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Formation of Pyrazines and a Novel Pyrrole in Maillard Model Systems of 1,3-Dihydroxyacetone and 2-Oxopropanal

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Alkylpyrazines are a very important class of Maillard flavor compounds, but their mechanism of formation is complex and consists of different pathways. The model reaction of 20 different amino acids with 1,3-dihydroxyacetone, as a precursor of 2-oxopropanal, was studied by means of SPME-GC-MS to investigate the involvement of the amino acid side chain in the substitution pattern of the resulting pyrazines. 2,5-Dimethylpyrazine was quantitatively the most important pyrazine formed from all of the amino acids. The amino acid side chain is not involved in its formation. The substituents of other less abundant pyrazines resulted mainly from the incorporation of the Strecker aldehyde or aldol condensation products in the intermediate dihydropyrazine. The importance of different reaction mechanisms was evaluated, taking into account the pattern of pyrazines identified. In the solvent extracts of aqueous model reactions of 2-oxopropanal with amino acids, the main reaction product was not a pyrazine but a novel pyrrole. This pyrrole was identified as 2,5-diacetyl-3-methyl-1*H*-pyrrole by means of spectral analysis, secured by chemical synthesis. A reaction mechanism for its formation was proposed and evaluated. The influence of various reaction conditions on the formation of 2,5-diacetyl-3-methyl-1*H*-pyrrole and 2,5-dimethylpyrazine in the model reaction of alanine with 2-oxopropanal was studied. These results underscore the importance of the ratio of the different reagents and the presence of water in the resulting flavor formation in the Maillard reaction.

KEYWORDS: Pyrazines; Maillard reaction; 2,5-diacetyl-3-methyl-1*H*-pyrrole; model reaction; flavor

INTRODUCTION

Pyrazines comprise a group of heterocylic nitrogen-containing compounds that contribute significantly to the unique roasted aroma of many heated food products (*1, 2*). Formed usually at temperatures above 100 °C, alkylpyrazines are important products of the Maillard reaction, or nonenzymatic browning (*3*). This reaction, initiated with the condensation of a reducing carbohydrate with an amino compound, is mostly responsible for the formation of flavor and color in processed food products. Several trialkylated pyrazines such as 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine display very low odor thresholds (0.01 ng/L of air) (*4*) and are impact flavor compounds of, for example, coffee (*5*), roasted sesame seeds (*6*), and roasted beef (*7*).

The formation of pyrazines has been studied in a wide range of model reactions to gain insight into the reaction mechanisms leading to their formation (*2*). The most accepted mechanism for pyrazine formation involves the condensation reaction of two α-aminocarbonyl compounds with the formation of a dihydropyrazine, which oxidizes spontaneously to the corresponding pyrazine (*8*). The ease with which dihydropyrazines undergo oxidation with atmospheric oxygen to form pyrazines is well-known (see, e.g., ref *9*). The initial α-aminocarbonyl compounds result mainly from the Strecker degradation between an amino acid and an α-dicarbonyl compound, being a product of carbohydrate degradation. When the intermediate dihydropyrazine reacts with a carbonyl compound, an alkylpyrazine with an additional substituent is formed and the oxidation step is not necessary (**Scheme 1A**) (*10*). Shibamoto and co-workers described the formation of pyrazines from model reactions of glucose and other carbohydrates with ammonium hydroxide as a source of ammonia (*10, 11*), thereby not involving the carbon skeleton of an amino acid. Alternatively, it was shown that decarbonylation and dehydration of amino acids can occur in such a way that pyrazines are formed from the thermal degradation of only the amino acid without a carbohydrate source (*12*). The many studies performed so far show that there is more than one chemical pathway involved in pyrazine

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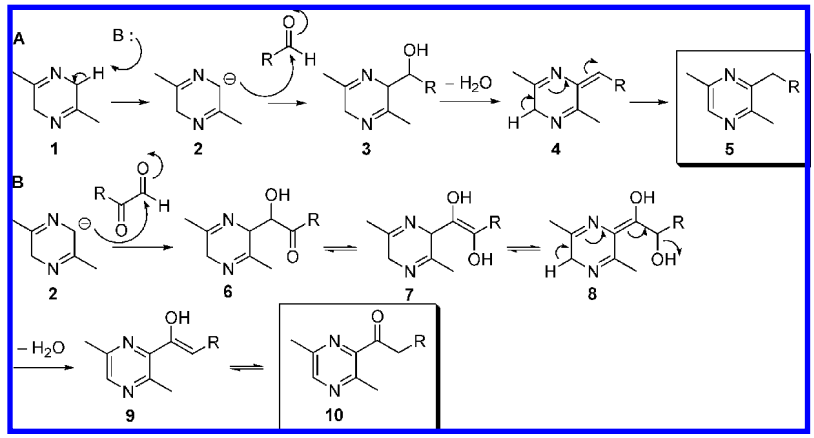
Scheme 1. Mechanism of Formation of 3-Alkyl-2,5-dimethylpyrazine (**5**) (**A**) and 3-Acyl-2,5-dimethylpyrazine (**10**) (**B**) from 3,6-Dimethyl-2,5-dihydropyrazine (**1**) and an Additional Carbonyl Compound



