Chemistry of 2-Acetyl-1-pyrroline, 6-Acetyl-1,2,3,4-tetrahydropyridine, 2-Acetyl-2-thiazoline, and 5-Acetyl-2,3-dihydro-4*H*-thiazine: Extraordinary Maillard Flavor Compounds

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1. Introduction

Flavor is one of the most important characteristics of a food product, especially when taking consumer acceptance as a parameter. At the origin of flavor in food products are enzymatic reactions of polyphenols, lipids, and proteins, but in processed food products, the Maillard reaction is of particular importance for flavor formation. Carbohydrate fragments, formed mainly by retro-aldol reactions, react with amino acid residues with the formation of, among other compounds, a wide variety of heterocyclic flavor compounds. During years of research, a vast quantity of Maillard flavor compounds has been identified, and among these, some very potent flavors were discovered. 2-Acetyl-1-pyrroline **1** and 6-acetyl-1,2,3,4-tetrahydropyridine **3** (which occurs in tautomeric equilibrium with 6-acetyl-2,3,4,5-tetrahydropyridine **2**) (Chart 1) are the most important flavor compounds of cooked rice and bread, and their flavor significance has been shown in many other food products. In particular, 2-acetyl-1-pyrroline and to a lesser extent 6-acetyl-1,2,3,4-tetrahydropyridine were detected as key aroma compounds in a large variety of cereal products and in food products of vegetable and animal origin. Numerous model experiments revealed a fascinating and peculiar chemistry of formation for these flavor compounds. The development of adequate synthetic routes was a challenge for many organic chemists. The extraordinary flavor properties of these compounds are related with the α-iminoketone or α-acylenamine structural element as part of a ring system. The sulfur-containing analogues 2-acetyl-2-thiazoline **4** and 5-acetyl-2,3-dihydro-4*H*-thiazine **5** exhibit similar popcorn-like flavor properties. A review of the chemistry of these important flavor compounds, their occurrence and significance, the mechanisms of formation, their biological origin, the developed synthetic routes, and their applications in the food industry is presented here.

2. Occurrence and Significance

2.1. Sources of 2-Acetyl-1-pyrroline

2-Acetyl-1-pyrroline **1** [2-AP; IUPAC name 5-acetyl-3,4-dihydro-2*H*-pyrrole; also 1-(3,4-dihydro-2*H*-pyrrol-5-yl)-ethanone] was identified for the first time as the most important flavor compound of cooked rice.[1] Since then, the flavor compound has been identified in a great variety of processed and cooked food products, especially in various rice varieties, both fragrant and nonfragrant varieties.[2] The content of 2-AP was shown to be positively correlated with

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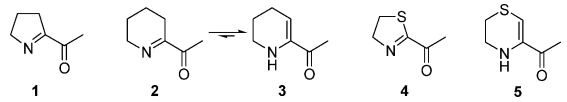
Norbert De Kimpe obtained his diploma of chemical agricultural engineer in 1971, Ph.D. degree in 1975, and habilitation degree in 1985, all from Ghent University, Ghent (Belgium). He performed postdoctoral research work at the University of Massachusetts, Harbor Campus, at Boston (1979) and at the Centre National de Recherche Scientifique (CNRS) in Thiais, Paris (France) (1983), where he worked on unstable nitrogen-substituted sulfenyl derivatives and electron-deficient carbenium ions, respectively. He made his scientific career at the Belgian National Fund for Scientific Research, where he went through all stages up to the position of Research Director. During this career he was affiliated with the Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences at Ghent University, where he took up teaching positions since 1987. He is now Professor in Organic Chemistry at the latter institution. He was a guest professor at the Centre Universitaire de Recherche sur la Pharmacopée et la Médecine Traditionelle in Butare (Rwanda, Central Africa) and the Universities of Perpignan (France), Helsinki (Finland), Leuven (Belgium), Siena (Italy), Barcelona (Spain), Sofia (Bulgaria), Buenos Aires (Argentina), and Pretoria (South Africa). He was awarded the degree of Doctor honoris causa from the Russian Academy of Sciences in Novosibirsk (Russia) in 1998. He is the author of 378 articles in international peer-reviewed journals. His research interests include (1) the synthesis of heterocyclic compounds, with focus on agrochemicals, pharmaceuticals, and natural products, (2) flavor chemistry, and (3) the bioassay-guided isolation of physiologically active natural products from medicinal plants.

desirable rice flavor characteristics.[3] 2-Acetyl-1-pyrroline appeared to be the most important discriminator, on quantitative and olfactive considerations, to differentiate between

Chart 1



scented and nonscented rice.[4] Besides in cooked rice, 2-AP has also been identified among the volatiles of various other cooked cereals and cereal products: bread crust,[5] toasted bread,[6] corn tortillas,[7] popcorn,[8] cooked sweet corn products,[9] extrusion cooked maize flour,[10] rice cakes,[11] puff pastries,[12] and cooked acha (a cereal indigenous to the Sahel region in Africa).[13] In all cases, 2-AP, though mostly present in low concentrations, contributes in great measure to the cereal odor notes of the food products. 2-Acetyl-1-pyrroline was shown to be a potent odorant of boiled potatoes,[14] is one of the key aroma compounds in roasted wild mango seeds,[15] roasted sesame seeds,[16] pan-fired green teas,[17] cured tobacco leaves,[18] boiled or fried mung bean,[19] and taro (a tropical root crop),[20] and is formed during the heating of bacuri and cupuaçu fruit pulp.[21,22] It was detected in pale lager beer,[23] peanut and pumpkin seed oils,[24] honey,[25] several soy-based products,[26] and among the volatiles of cooked mushrooms (*Pleurotus* sp.) (mistakenly identified as 2-acetyl-2-pyrroline).[27] Also, products of nonvegetable origin contained 2-AP as a highly aroma-active constituent: dry and fresh milk, [28,29] Camembert and Swiss Gruyere cheese,[30,31] rennet casein,[32] liquid Cheddar whey,[33] Iberian dry-cured ham,[34,35] Mediterranean dried sausages,[36] cooked beef,[37] boiled carp fillet,[38] boiled salmon, cod and trout,[39,40] cooked blue crab claw meat,[41] cooked lobster tail meat,[42,43] boiled prawns,[44] ripened anchovy,[45] crayfish-processing byproducts,[46] tuna sauce,[47] stored sardines,[48] and enzyme-hydrolyzed oyster cooker effluent.[49]

These findings led to the assumption that 2-AP is a resultant of the Maillard reaction and that it is formed during the cooking or processing of rice and other food products. This thermal formation, together with the instability of the compound, complicated the development of adequate analytical procedures. The determination of important but low-concentrated volatiles depends greatly on the development of sensitive analytical techniques. To obtain truthful quantitative determinations of 2-AP concentrations in food products, care must be taken to avoid losses due to the instability of the compound on one hand and not to overestimate the 2-AP quantities by additional formation upon heat treatment of precursors on the other hand.

