 h at 100 °C yielded 0.5% of PTHP (sum of both tautomers), thereby corroborating the key role of 1-pyrroline and HB in PTHP formation.

The formation of HB from glucose can be explained by different pathways. We followed the idea that acetaldehyde and glycolaldehyde might be the precursors of HB (Figure 5). Both compounds are well-known degradation products of carbohydrates. It is suggested that glycolaldehyde and acetaldehyde yield, by an Aldol-type reaction, 2,3-dihydroxybutanal (**I** in Figure 5), which after enolization and elimination of water would result in 2-oxobutanal (**III** in Figure 5). This α-diketo compound might be reduced, for example, by a disproportionation with an ene-diol such as **II** (Figure 5), to yield HB (**IV**). To confirm this proposal, a mixture of acetaldehyde and hydroxyacetaldehyde was reacted in aqueous solution. The results (Table 3) showed that 2-oxobutanal is formed in very high yields. The complementary reaction pathway (right side in Figure 5) leading to 2,3-butanedione is obviously not favored, because a 30 times lower amount of this dione was generated (Table 3).

In summary, these results establish the key role of 1-pyrroline also in the formation of the 2-propionyltetrahydropyridines.

Formation of PP. In Table 4, the carbon isotope ratios in PP formed from proline in the presence of either [$^{13}C_6$]-glucose (B in Table 4) or unlabeled glucose (A) are contrasted. The main molecular ion in the labeled PP (B; *m/z* 128) was shifted up by 3 mass units compared

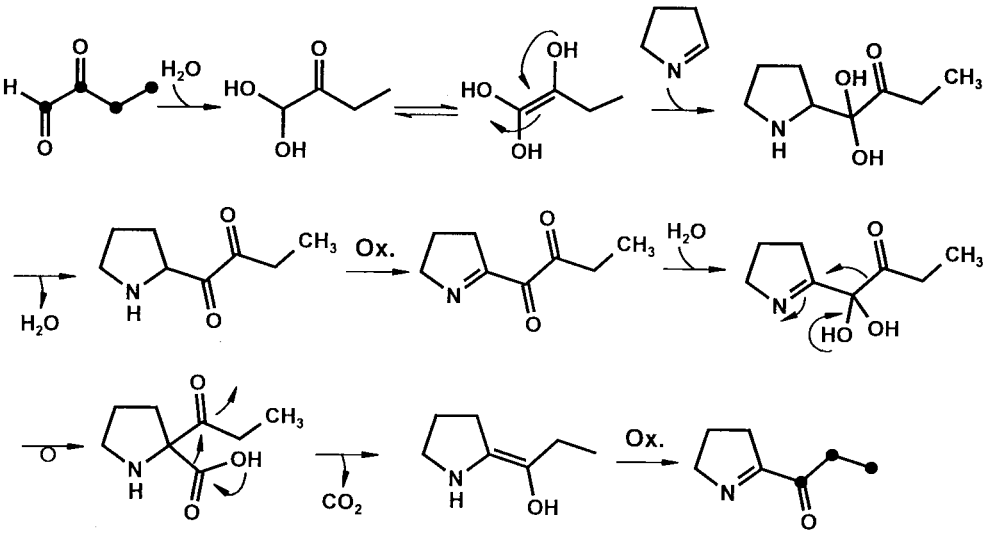

Figure 7. Hypothetical reaction pathway leading from 1-pyrroline and 2-oxobutanal to PP: ●, carbon-13 label (from U-^{13}C-labeled glucose).

Table 4. Carbon Isotope Ratio in 2-Propionyl-1-pyrroline (PP) Generated from Proline and Glucose or [$^{13}C_6$]Glucose[a]

	rel distribution (%) in PP generated in	
m/z	(A) glucose	(B) [$^{13}C_6$]glucose
123	0.9	<0.1
124	43.7	<0.1
125	51.4	<0.1
126	4.0	2.7
127	0.1	35.5
128	<0.1	57.3
129	<0.1	4.3
130	<0.1	0.2

[a] Cf. Table 2.

with the unlabeled PP (*m/z* 125). These data clearly indicate that three carbon atoms of the labeled glucose had been incorporated in the flavor compound. From a comparison of the MS/EI of the unlabeled PP with that of the [$^{13}C]_3$PP (Figure 6) it can be derived that the three labeled carbons are present in the propionyl group as represented by the fragments *m/z* 60 (labeled) and *m/z* 57 (unlabeled). There is, however, no labeling in the pyrroline ring (*m/z* 69/68 in either isotopomer).