formation in complex Maillard systems. Even a very simple model system produces a large number and variety of alkylpyrazines. The availability of various precursors will determine which route predominates. Understanding the dependence of alkylpyrazine formation on the amino acids present is useful, for example, to predict changes in the alkylpyrazine profile of a food product when an amino acid is added (see, e.g. ref *13*). Therefore, a detailed study of the formation of different pyrazines from the reaction of carbohydrate fragments with various α-amino acids was performed. The reactions were performed with 2-oxopropanal, one of the most important carbohydrate fragments and, as such, an important precursor of pyrazines in food (*14*). However, because 2-oxopropanal is very reactive and, upon heating, prone to self-condensation and other side reactions, the in situ generation of 2-oxopropanal by dehydration of 1,3-dihydroxyacetone can result in less complicated reaction mixtures and allow as such a more straightforward study of the mechanisms involved (*15*). Therefore, the reaction of 1,3-dihydroxyacetone with each of 20 different α-amino acids was also performed in the absence of water. As such, the expected influence of water on these transformations could also be verified.

MATERIALS AND METHODS

Chemicals. 1,3-Dihydroxyacetone (DHA, dimer 98%), 2-oxopropanal (40% wt in water), phenylglyoxal, 2,3-pentanedione, glyoxal, alanine, proline, arginine, lysine monohydrate, hydrogen peroxide (30% in water), 2-acetyl-1-methylpyrrole, 2,5-dimethylpyrazine, ethylpyrazine, silica gel (0.035–0.070 mm, pore diameter ca. 6 nm), and chloroform-*d* (0.03 v/v % TMS, 99.8+ atom % D) were from Acros Organics (Geel, Belgium). Hydroxyacetone, 2(3)-*tert*-butyl-4-hydroxyanisole, 2-mercaptophenol, tetrafluoroboric acid−diethyl ether complex (54%), 3-methyl-1*H*-pyrrole, glycine, valine, asparagine, aspartic acid, glutamine, glutamic acid, threonine, histidine, methionine, and tryptophan were from Sigma-Aldrich (Bornem, Belgium). Serine, phenylalanine, cysteine, leucine, and isoleucine were from Janssen Chimica (Geel, Belgium). Tyrosine was from Difco Laboratories (BD, Erembodegem, Belgium).

Model Reactions. For the small-scale model reactions, 5 mmol of amino acid was mixed and ground with 5 mmol of 1,3-dihydroxyacetone in a 20 mL headspace vial closed with a magnetic crimp cap with septum (Gerstel, Mülheim a/d Ruhr, Germany). The reaction mixtures (without solvent) were heated in an oil bath at 90 °C for 30 min and rapidly cooled in an ice bath afterward.

For the other model reactions, 10 mmol of amino acid was dissolved/dispersed in 10 mL of phosphate buffer (1 M, pH 7.0), and equimolar amounts of dicarbonyl compound were added (for 2-oxopropanal: 1800 μL of 40% in water). The pH was adjusted to 7 with 1 N NaOH, and the mixture was heated at 100 °C for 30 min in an oil bath. After this time, the reaction mixtures were immediately cooled in an ice bath.

Analysis of Flavor Compounds. The volatiles formed from the 1,3-dihydroxyacetone model reactions were directly sampled by means of headspace SPME during 30 min at 30 °C with a 50/30 μm DVB/Car/PDMS fiber (Supelco, Bornem, Belgium) and desorbed during 2 min at 250 °C in the GC-MS inlet. SPME extraction and desorption were performed automatically by means of a Multipurpose Sampler (MPS-2, Gerstel).

For the other model reactions, the pH was adjusted to 9.0 with 2 N NaOH, and the mixture was extracted three times with 10 mL of chloroform. After drying ($MgSO_4$), filtration, and partial evaporation of the solvent (to approximately $^1/_{10}$ of its volume), 150 μL of a standard solution of 2-acetyl-1-methylpyrrole (1% in dichloromethane) was added as internal standard. The extracts were analyzed by means of GC-MS.