Buttery and co-workers determined the 2-AP concentration in 10 different rice varieties.[2a] This was done by steam distillation−continuous extraction of uncooked rice and resulted in concentrations of 6−90 ppb, expressed relative to the dry weight of the rice. Within the same variety, brown rice contained somewhat higher 2-AP concentrations than white rice, indicating that the degree of milling influences the 2-AP content. Using an acid-phase solvent extraction, the effect of other interfering compounds could be lessened.[2b] Still, a recovery of only 28% 2-AP was reached. Taking this into account and making use of an internal standard, 2-AP concentrations for brown aromatic rice varieties of 560−760 ppb were calculated. Different research groups refined the analytical method for the determination of 2-AP. Following extraction of 2-AP from rice by microscale steam distillation−solvent extraction, GC-MS analysis was performed in the more sensitive SIM (selected ion monitoring) mode. This led to experimental concentrations of 76−156 ppb of 2-AP in rice.[2e] Solid-phase microextraction (SPME)

was used for the screening of several rice varieties for the presence of 2-AP. SPME-GC-MS was proven to be a very useful technique for the screening and qualitative differentiation among rice varieties but not for the quantification of 2-AP.[2h] Schieberle and co-workers developed stable isotope dilution assays for the quantification of 2-AP in bread crust[5] and in freshly popped corn.[50] In wheat bread crust the concentration of 2-AP was determined to be 78 μg/kg, while in freshly popped corn 24 μg 2-AP/kg was found. In other heated food products, 2-AP concentrations in the range of 1−10 ppb were found.[7,9,11,38] Calculation of the so-called flavor dilution factor by aroma extract dilution analysis in different food products revealed the extraordinary importance of 2-AP in the aroma of a variety of food products.[2i,38,42]

In later investigations, however, 2-acetyl-1-pyrroline also revealed its presence in raw plant material. Buttery and coworkers already noticed that the characteristic 2-AP odor was present in uncooked rice, but they could not confirm this by extraction.[2a] Mahatheeranont et al. quantified 2-AP in uncooked Thai rice (0.3 ppm) using a nonthermal solvent extraction method.[51] Later, concentrations of 2-AP in fresh uncooked brown aromatic rice (KDML 105) were found to be as high as 3 ppm.[52] Yoshihashi used a stable isotope dilution assay to quantify 2-AP in aromatic rice under different conditions and in various parts of the plant.[53] The results revealed that 2-AP was not formed during the cooking or postharvest processes of aromatic rice and confirmed the biological origin of 2-AP.

Also, in Pandan leaves (*Pandanus amaryllifolius*),[54] raw Myabi muskmelon fruit,[55] and chempedak fruit and jackfruit,[56] 2-AP was identified. 2-Acetyl-1-pyrroline was detected among the volatiles of dry spinach[57] and raw French beans.[58] In both cases, the concentration of 2-AP and as a result its importance in the overall aroma increased upon cooking of the vegetables. The dried flowers of the plant *Vallaris glabra* (so-called 'bread flowers') contain the highest reported concentrations of 2-acetyl-1-pyrroline, namely, 26 ppm.[59] In Pandan leaves, which are traditionally added during cooking of common rice to impart a scented rice aroma, 2-AP is present at a concentration of 1 ppm, which is more than 10 times higher than the 2-AP concentration in scented rice varieties.[54] Other sources report concentrations of 10 ppm 2-AP in fresh *Pandanus amaryllifolius* leaves.

In contrast to its mostly desired presence, 2-AP was shown to be responsible for a 'mousy' off-flavor that developed when raw pearl millet grits were wetted and slowly dried.[60] 2-Acetyl-1-pyrroline was also identified as one of the components responsible for the development of a 'mousy' off-flavor in wine.[61] Its presence there was shown to be of microbial origin and will be discussed below (section 4.2).

Confusing reference has been made in the literature to the presence of 2-AP among the volatiles of the urine of tigers, important for territorial and sexual statements. However, this does not concern 2-AP itself but another unidentified rice flavor compound.[62]

2.2. Sources of 6-Acetyl-1,2,3,4-tetrahydropyridine

Already in 1963, Wiseblatt and Zoumut isolated a substance with a bread-like flavor after boiling of fermented liquid brews, containing only glucose and yeast in an inorganic aqueous buffer solution.[63] A structure identification was, however, only published in 1969, when Hunter et al.[64] isolated the bread flavor compound from a modified Wiseblatt reaction of proline with 1,3-dihydroxyacetone in the presence of sodium bisulfite and identified it as 6-acetyl-1,2,3,4-tetrahydropyridine **3** (occurring in tautomeric equilibrium with 6-acetyl-2,3,4,5-tetrahydropyridine **2**) (Chart 1). Regular mistakes are found concerning the name of this compound. According to the IUPAC nomenclature rules, compound **2** is named 1-(3,4,5,6-tetrahydropyridin-2-yl)-ethanone and compound **3** is named 1-(1,4,5,6-tetrahydropyridin-2-yl)ethanone.

Table 1. Concentrations (conc) and Odor Activity Values (OAV) of 2-Acetyl-1-pyrroline 1 and 6-Acetyl-1,2,3,4-tetrahydropyridine 3 in Different Food Products[104] [a]

	2-AP		6-ATHP	
food product	conc (μg/kg)	OAV	conc (μg/kg)	OAV
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		
basmati rice	610	83516		
cooked sweet corn	44	6027		

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6-Acetyl-1,2,3,4-tetrahydropyridine has a typical roasty odor, resembling the flavor of crackers and popcorn, and is regarded nowadays as a very important Maillard flavor compound. Spraying week-old bread with an aqueous solution containing only 6 ppm of the sodium bisulfite complex of this compound returned a desirable fresh-bread odor to the product.[64] 6-Acetyl-1,2,3,4-tetrahydropyridine (6-ATHP) contributes to the aroma of several baked products: potato chips,[65] bread crust,[66] popcorn,[8] corn tortillas,[67] toast,[6] and rice cakes.[68] Both 2-AP and 6-ATHP contribute significantly to the flavor of bread crust, although 2-AP has the highest odor unit in wheat bread crust and 6-ATHP dominates in rye bread crust.[66] Both flavor compounds are found in breadcrumbs in 30-fold lower concentrations than in the crust. This is due to the lower water activity in the outside crust, stimulating Maillard reactions. 6-Acetyl-1,2,3,4-tetrahydropyridine was also detected in wort and beer, food products containing high concentrations of proline.[69] Analysis of the volatile constituents of *Semnostachya menglaensis* Tsui, a rare Chinese odorous plant, revealed the presence of 6-acetyl-1,2,3,4-tetrahydropyridine **3** and 6-acetyl-2,3,4,5-tetrahydropyridine **2**.[70] The main volatiles, however, were the higher homologues of these aroma compounds, 6-propionyl-1,2,3,4-tetrahydropyridine and 6-propionyl-2,3,4,5-tetrahydropyridine.