In recent investigations we had established 1-pyrroline and 2-oxopropanal as the key intermediates in the formation of AP (Schieberle, 1995; Hofmann and Schieberle, 1998b). The reaction pathway proposed suggests that in the course of the reaction, one carbon atom of the 2-oxopropanal is lost as carbon dioxide. Because it has already been shown that the reaction of 2-oxobutanal and 1-pyrroline yields PP (Schieberle, 1991), a similar reaction mechanism can be proposed starting from 1-pyrroline and 2-oxobutanal as the key intermediates in PP formation (Figure 7). As indicated in the figure, the $^{13}C_4$-labeled 2-oxobutanal (generated from the labeled glucose) will lose the aldehyde carbon in the course of the reaction. The oxidation steps assumed in the reaction pathway have recently been established based on a new synthesis of AP and ATHP (Hofmann and Schieberle, 1998a).

Conclusions. The results have shown that 2-propionyl-1-pyrroline and the two tautomers of 2-propionyltetrahydropyridine are further significant roast odorants generated upon heating the amino acid proline in the presence of carbohydrates. On the basis of the results of the model studies presented, it can be assumed that these roast odorants are formed in foods from 1-pyrroline, the Strecker degradation product of proline, by similar mechanisms as recently confirmed for 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine. The specific precursor for PP is 2-oxobutanal, and that for PTHP is hydroxy-2-butanone. In a food, the latter two intermediates are supplied by cleavage of carbohydrate skeletons.

LITERATURE CITED

Buttery, R.; Ling, L. C.; Stern, D. J. Studies on popcorn aroma and flavor volatiles. *J. Agric. Food Chem.* **1997**, *45*, 837−843.

De Kimpe, N.; Keppens, M. Novel syntheses of the major flavor components of bread and cooked rice. *J. Agric. Food Chem.* **1996**, *44*, 1515−1519.

Hofmann, T.; Schieberle, P. New and convenient syntheses of the important roasty, popcorn-like smelling food aroma compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine from their corresponding cyclic α-amino acids. *J. Agric. Food Chem.* **1998a**, *46*, 616−619.

Hofmann, T.; Schieberle, P. 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. *J. Agric. Food Chem.* **1998b**, *46*, 2270−2277.

Hunter, I. R.; Walden, M. K.; Scherer, J. R.; Lundin, R. E. Preparation and properties of 1,4,5,6-tetrahydro-2-acetopyridine, a cracker-odor constituent of bread aroma. *Cereal Chem.* **1969**, 189−195.

Lane, M. C.; Nursten, H. E. The variety of odors produced in Maillard model systems and how they are influenced by the reaction conditions. In *The Maillard Reaction in Food and Nutrition*; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 141−158.

Nickerson, G. B.; Likens, S. T. Gas chromatographic evidence for the occurrence of hop oil components in beer. *J. Chromatogr.* **1966**, *2*, 677−678.

Roberts, D. D.; Acree, T. E. Gas chromatography olfactometry of glucose-proline Maillard reaction products. In *Thermally Generated Flavors*; Parliment, T. H., Morello, M. J., McGorrin, R., Eds.; American Chemical Society: Washington, DC, 1994; pp 71−79.

Schieberle, P. Formation of 2-Acetyl-1-pyrroline and Other Important Flavor Compounds in Wheat Bread Crust. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C. T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; S. 268−275.

Schieberle, P. Studies on Bakers Yeast as Source of Maillard-Type Bread Flavour Compounds. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Advances in Life Sciences: Basel, 1990a; pp 187−196.

Schieberle, P. The role of free amino acids present in yeast as precursors of the odorants 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine in wheat bread crust. *Z. Lebensm. Unters. Forsch.* **1990b**, *191*, 206−209.

Schieberle, P. Primary odorants in popcorn. *J. Agric. Food Chem.* **1991**, *39*, 1141−1444.

Schieberle, P. Quantification of important roast-smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. *J. Agric. Food Chem.* **1995a**, *43*, 2442−2448.

Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Gaonkar, A. G., Ed.; Elsevier Science: Amsterdam, 1995b; pp 403−431.

Schieberle, P.; Hofmann, T. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem.* **1997a**, *45*, 227−232.

Schieberle, P.; Hofmann, T. Characterization of key odorants in dry-heated cysteine/carbohydrate mixtures—Comparison with aqueous reaction systems. *Proceedings of the 214th National Meeting of the American Chemical Society*, Sept 7−11, 1997, Las Vegas; American Chemical Society: Washington, DC, 1997b.

Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of (*E*)-β-damascenon in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.

Tressl, R.; Grünewald, K. G.; Helak, B. Formation of flavour components from proline and hydroxyproline with glucose and maltose and their importance to food flavour. In *Flavour '81*; Schreier, P., Ed.; Walter de Gruyter: Berlin, 1981; pp 397−416.

Received for review December 29, 1997. Revised manuscript received April 22, 1998. Accepted April 24, 1998.

JF971101S