GC-MS. For the analysis of the flavor compounds a Hewlett-Packard 6890 GC Plus coupled with a HP 5973 MSD (mass selective detector, quadrupole type), equipped with a CIS-4 PTV (programmed temperature vaporisation) injector (Gerstel), and a HP5-MS capillary column (30 × 0.25 mm i.d.; coating thickness = 0.25 μm) was used. Working conditions for liquid injections were as follows: injector, 250 °C; transfer line to MSD, 250 °C; oven temperature, start at 50 °C, programmed from 50 to 150 at 2 °C min^{-1} and from 150 to 220 at 20 °C min^{-1}, held for 5 min; carrier gas (He), 1.2 mL min^{-1}; split 1/10; ionization EI, 70 eV. For the analysis of SPME extracts, the oven temperature was programmed at 35 °C, held for 5 min, programmed from 35 to 80 at 2 °C min^{-1} and from 80 to 250 at 20 °C min^{-1}, and held for 5 min, and the carrier gas (He) flow was 1 mL min^{-1}. Substances were identified by comparison of their mass spectra and retention times with those of reference substances and by comparison with the Wiley (6th) and the NIST Mass Spectral Library (version 1.6d, 1998). Linear retention indices (LRI) were calculated and compared with literature values (*4, 16*). When only MS data were available, identities were considered to be tentative. Quantification was performed, after calculation of the response factors, by means of an internal standard 2-acetyl-1-methylpyrrole.

Synthesis of 2,5-Diacetyl-3-methyl-1*H*-pyrrole. First, 2-methyl-1,3-benzoxathiolium tetrafluoroborate was prepared starting from *o*-mercaptophenol (16 mmol), equimolar amounts of acetic acid, and 54% tetrafluoroboric acid−diethyl ether complex (8 mL) as condensing agent. The mixture was heated at 35 °C for 2 h. After cooling in an ice bath, dry diethyl ether (64 mL) was added to precipitate the salt, and the mixture was kept in the freezer for 1 h to enhance the precipitation. The precipitate was a highly hygroscopic orange salt that was recovered by filtration under N_2 atmosphere. The salt was washed with dry diethyl ether (three times, 10 mL) and dried by evaporation in vacuo. Yields obtained were 80% (crude reaction mixture). To a mixture of 3-methyl-1*H*-pyrrole (2 mmol) and dry pyridine (ratio 3:1, respectively) in dry acetonitrile (3 mL) was added 2-methyl-1,3-benzoxathiolium salt (3.5

Table 1. Pyrazines (GC-MS Peak Area × 10^{-6}) Detected in the Headspace of Model Reactions of 1,3-Dihydroxyacetone with Various Amino Acids (90 °C, 30 min)

compound	LRI[a]	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
methylpyrazine	818	1.20	14.18	0.60					3.35				10.34				38.48				
2,5(6)-dimethylpyrazine	912	84.8	493.9	164.3	15.6	36.5	29.5	9.5	330.5	25.4	16.6	11.2	385.5	15.3	27.9	1.6	145.8	22.1	18.4	8.0	11.1
2-ethyl-6-methylpyrazine	1000	5.70							11.58								6.14				
trimethylpyrazine	1003		148.63						18.65									1.55			
3-ethyl-2,5-dimethylpyrazine	1082	3.63	44.97			0.40			3.55				33.24				7.67				
2-ethyl-3,5-dimethylpyrazine	1088	5.29																			
2-methyl-propylpyrazine[b]	1092	5.82																			
3-ethenyl-2,5-dimethylpyrazine	1103		11.94																		
2-acetyl-5-methylpyrazine	1126								1.46												
2-acetyl-6-methylpyrazine	1132	0.29					0.60		0.46												2.58
2-methyl-(*E*-1-propenyl)-pyrazine[b]	1163	6.89																			
2-acetyl-3,5-dimethylpyrazine	1173	0.93							4.19												
2,5-dimethyl-3-(2-methylpropyl) pyrazine	1187																				0.63
2-propanoyl-5-methylpyrazine	1194	2.45			1.78																
2-propanoyl-6-methylpyrazine	1198	12.3			2.00			0.68										0.57			

[a] Except for 2-ethyl-6-methylpyrazine and the acetyl- and propanoyl-substituted pyrazines, for which no reference values are found and which are thus tentatively identified, the LRI values (DB-5 stationary phase) correspond to literature data (*4*). [b] Elution order of the isomers is not determined in ref *4*.