In Table 1 a comparison is presented of the concentrations and odor activity values of 2-AP and 6-ATHP in various food products.

2.3. 2-Acetyl-2-thiazoline and 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine, Sulfur-Containing Popcorn-like Flavor Compounds

Sulfur-containing flavor compounds constitute a major class of aroma volatiles found in vegetables, cooked meat, and other processed foods. While the sulfur-containing volatiles in vegetables such as *Allium* species are formed by enzymatic reactions,[71] sulfur-containing flavors found in meat products are normally formed through thermal processing of food systems containing cysteine or cystine. The sulfur-containing analogues of 2-AP and 6-ATHP, 2-acetyl-2-thiazoline **4** (2-AT) and 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** (5-ADHT) (Chart 1), display similar roasty, popcorn-like odor characteristics and low odor thresholds.

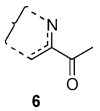
2-Acetyl-2-thiazoline **4** was identified for the first time among the flavor volatiles of beef broth[72] and was later reported as one of the character-impact flavor compounds of roasted beef.[73] In beef, roasted for a short time, concentration levels of 2-acetyl-2-thiazoline **4** of 14−28 μg/kg were determined using stable isotope dilution assays.[74] 2-Acetyl-2-thiazoline was detected as a potent odorant of several processed meat products, such as chicken broth,[75] cooked chicken,[76] stewed beef juice,[77] and cooked meat patties.[78] In addition, the flavor compound was detected among the aroma volatiles of boiled trout, [40,79] cooked mussels,[80,81] turbot,[82] boiled carp fillet,[83] cooked clams,[84] fresh goat cheese,[85] Cheddar cheese,[86,87] ice cream,[88] milk,[28,89] pan-fired green tea,[17] roasted white sesame seeds,[16] wine,[90] rambutan fruit,[91] lychee fruit,[92] cooked mushrooms (*Pleurotus* sp.),[27] sweet corn products,[9] and heated yeast extracts.[93]

5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** was initially identified from a model reaction of ribose with cysteine.[94] This compound has not yet been identified in food systems.

2.4. Flavor Properties

On the basis of their organoleptic properties, so-called 'α-acetyl-*N*-heterocycles' are considered a separate class of Maillard reaction products.[95] They generally have low to very low odor thresholds and a pleasant roasty cracker-like odor, which is attributed to the α-iminoketone or α-acylenamine structural element as part of a ring system (Chart 2).[96]

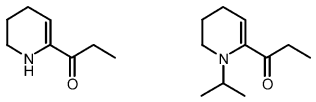
Chart 2



6

This structural requirement does not apply to *N*-alkylated α-acetyl cyclic enamines, which do not show the roasty flavor characteristics at all.[97] For instance, 6-propionyl-1,2,3,4-tetrahydropyridine **7** displays Maillard flavor characteristics, while 1-isopropyl-6-propionyl-1,2,3,4-tetrahydropyridine **8** does not (Chart 3).[97]

Chart 3



7 **8**

Because of these properties and since these 2-acetylazaheterocycles are generally formed in higher concentrations than other acylazaheterocycles, they are of utmost importance in the flavor of heated food products and in process flavorings.

Within this group of flavor compounds, many publications have focused on 2-AP **1** and 6-ATHP **3** because of their significance and extremely low odor thresholds. These odor thresholds were determined in different media, as reported in Table 2.

Table 2. Odor Thresholds of 2-Acetyl-1-pyrroline 1 and 6-Acetyl-1,2,3,4-tetrahydropyridine 3 in Different Media

	2-AP	6-ATHP
air	0.02 ng/L[8]	0.06 ng/L[8]
water	0.1 μg/kg[2]	1.0 μg/kg[67]/1.6 μg/kg[6]
starch	0.0073 μg/kg[6]	0.054 μg/kg[6]
sunflower oil	0.1 μg/kg[30]	

The presence of a longer alkyl chain or an aromatic ring system significantly increases the odor threshold of comparable 2-acetylazaheterocycles (Tables 3 and 4). This is clearly demonstrated in the odor threshold of 2-acetyl-1*H*-pyrrole **8**, which does not smell roasty and has an odor threshold of >2000 ng/L air. 2-Propionyl-1-pyrroline **12**, with an alkyl chain of one carbon atom more than 2-acetyl-1-pyrroline **1**, has a low odor threshold similar to 2-acetyl-1-pyrroline **1** and a roasty, popcorn-like odor. Higher homologues of 2-propionyl-1-pyrroline **12**, however, such as 2-butanoyl-1-pyrroline **13** and 2-hexanoyl-1-pyrroline **14**, do not smell roasty and possess very high odor thresholds.[8] The same accounts for the higher homologues of 5-acetyl-2,3-dihydro-4*H*-thiazine.[98] 2-Propionyl-2-thiazoline has a similar low odor threshold (0.07 ng/L air) as 2-acetyl-2-thiazoline, but higher homologues of this compound have not been synthesized.

3. Mechanism of Formation

3.1. Studies on the Mechanism of Formation of 2-Acetyl-1-pyrroline

Tressl et al. reported that small amounts of 2-AP were formed when model mixtures of proline and monosaccharides were heated.[99] Schieberle showed that heat treatment of a ground yeast/sucrose mixture represented an important source of 2-AP formed during the bread baking process.[100] Precursor studies demonstrated the formation of 2-AP in heated model systems of proline with sugars and sugar degradation products and especially their phosphorylated derivatives. Conversion of the latter activated derivatives, such as 1,3-dihydroxyacetone phosphate, into higher amounts of 2-AP holds a mechanistic rationale (vide infra).

Experiments with labeled carbohydrates indicated that in 2-AP **1**, generated from proline and U-^{13}C-glucose, the label was present only in both carbons of the acetyl group.[100] From the reaction of proline and 1-^{13}C-glucose, Rewicki et al. detected a 1:1 mixture of unlabeled and singly labeled 2-AP (with the label on the methyl group).[101] From these results, they proposed a mechanism as is displayed in Scheme 1. From 1-deoxy-2,3-glucosone, two isomers of 'diacetyl-formoin' (**18** and **19**) are derived with two equivalent sites of reactivity. Aldol addition of 1-pyrroline **22**, a degradation product of proline, to the dihydro form of diacetyl-formoin (**20** and **21**) is followed by retro-aldol cleavage, yielding 2-acetylpyrrolidine **26** that oxidizes to 2-acetyl-1-pyrroline **1** with the expected extent and position of labeling.

Labeling of proline demonstrated that the carbon of the carboxyl group of proline was absent in 2-AP.[100] These findings as well support the hypothesis that 2-AP in thermally degraded proline/glucose mixtures is formed by 'acylation' of 1-pyrroline by a two-carbon sugar fragment, among which 2-oxoaldehydes were shown to be the most effective.

Various model experiments revealed that phosphate ions are essential in the realization of high yields since replacement of a phosphate buffer by a malonate buffer decreased the yields of model reactions to about one-third.[50] Triose phosphates occurring in yeast were identified as 2-AP precursors.[100] The high yield obtained from the reaction of 1,3-dihydroxyacetone phosphate **27** with proline[100] can be explained by the generation of 2-oxopropanal **30**, which is the actual active sugar fragment in the formation of 2-AP **1** (Scheme 2). The enol 1,3-dihydroxyacetone phosphate, i.e., compound **28**, is able to expel phosphate as a leaving group much better than the analogous hydroxyl group of 1,3-

Table 3. Odor Threshold Values of Various 2-Acetylazaheterocycles[8,65,74,94]

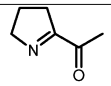
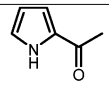
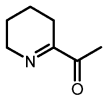
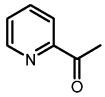
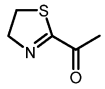
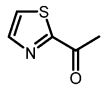
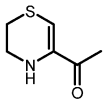
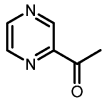
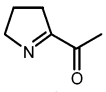
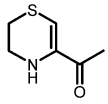
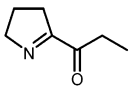
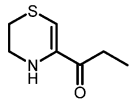
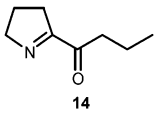
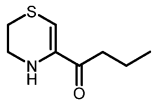
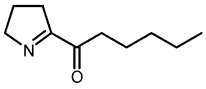
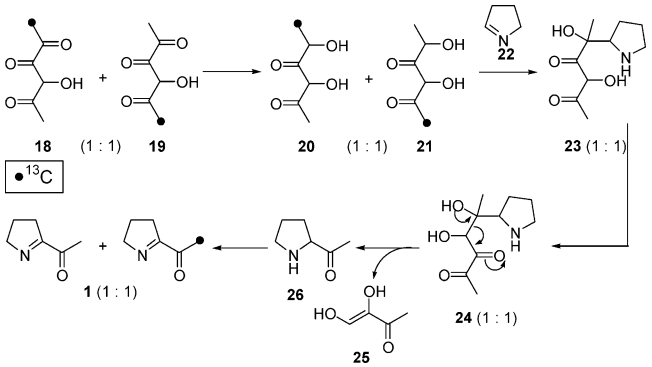
Flavor compound	Odor threshold (μg/L H_2O)	Aromatic flavor compound	Odor threshold (μg/L H_2O)
 **1**	0.1	 **9**	170,000
 **3**	1.6	 **10**	19
 **4**	1	 **11**	10
 **5**	1.7	 **12**	62

Table 4. Odor Threshold Values of 2-Acylazaheterocycles with Varying Alkyl Chain Lengths[8,98]

2-Acyl-1-pyrroline homologues	Odor threshold (ng/L air)	5-Acyl-2,3-dihydro-4*H*-thiazine homologues	Odor threshold (ng/L air)
 **1**	0.02	 **5**	0.05
 **13**	0.02	 **16**	0.1
 **14**	> 2,000	 **17**	> 2,000
 **15**	> 2,000		

Scheme 1. Hypothetical Formation of a 1:1 Mixture of 2-Acetyl-1-pyrroline/2-[2-^{13}C]acetyl-1-pyrroline from [1-^{13}C]-Glucose and l-Proline, on the basis of Labeling Studies[101]

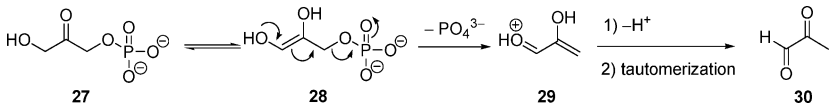


dihydroxyacetone. In this way, the protonated enol structure **29** is generated which affords the reactive sugar degradation compound **30**.

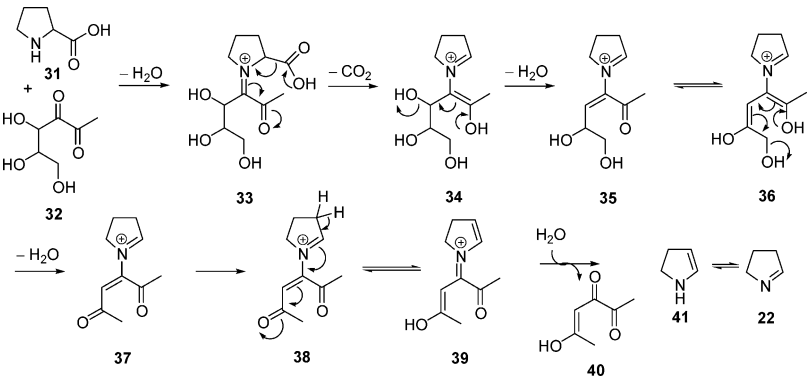
When reacting equimolar amounts of 1-pyrroline **22** and 2-oxopropanal **30** in an aqueous buffer solution, yields of 2-AP **1** of 5 mol % were accomplished.[104] 2-Acetyl-1-pyrroline constituted 72% of the volatile fraction in 1-pyrroline/2-oxopropanal model reactions.[100] The reaction of 1-pyrroline **22** with two other α-oxoaldehydes, namely, 1,2-butanedione and phenylglyoxal, yielded 2-propionyl-1-pyrroline **13** and 2-benzoyl-1-pyrroline in yields comparable with the formation of 2-AP **1** from 2-oxopropanal **30**.[50] These results confirm that 1-pyrroline **22** and 2-oxopropanal **30** are most probably the active reagents in the formation of 2-AP **1**.