equiv with respect to 3-methyl-1*H*-pyrrole) in one portion and under stirring. The reaction was carried out for 1 h at room temperature, and it was stopped by pouring the mixture into water (55 mL). 3-Methyl-2,5-bis(2-methylbenzo[1,3]oxathiol-2-yl)-1*H*-pyrrole was extracted with chloroform (two times, 83 mL) and washed with 5% NaOH solution (two times, 44 mL) and finally with water (44 mL). The extract was dried ($MgSO_4$) and the solvent removed by evaporation in vacuo. The hydrolysis of 3-methyl-2,5-bis(2-methylbenzo[1,3]oxathiol-2-yl)-1*H*-pyrrole (1.5 mmol) was performed with aqueous H_2O_2 (30%) (6 mmol) and acetic acid (8 mL). The reaction was stopped after 30 min at 40–45 °C, and the reaction product was compared on TLC with the unhydrolyzed compound (petroleum ether/ethyl acetate 1:1). The hydrolyzed mixture was poured in 10 mL of water and extracted with chloroform (three times, 10 mL). The extract was washed with a saturated solution of $NaHCO_3$ until complete neutralization of acetic acid, with 5% aqueous NaOH, then with water, and dried ($MgSO_4$). This resulted in a complex reaction mixture, mainly due to monoacetylation of 3-methyl-1*H*-pyrrole in different positions. The synthesized compound could not be completely purified by means of flash chromatography and preparative TLC, but its purity was increased to 78%, which allowed the comparison of spectral data with the isolated compound. Also by GC-MS analysis, the presence of 2,5-diacetyl-3-methyl-1*H*-pyrrole as the main constituent was confirmed.

Column Chromatography. The different compounds in the extracts were separated by chromatography over a short silica column (20 cm, i.d. = 2 cm) using a solvent mixture of hexane and ethyl acetate (7:3). Spots were detected on TLC by visualization under UV.

NMR Spectroscopy. High-resolution ^{1}H NMR and ^{13}C NMR spectra were taken in $CDCl_3$ as solvent (tetramethylsilane as internal standard) with a JEOL Eclipse FT NMR spectrometer.

2,5-Diacetyl-3-methyl-1H-pyrrole: yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$), δ 2.40 (3H, s, C$\underline{H}_3$), 2.45 (3H, s, C$\underline{H}_3$CO), 2.50 (3H, s, C$\underline{H}_3$CO), 6.68 (1H, s, C$\underline{H}$), 9.75 (1H, br s, N$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$), δ 14.1 (C$\underline{H}_3$), 26.2 (C$\underline{H}_3$CO), 28.7 (C$\underline{H}_3$CO), 118.4 (C$\underline{H}$CN), 126.7 (C$\underline{C}$N), 132.3 (C$\underline{C}$N), 132.6 (C$\underline{C}$N), 189.1 (C═O), 190.0 (C═O); IR cm^{-1} 1630 (C═O), 1256; MS (70 eV), *m/z* (%) 150 (100), 165 (89), 132 (23), 108 (21), 43 (13), 53 (7); LRI (DB-5) 1495.

2,5-Diacetyl-3-hydroxy-4-methyl-1H-pyrrole: MS (70 eV), *m/z* (%) 181 (100), 166 (96), 124 (21), 43 (18), 148 (12), 153 (10), 138 (9), 110 (6), 83 (6); LRI (DB-5) 1644.

RESULTS AND DISCUSSION

In a first instance, 1,3-dihydroxyacetone was reacted on a small scale with each of 20 different amino acids in dry reaction conditions (90 °C, 30 min), after which the volatiles were sampled by means of headspace SPME-GC-MS. Pyrazines were the most important group of volatiles detected. Fifteen different pyrazines were identified and are reported in **Table 1**. Analysis of the pyrazines identified for the different amino acids enables the evaluation of the relative importance of the different reaction mechanisms postulated in the literature.

All amino acids yielded 2,5(6)-dimethylpyrazine upon reaction with 1,3-dihydroxyacetone, a precursor of 2-oxopropanal. It must be noted that complete chromatographic separation of 2,5-dimethylpyrazine and 2,6-dimethylpyrazine is not possible under the chromatographic conditions described (DB-5 stationary phase). Both compounds elute with a difference in retention index of 1 unit, and their mass spectra are identical (*16*). Unambiguous identification of a single chromatographic peak at this specific retention and with this specific mass spectrum is impossible. Therefore, both compounds will be considered together in the following discussions. The formation of 2,5(6)-dimethylpyrazine occurs via the well-known condensation of two α-aminoketones, resulting from the Strecker degradation of 2-oxopropanal with an amino acid. Condensation of two

Scheme 2. Hypothetical Formation Mechanism of 2-Acyl-5-methylpyrazine (**23**) from the Condensation of 1-Amino-2-propanone (**21**) and the α-Aminoketone (**20**) Derived from 1,3-Dihydroxyacetone (**11**) and an Aldehyde (R = H, CH_3)

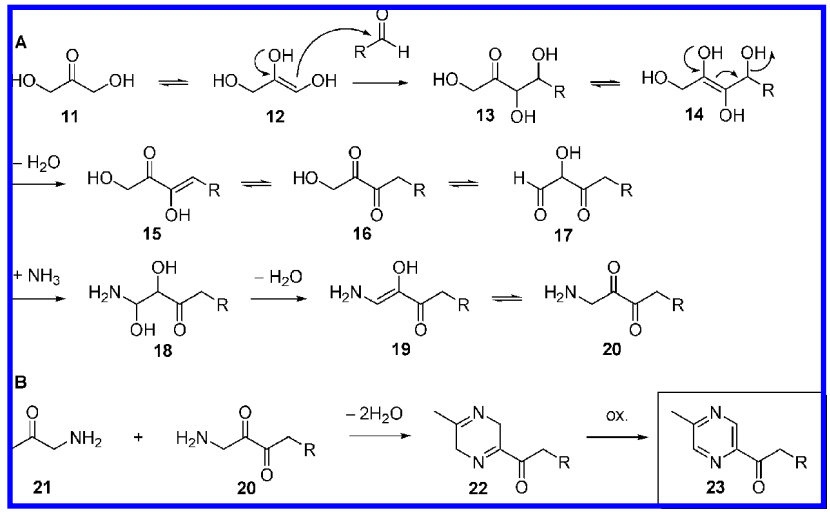


Table 2. Yields of 2,5-Diacetyl-3-methyl-1*H*-pyrrole (**34**) from the Model Reaction of 2-Oxopropanal (**24**) with Various Amino Acids (1 M, 100 °C, 30 min, Phosphate Buffer)

amino acid	yield of **34** (%)
alanine	0.02
asparagine	0.36
aspartic acid	1.56[a]
glutamic acid	1.81
leucine	0.16
phenylalanine	0.04[a]
tryptophan	0.02

[a] Because of low solubility, a 0.5 M solution was used instead of 1 M.

molecules of 1-aminopropan-2-one or 2-aminopropanal followed by spontaneous oxidation yields 2,5-dimethylpyrazine, whereas 2,6-dimethylpyrazine results from the condensation of 1-aminopropan-2-one with 2-aminopropanal. This last one, however, is less abundant because the aldehyde carbonyl function of 2-oxopropanal is more reactive than the keto function. The formation of 2,5(6)-dimethylpyrazine is independent of the amino acid side chain, although the reactivity of each amino acid in the Strecker degradation determines the extent to which it is formed. Only the more stable Strecker aldehydes were detected in the headspace of the reaction mixtures (especially those of isoleucine, leucine, methionine, phenylalanine, and valine). From **Table 1** it can be derived that arginine, lysine, and glycine yielded the highest amounts of 2,5(6)-dimethylpyrazine. These amino acids are generally known as highly reactive compounds because of the presence of a nucleophilic amino function in their side chain (in the case of lysine and arginine) or because of the flexibility of the molecule due to the absence of a side chain (in the case of glycine). A different study, comparing the reactivity in pyrazine formation of various amino acids in a model system consisting of wheat starch, glucose, and labeled glycine in water, found quite different results. Arginine, for example, yielded the lowest amount of 2,5(6)-dimethylpyrazine and phenylalanine the highest in the presence of glycine (*17*). These different results illustrate the importance of the matrix in the outcome of such model reactions.

Besides 2,5(6)-dimethylpyrazine, 14 other alkyl- and acylpyrazines were identified in the model mixtures. The largest variety in pyrazines was detected from the model reactions of 1,3-dihydroxyacetone with alanine, glycine, arginine, and serine. The reaction of proline, with a secondary amino function, with 1,3-dihydroxyacetone yielded very little pyrazines [only 2,5(6)-dimethylpyrazine in low amounts]. This amino acid, however, yields other interesting flavor compounds, such as 6-acetyl-1,2,3,4-tetrahydropyridine (*18*). In all of these model mixtures, the amino acid side chain determined the substitution pattern of the resulting pyrazines to a greater or lesser extent, as will be discussed.