To determine the origin of 1-pyrroline in bread crust, Schieberle and co-workers separately reacted the most important amino acids of yeast with 2-oxopropanal in model experiments. The results revealed that 2-AP was formed from proline and ornithine in comparable amounts.[102] Both amino acids are able to form 1-pyrroline **22**, the key intermediate in the formation of 2-AP **1**. 1-Pyrroline **22** results from the Strecker degradation of proline **31**, catalyzed by α-dicarbonyl

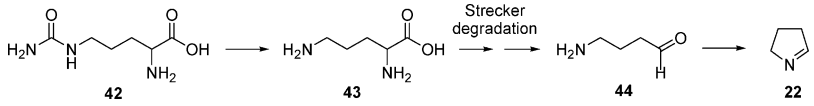
Scheme 2. Generation of 2-Oxopropanal 30 from 1,3-Dihydroxyacetone Phosphate 27



Scheme 3. Hypothetical Mechanism of Formation Leading from Proline 31 and 1-Deoxyosone 32 to 1-Pyrroline 22[50]



Scheme 4. Formation of 1-Pyrroline 22 from Citrulline 42 and Ornithine 43[102]



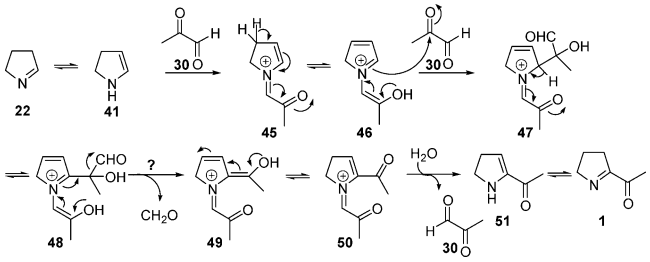
compounds, such as 2-oxopropanal **30** or a deoxyosone, e.g., 1-deoxyosone **32**, an intermediate generated from the dehydration of fructose (Scheme 3).[50] The reaction starts with the formation of iminium ion **33**, followed by decarboxylation and water elimination leading to intermediate **35**. A second water molecule is eliminated in a retro-Michael reaction, yielding intermediate **37**, from which 1-pyrroline **22** can be generated via hydrolysis of iminium ion **39**.

When the nucleophilic attack of proline **31** is aimed at the carbonyl group of carbon-2 of 1-deoxyosone **32** instead of carbon-3, a similar pathway can be constructed for the formation of 1-pyrroline **22**.

Citrulline **42** and ornithine **43**, however, are also possible precursors of 1-pyrroline **22** through cyclization of 4-aminobutanal **44** via a Strecker degradation protocol (Scheme 4).[102]

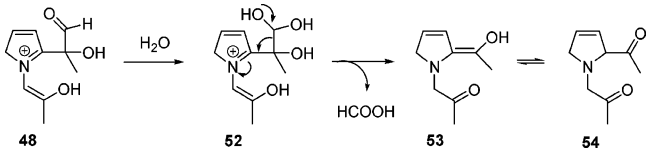
From these results, a reaction mechanism was proposed for the formation of 2-acetyl-1-pyrroline **1**, initiated by the formation of an iminium species between the tautomer 2-pyrroline **41** and 2-oxopropanal **30** (Scheme 5).[50] Tautomerism and subsequent nucleophilic attack of intermediate **46** to a second molecule of 2-oxopropanal **30** provides iminium ion **47**, which, upon deformylation according to the authors, is hydrolyzed into 2-oxopropanal **30** and 2-acetyl-2-pyrroline **51** that tautomerizes into 2-acetyl-1-pyrroline **1**.

Scheme 5. Hypothetical Mechanism of Formation of 2-Acetyl-1-pyrroline 1 from 1-Pyrroline 22 and 2-Oxopropanal 30 According to Schieberle[50]



Several questions arise concerning this reaction pathway. First, nucleophilic attack of the intermediate **46** may as well be aimed at carbon-1 of 2-oxopropanal **30**, and the products of this pathway are not described. Second, elimination of the formyl group cannot occur as described because the formyl group is not a leaving group. Alternatively, deformylation could occur by attack of water onto the formyl group of intermediate **48** and subsequent transformation into, for instance, compound **54**, as shown in Scheme 6.

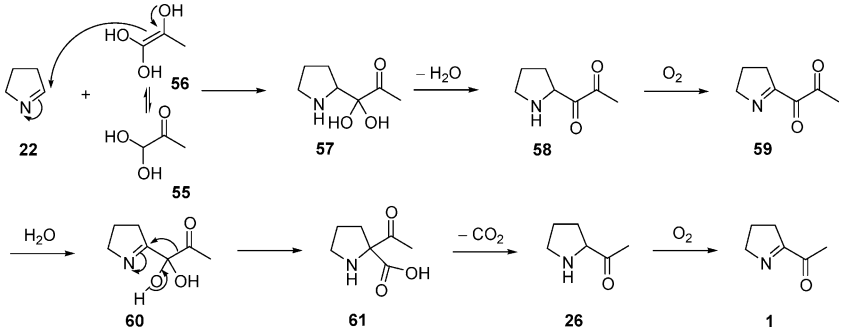
Scheme 6. Alternative to the Deformylation of Intermediate 48 (Scheme 5) Leading to Compound 54



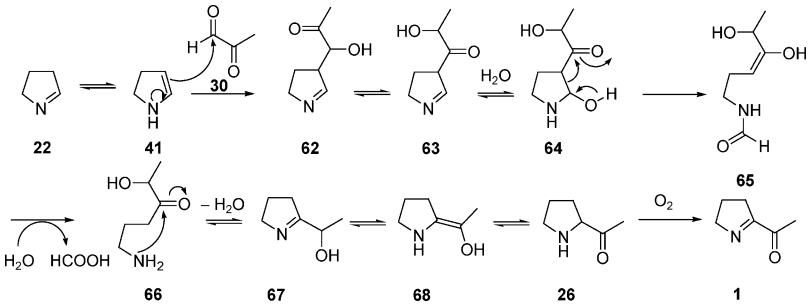
Since higher amounts of 2-AP **1** were formed from the reaction of 2-oxopropanal **30** and 1-pyrroline **22** under aqueous conditions, the hydrated 2-oxopropanal **55** was proposed as the reactive species. In addition, it was shown that 2-acetylpyrrolidine **26** could be easily oxidized to 2-AP **1** in high yields.[103] From these findings, a new reaction mechanism was proposed (Scheme 7).[104] 1-Pyrroline **22** condenses with hydrated 2-oxopropanal **56** to generate 2-(1,2-dioxopropyl)-pyrrolidine **57**, which spontaneously oxidizes with air oxygen to the corresponding 1-pyrroline **59**, the latter undergoing an addition of water to the central reactive carbonyl function (hydrate formation) and subsequent semibenzilic rearrangement. The β-ketoacid **61** thus formed decarboxylates to 2-acetylpyrrolidine **26**, affording 2-acetyl-1-pyrroline **1** upon spontaneous air oxidation.