In general, the formation of a substituted pyrazine starts from two α-dicarbonyl compounds, which are converted to the corresponding α-aminoketones. Whereas initially only 2-oxopropanal is present, several other carbonyl compounds are formed during the course of the reaction, such as the Strecker aldehydes and aldol condensation products. For instance, the condensation of 2-oxopropanal with formaldehyde, the Strecker aldehyde resulting from glycine, results in the formation of 2-oxo-3-butenal. Condensation of the α-aminoketones resulting from 2-oxopropanal and 2-oxo-3-butenal yields 3-methyl-6-vinyl-2,5-dihydropyrazine and 2-ethyl-6-methylpyrazine upon rearrangement of the double bonds. As in this case, the oxidation step of the dihydropyrazine is not necessary in the presence of an α,*β*-unsaturated carbonyl function because rearrangement of the double bonds with the side chain yields the corresponding pyrazine. The formation of 2-methyl-5-(1-*Z*-propenyl)pyrazine from alanine can be explained by condensation of the aminoketones resulting from 2-oxopropanal and 2-oxo-3-pentenal (the aldol condensation product of 2-oxopropanal with acetaldehyde), followed by spontaneous oxidation.

Instead of the oxidation step, a proton may be abstracted from 3,6-dimethyl-2,5-dihydropyrazine **1** followed by a nucleophilic attack across a carbonyl compound (e.g., the Strecker aldehyde) and elimination of water. Thus, an additional substituent can be incorporated on the pyrazine (**Scheme 1A**). This reaction mechanism was first suggested by Shibamoto et al. for the formation of ethylpyrazines from acetaldehyde (*10*). According to this mechanism, the formation of 3-ethyl-2,5(6)-dimethylpyrazine from alanine, of trimethylpyrazine from glycine, and of 2,5-dimethyl-3-(2-methylpropyl)pyrazine from valine can be explained by the addition of 3,6-dimethyl-2,5-dihydropyrazine **1** to the respective Strecker aldehyde (**Table 1**). Experiments of glucose and fructose with ^{13}C-labeled alanine also showed the importance of this pathway because the alkylpyrazine carbon atoms resulted from the carbohydrate unit as well as from the amino acid (*19*). For example, 2,5-dimethylpyrazine was found to be completely unlabeled and thus incorporated all carbon

Scheme 3. Hypothetical Formation Mechanism of 2,5-Diacetyl-3-methyl-1*H*-pyrrole (**34**) from the Condensation of 1-Amino-2-propanone (**21**) and Two Molecules of 2-Oxopropanal (**24**)

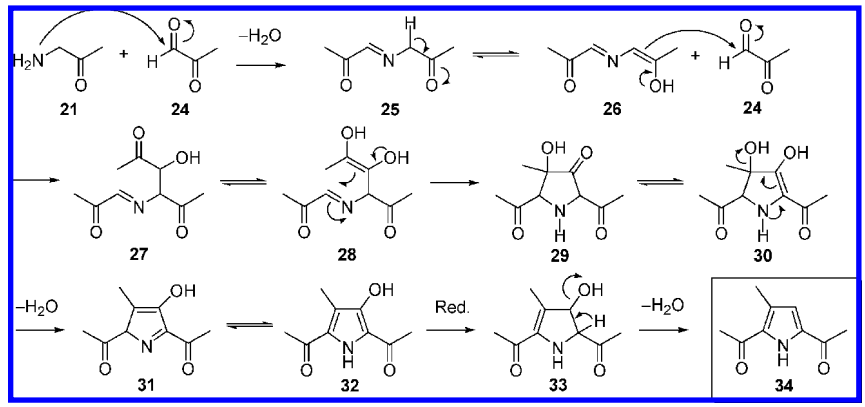


Table 3. Yields of 2,5-Diacetyl-3-methyl-1*H*-pyrrole (**34**) and 2,5(6)-Dimethylpyrazine (**35**) from the Model Reaction of Alanine (**36**) with 2-Oxopropanal (**24**)

time (min)	temp (°C)	ratio **36**:**24**	buffer	initial pH	yield of **34** (%)	yield of **35** (%)
30	130	1:1	NaH_2PO_4/Na_2HPO_4	7	0.020 ± 0.0073	0.02
30	130	1:1	$NaHCO_3/H_2CO_3$	7	0.055 ± 0.0094	0.011 ± 0.00064
30	130	1:1	CH_3COOH/CH_3COONa	4	1.6 ± 0.040	0.042 ± 0.005
10	100	1:1	CH_3COOH/CH_3COONa	4	0.080 ± 0.039	nd[a]
30	100	1:1	CH_3COOH/CH_3COONa	4	1.1 ± 0.087	0.020 ± 0.00025
60	100	1:1	CH_3COOH/CH_3COONa	4	1.3 ± 0.045	0.026 ± 0.0030
30	100	2:1	CH_3COOH/CH_3COONa	4	0.50 ± 0.019	0.013 ± 0.0029
30	100	1:3	CH_3COOH/CH_3COONa	4	3.7 ± 0.074	0.016 ± 0.0040

[a] Not detected.

atoms from the sugar, whereas 3-ethyl-2,5-dimethylpyrazine was 100% labeled in the ethyl side chain (*19*). Also by means of labeling, Low et al. (*13*) demonstrated the importance of this pathway (**Scheme 1A**) in a potato model system, especially for the higher substituted alkylpyrazines of higher molecular weight or with branched-chain substituents.

When the nucleophilic attack of the dihydropyrazine is aimed at an α-dicarbonyl compound, acylated pyrazines can be formed (**Scheme 1B**). However, the acylated pyrazines detected in the model reactions described (**Table 1**) are mainly disubstituted. Therefore, their formation from 3,6-dimethyl-2,5-dihydropyrazine **1**, the most abundant dihydropyrazine, is unlikely, because only one acyldimethyl-substituted pyrazine, **10**, was identified. Another hypothesized mechanism for the formation of 2-acyl-5-methylpyrazines **23** is shown in **Scheme 2** and starts with the condensation reaction of 1,3-dihydroxyacetone **11** with an aldehyde, followed by reaction with ammonia on one of the oxygenated carbons to form the corresponding α-aminoketone **20**, in an Amadori-type rearrangement (**Scheme 2A**). Condensation of this aminoketone **20** with 1-amino-2-propanone **21**, followed by spontaneous oxidation, yields 2-acyl-5-methylpyrazine **23** (**Scheme 2B**). Following this reaction mechanism, glycine (via its Strecker aldehyde formaldehyde) yields 2-acetyl-5(6)-methylpyrazine, whereas alanine (via its Strecker aldehyde acetaldehyde) yields 2-propanoyl-5(6)-methylpyrazine, as **Table 1** confirms. In **Table 1**, however, it can also be seen that the model reactions of 1,3-dihydroxyacetone with other amino acids yield 2-acyl-5(6)-methylpyrazines as well.

Another possible pathway for pyrazine formation involves the degradation of amino acids with the formation of free ammonia. Model studies have shown that especially glutamine releases considerable amounts of ammonia upon moderate heating (110 °C) (*20*). At higher temperatures (180 °C), also asparagine, cysteine, and aspartic acid yielded relatively high amounts of ammonia (*20*). Reaction of α-hydroxycarbonyl compounds (e.g., 1,3-dihydroxyacetone and aldol condensation products derived thereof) with ammonia may directly yield α-aminoketones outside the Strecker degradation, in an Amadori type rearrangement (*11*). This way, for instance, aminoacetaldehyde results from hydroxyacetaldehyde (Strecker aldehyde of serine) and condenses with 1-aminopropan-2-one (or 2-aminopropanal) with the formation of methylpyrazine from serine and 2-oxopropanal.

In conclusion of this part of the discussion, the present results show that the use of this simple model system allowed the formation of most of the alkylpyrazines detected to be explained on the basis of reaction mechanisms that have been proposed in the past. The formation of 2,5(6)-dimethylpyrazine, the structure of which is independent of the amino acid involved, greatly exceeded the formation of other pyrazines. Amino acid side chains were rarely incorporated in pyrazine structures. To some extent the Strecker aldehydes or aldol condensation products were incorporated in the pyrazine skeleton at the intermediate dihydropyrazine stage. In addition to this, several other alkylpyrazines were detected for which specific hypothetical formation mechanisms could be formulated. However, the substitution pattern of all the pyrazines identified in this model system could not be completely predicted. For instance, the high amounts of the alkylpyrazines, such as trimethylpyrazine, formed from arginine are difficult to explain on the basis of its structural features. The high reactivity of arginine probably leads to accelerated degradation and condensation reactions, and as a result pyrazines are formed with substitution patterns that cannot be traced back to the amino acid side chain.

In comparison with the 1,3-dihydroxyacetone model reactions (in dry reaction conditions), 2-oxopropanal was heated in an aqueous solution with alanine (1 M, 90 °C, 30 min, pH 7). As by SPME-GC-MS analysis only low amounts of flavor compounds were detected, a solvent extraction of the reaction mixture was performed. In this solvent extract 2,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and some furanones were identified with an unknown compound as the main reaction

product. Whereas this unknown compound, with a relatively long retention (LRI 1495), was difficult to detect by SPME (probably due to the low volatility), it was the main constituent of the solvent extract.