Labeling experiments showed that 2-AP mainly incorporated two carbon atoms from glucose, as discussed above, but a minor amount of 2-AP incorporated three carbon atoms from glucose. The finding that 2-AP was equally formed from 2-methyl-1-pyrroline, though in somewhat lower

Scheme 7. Proposed Reaction Mechanism for the Formation of 2-Acetyl-1-pyrroline 1 from 1-Pyrroline 22 and 2-Oxopropanal Hydrate 55 on the basis of Labeling Experiments and the Oxidation of 2-Acetylpyrrolidine 26[104]



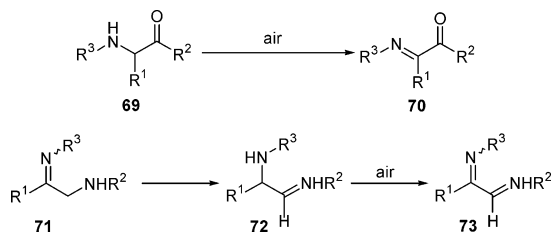
Scheme 8. Alternative Reaction Pathway Leading from 1-Pyrroline 22 and 2-Oxopropanal 30 to 2-Acetyl-1-pyrroline 1 on the basis of Labeling Experiments[104]



amounts, suggests that the carbon-2 of 1-pyrroline can be lost during the reaction with 2-oxopropanal. This could be explained in an alternative reaction pathway for the formation of 2-AP **1** from the reaction of 1-pyrroline **22** with 2-oxopropanal **30** (Scheme 8). This pathway starts with a nucleophilic attack of the tautomeric 2-pyrroline **41** at carbon-1 of 2-oxopropanal **30**. Addition of water to the imine **63** formed, followed by ring opening and subsequent hydrolysis of the generated *N*-substituted formamide **65**, yields 6-amino-2-hydroxy-3-hexanone **66**. Upon cyclization 2-(1-hydroxyethyl)-1-pyrroline **67** is formed. This compound tautomerizes to 2-acetyl-pyrrolidine **26** via 1-(2-pyrrolidinylidene)ethanol **68**.[104] Spontaneous air oxidation yields 2-acetyl-1-pyrroline **1**.

Both pathways establish 2-acetylpyrrolidine **26** as the direct precursor of 2-acetyl-1-pyrroline **1** that is formed by spontaneous oxidation. The air oxidation of 2-acetylpyrrolidine **26** to 2-acetyl-1-pyrroline **1** is similar to the known oxidation of α-aminoketones **69** and α-aminoimines **71** to α-iminoketones **70** and α-diimines **73**, respectively,[105] and can be extended as shown in Scheme 9. There is proven

Scheme 9. Air Oxidation of α-Aminoketones 69 and α-Aminoimines 71



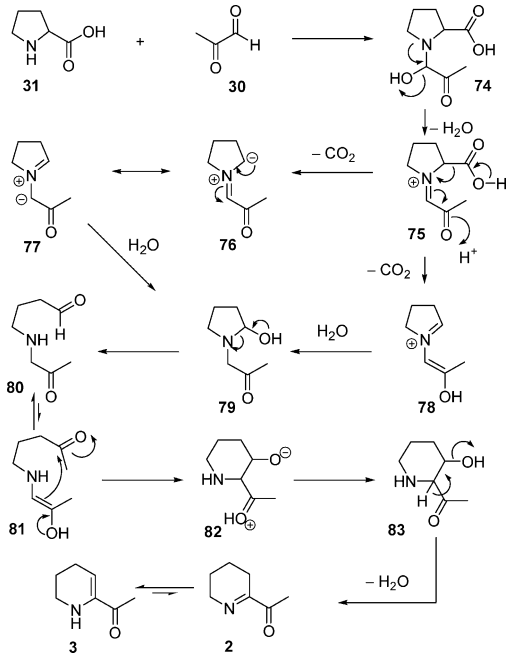
evidence that α-aminoketimines **71** tautomerize first to α-aminoaldimines **72**, which are further susceptible to oxidation to α-diimines **73**. Tautomers **72** can be isolated as end products when the reaction is performed under nitrogen atmosphere.

3.2. Studies on the Mechanism of Formation of 6-Acetyl-1,2,3,4-tetrahydropyridine

6-Acetyl-1,2,3,4-tetrahydropyridine **3** was first isolated from the model reaction of proline with 1,3-dihydroxyacetone in dry reaction conditions.[64] This model reaction was later studied in detail and yielded 2.7 mol % of 6-ATHP in optimal reaction conditions.[106] The yield in water solution was significantly lower than in dry reaction conditions. Addition of sodium bisulfite to the reaction mixture significantly increased the yield of 6-ATHP, ascribed to a stabilizing effect. Various other model experiments of proline with sugars or their degradation products yielded 6-ATHP.[69] Yields are generally quite low, but due to the low odor threshold, an intense flavor develops during the reaction. Unlike 2-acetyl-1-pyrroline, which is formed from proline or ornithine when reacted with the sugar degradation product 2-oxopropanal, 6-ATHP is formed exclusively from proline and preferentially from fructose as compared to glucose or 2-oxopropanal.[50]

For the formation of 6-acetyl-1,2,3,4-tetrahydropyridine **3**, the so-called Hodge mechanism has long served as the standard mechanism.[107] This mechanism is described in numerous textbooks in which it remained unquestioned for several decades. It was proposed in 1972 and starts with the nucleophilic addition of the proline **31** nitrogen atom at carbon-1 of 2-oxopropanal **30** as displayed in Scheme 10. Elimination of water from adduct **74** yields iminium species **75**. Decarboxylation results into the azomethin ylide **76**, which is in mesomeric equilibrium with the resonance form **77**. Addition of water to **77** affords unstable adduct **79** that ring opens to *N*-2-oxopropyl-4-aminobutanal **80**. These last steps may also be represented as a concerted process for the conversion of **75** into **79** via **78**. Intramolecular aldol-type