Isolation of this compound by column chromatography followed by spectroscopic analysis led to the identification of the yellow-colored 2,5-diacetyl-3-methyl-1*H*-pyrrole, a compound that has not been identified before. The structural elucidation was based on a combination of ^{1}H NMR, which revealed the presence of three methyl groups, an amino group, and one additional aromatic proton, ^{13}C NMR, showing two conjugated acetyl functions, three aromatic signals, and one aliphatic one, and mass spectrometry. These data indicated the formation of a pyrrole, substituted with two acetyl groups in the α-position of the nitrogen, and one additional methyl group. For confirmation of the structural elucidation, the compound was synthesized on the basis of a known procedure for the synthesis of 2,5-diacetyl-1*H*-pyrrole (*21*). 3-Methyl-1*H*-pyrrole was reacted with an excess of 2-methyl-1,3-benzoxathiolium tetrafluoroborate as the acylating agent, followed by hydrolysis to obtain 2,5-diacetyl-3-methyl-1*H*-pyrrole. The GC retention and the spectral data of the synthesized 2,5-diacetyl-3-methyl-1*H*-pyrrole corresponded to the main compound isolated from the Maillard model reactions.

2,5-Diacetyl-3-methyl-1*H*-pyrrole was also identified in model reaction mixtures of 2-oxopropanal with other amino acids, in varying yields (**Table 2**). The α-amino acids not mentioned in **Table 2** did not yield detectable 2,5-diacetyl-3-methyl-1*H*-pyrrole concentrations. The high yields recorded upon reaction of aspartic and glutamic acid are probably due to a pH effect (see below), because in this case the buffer could not maintain the final pH at 7. Although the influence of the amino acid side chain on the yield of 2,5-diacetyl-3-methyl-1*H*-pyrrole was not studied in detail, these results indicate that the structure of the pyrrole formed does not depend on the amino acid side chain. A reaction mechanism was hypothesized as shown in **Scheme 3**. According to this reaction pathway, 2,5-diacetyl-3-methyl-1*H*-pyrrole **34** results from the condensation of one molecule of 1-amino-2-propanone **21** with two molecules of 2-oxopropanal **24**. A reduction step is required in the reaction sequence, but is plausible in Maillard reaction mixtures due to the presence of several reductones (*15, 22*). The reaction intermediate 2,5-diacetyl-3-hydroxy-4-methyl-1*H*-pyrrole **32** was tentatively identified (on the basis of its mass spectrum) in trace amounts, and its presence confirms the hypothesized reaction pathway. Considering this reaction mechanism, the formation of 2,5-diacetyl-3-methyl-1*H*-pyrrole occurs in competition with the formation of 2,5-dimethylpyrazine. An excess of 2-oxopropanal (ratio 2-oxopropanal:amino acid ≥ 3:1) combined with reducing conditions leads to the preferential formation of 2,5-diacetyl-3-methyl-1*H*-pyrrole. In **Table 3**, the influence of various reaction conditions on the yields (with respect to alanine) of 2,5(6)-dimethylpyrazine and 2,5-diacetyl-3-methyl-1*H*-pyrrole is shown. At 130 °C and pH 7 (phosphate or carbonate buffer), the yields of both compounds were very low. Performing the reaction in an acetate buffer at pH 4 increased the yields of 2,5(6)-dimethylpyrazine about twice, whereas the yields of 2,5-diacetyl-3-methyl-1*H*-pyrrole increased >30 times. Different steps in the reaction sequence can indeed be acid-catalyzed (elimination of water, nucleophilic addition). The yields of both compounds increased slightly with time between 10 and 60 min, although the difference between 30 and 60 min of reaction time was low. Increasing the reaction temperature from 100 to 130 °C had a more pronounced effect on the yield of 2,5(6)-dimethylpyrazine than on 2,5-diacetyl-3-methyl-1*H*-pyrrole. The increase in pyrazine formation with temperature has been shown repeatedly (see, e.g., ref *3*). Changing the ratio of alanine to 2-oxopropanal to 2:1 decreased the yields (with respect to alanine) of 2,5-diacetyl-3-methyl-1*H*-pyrrole and of 2,5(6)-dimethylpyrazine about 2-fold, indicating that the yields with respect to 2-oxopropanal remained more or less the same. A ratio of 1:3 of alanine to 2-oxopropanal, on the other hand, gave a considerably higher yield of 2,5-diacetyl-3-methyl-1*H*-pyrrole (about 3-fold), whereas the yield of 2,5(6)-dimethylpyrazine decreased. These results confirm the reaction mechanisms proposed and indicate that the ratio of the reagents in the reaction mixture determines the reaction outcome. 2,5-Diacetyl-3-methyl-1*H*-pyrrole will be preferentially formed in concentrated systems containing an excess of 2-oxopropanal under reducing conditions. No similar pyrroles with a different substitution pattern were detected when alanine was reacted with some other α-dicarbonyl compounds (glyoxal, phenylglyoxal, and 2,3-pentanedione).

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