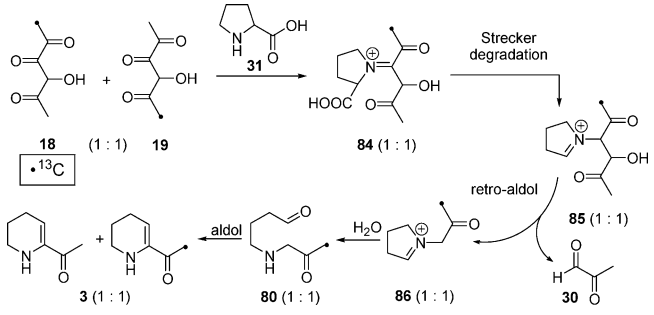
Scheme 10. Hypothetical Mechanism of Formation of 6-Acetyl-1,2,3,4-tetrahydropyridine 3 Proposed by Hodge et al.[107]



condensation of keto aldehyde **80** affords intermediate **73**, from which 6-acetyl-1,2,3,4-tetrahydropyridine **3** is formed by elimination of water.

Experiments performed by Rewicki and co-workers with labeled U-^{13}C-glucose and proline indicated the presence of three carbon labels in 6-ATHP, two in the acetyl group and one labeled carbon atom in the ring.[108] When proline was reacted with 1-^{13}C-glucose, a mixture of unlabeled 6-ATHP (60%) and singly labeled 6-ATHP with the label on the methyl group was obtained.[101] The authors hypothesized a reaction pathway similar to the pathway for 2-AP described in Scheme 1, starting from two equivalent isomers of 'diacetyl-formoin' (**18** and **19**), which react with proline **31** with the formation of compound **84** (Scheme 11). Strecker

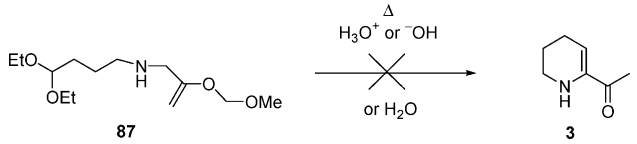
Scheme 11. Formation of a 1:1 Mixture of 6-Acetyl/6-[6-^{13}C]acetyl-1,2,3,4-tetrahydropyridine from [1-^{13}C]-Glucose and Proline on the basis of Labeling Studies[101]



degradation and retro-aldol cleavage lead to compound **86**, which undergoes hydrolytic ring opening to *N*-2-oxopropyl-4-aminobutanal **80**, yielding 6-ATHP **3** upon cyclization. This mechanism is similar to the Hodge mechanism presented in Scheme 10, and both mechanisms proceed via the common intermediate *N*-2-oxopropyl-4-aminobutanal **80**.

To investigate the intermediacy of this labile *N*-2-oxopropyl-4-aminobutanal **80** a doubly protected form of this compound **87** was synthesized. This diprotected *N*-2-oxopropyl-4-aminobutanal **87** was then subjected to a broad variety of hydrolytic conditions (acidic, basic, neutral, and combinations thereof), which are known to yield the free aldehyde and amine, but in the complex reaction mixtures obtained there was no formation of even the slightest trace of 6-ATHP **3** (Scheme 12).[109] Accordingly, it was concluded

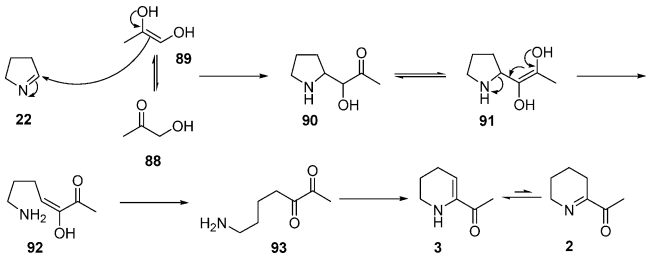
Scheme 12. Failure of the Diprotected *N*-2-oxopropyl-4-aminobutanal 87 to Yield 6-ATHP 3 under Diverse Hydrolytic Conditions[109]



that the so-called Hodge mechanism is most probably not operative.

Reaction of 1-pyrroline **22** and 1-hydroxy-2-propanone **88**, both Strecker degradation products of the reaction of proline **31** with an α-dicarbonyl compound, yielded significant amounts of 6-ATHP under basic conditions. From these findings, a different mechanism was proposed for the formation of 6-acetyl-1,2,3,4-tetrahydropyridine **3**, starting from 1-pyrroline **22** and 1-hydroxy-2-propanone **88** (Scheme 13).[104] The pathway starts with the attack of carbon-1 of

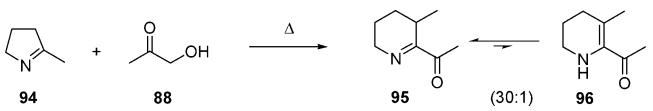
Scheme 13. Proposed Reaction Mechanism for the Formation of 6-Acetyl-1,2,3,4-tetrahydropyridine 3 from the Condensation of 1-Pyrroline 22 and 1-Hydroxy-2-propanone 88 on the basis of the Synthesis of Intermediate 90[104]



enolized 1-hydroxy-2-propanone **89** at carbon-2 of 1-pyrroline **22**. This step might explain the preferred formation of 6-ATHP at higher pH values. The intermediate 2-(1-hydroxy-2-oxopropyl)pyrrolidine **90** thus formed was synthesized and shown to yield 6-ATHP **3** upon heating.[104] The ring enlargement proceeds via ring opening to 7-aminoheptane-2,3-dione **93**, which finally cyclizes to 6-ATHP **3**. Such cyclizations have been used later in syntheses of this Maillard compound (cf. section 5).

Performing the reaction of 1-hydroxy-2-propanone **88** with 2-methyl-1-pyrroline **94** instead of 1-pyrroline **22** confirmed this pathway, since ring enlargement yielded 2-acetyl-3-methyl-3,4,5,6-tetrahydropyridine **95** (Scheme 14). This

Scheme 14. Formation of 2-Acetyl-3-methyl-3,4,5,6-tetrahydropyridine 95 from the Reaction of 2-Methyl-1-pyrroline 94 with 1-Hydroxy-2-propanone 88[103]



compound showed the same popcorn-like odor at a similar low odor threshold as 6-ATHP, but the tautomeric equilib-

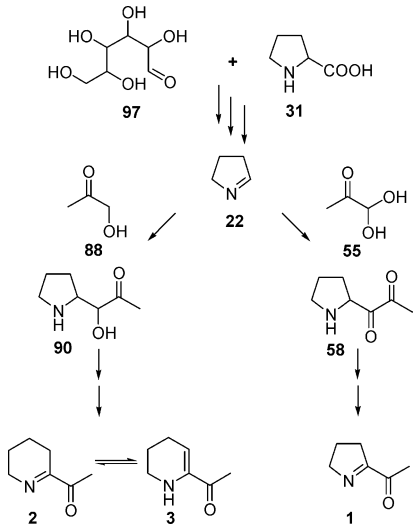
rium was shifted strongly to the imine tautomer **95**, stabilized by the electron-donating effect of the methyl group.

A pathway for the formation of 6-ATHP from lysine via 2,3,4,5-tetrahydropyridine, analogous to the formation of 2-AP from ornithine via 1-pyrroline, could not be established.[102] Possibly, the intermediate 2,3,4,5-tetrahydropyridine is not stable and rapidly trimerizes.[144]

3.3. Common Reaction Pathway for the Formation of 2-Acetyl-1-pyrroline and 6-Acetyl-1,2,3,4-tetrahydropyridine

Both flavor compounds 2-acetyl-1-pyroline **1** and 6-acetyl-1,2,3,4-tetrahydropyridine **3** are essentially formed from the same precursors: 1-pyrroline and carbohydrate fragments. The relative concentrations of both odorants in proline model systems and thermally processed proline-rich food products depend on the predominant carbohydrate cleavage product present. If high amounts of 2-oxopropanal **30** are present, 2-AP **1** will be preferably formed from the hydrated compound **55**, whereas in the presence of the reduction product 1-hydroxy-2-propanone **88**, the formation of 6-ATHP **3** is favored (Scheme 15).

Scheme 15. Formation of 2-Acetyl-1-pyrroline 1 and 6-Acetyl-1,2,3,4-tetrahydropyridine 3 from 1-Pyrroline 22 and Carbohydrate Fragments[110]



When 2-oxopropanal **30** in low concentrations is reacted with proline, the formation of 6-ATHP **3** dominates the formation of 2-AP **1**. This is explained by the formation of 1-hydroxy-2-propanone **88** by Strecker reaction of 2-oxopropanal **30** in the presence of high amounts of free amino acids. When, on the contrary, high amounts of 2-oxopropanal **30** are present, 1-pyrroline **22** formed by Strecker degradation will preferably react with the excess 2-oxopropanal **30** present in the reaction mixture with the formation of 2-AP **1** as a consequence.[104]

When proline is reacted with 1,3-dihydroxyacetone (which forms 2-oxopropanal by elimination of water) in dry reaction conditions and in the presence of sodium bisulfite ('Hunter' reaction), only 6-ATHP is formed.[106] The absence of water and the reducing power of bisulfite (formation of 1-hydroxy-2-propanone **88**) lead to the exclusive formation of 6-ATHP (**3** and **2**) without a trace of 2-AP **1**. In reaction mixtures of carbohydrates and proline, 6-ATHP mostly predominates. Model reactions of proline with different monosaccharides, performed by Tressl et al.,[108] yielded 6-ATHP concentrations that were 7−40 (for glyceraldehyde) times higher than the corresponding 2-AP concentrations.

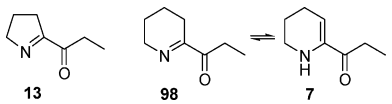
In the reaction with 2-oxopropanal **30**, ornithine **43** was shown to be an efficient precursor of 2-AP **1** but not of 6-ATHP **3**. Contrary to the Strecker degradation of the secondary amino acid proline **31** with 2-oxopropanal **30**, the Strecker degradation of ornithine **43** with 2-oxopropanal **30** does not involve formation of 1-hydroxy-2-propanone **88**, a necessary precursor of 6-ATHP **3**. Since baker's yeast contains 3.5 times more ornithine **43** than proline **31**, this explains the higher concentrations of 2-AP **1**, as compared to 6-ATHP **3**, found in bread crust.[5] In popcorn, where no ornithine is present, mainly 6-ATHP **3** is formed.[50]

The formation of these odorants in Maillard model systems consisting of glucose and proline was compared with model systems of the corresponding Amadori compound fructosyl-proline. In contrast to other odorants such as 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone, 2-AP **1** and 6-ATHP **3** were found to be formed preferentially from glucose/proline model systems as compared to the degradation of the Amadori product.[110] This is another indication that the formation of these flavor compounds does not imply the major Maillard reaction pathways through the Amadori compound and subsequent enolization reactions but that 2-AP and 6-ATHP are instead formed, as shown, by side reactions of sugar degradation products and 1-pyrroline.

3.4. Formation of 2-Propionyl-1-pyrroline and 6-Propionyl-2,3,4,5-tetrahydropyridine

2-Propionyl-1-pyrroline **13**, the higher homologue of 2-acetyl-1-pyrroline, has been identified as a key odorant in freshly popped corn[8] and heated glucose/proline model systems,[111] and it has been detected in Iberian ham[34] and peanut seed oil.[24] The higher homologues of 6-acetyl-1,2,3,4-tetrahydropyridine and 6-acetyl-2,3,4,5-tetrahydropyridine have been tentatively identified in thermally treated proline/glucose systems[101,111] and were identified as the main volatile constituents of *Semnostachya menglaensis* Tsui, a Chinese plant.[70] 6-Propionyl-1,2,3,4-tetrahydropyridine **7** and 6-propionyl-2,3,4,5-tetrahydropyridine **98** have been synthesized, and the cracker-like flavor properties were reported (Chart 4).[140]

Chart 4



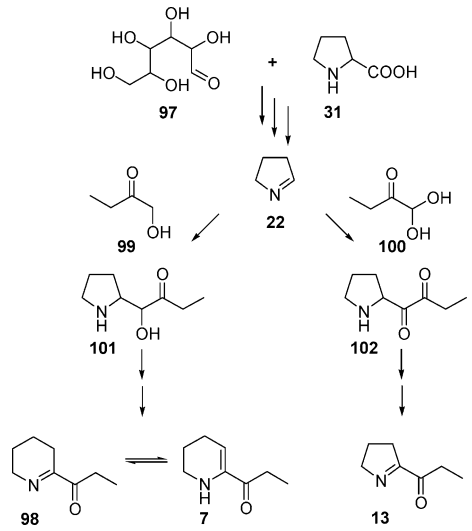
The formation of 6-propionyl-1,2,3,4-tetrahydropyridine **7** was proposed to proceed via an analogous pathway as described in Scheme 13 for 6-ATHP **3**, from 1-pyrroline **22** and 1-hydroxy-2-butanone **99** as the key intermediates. 1-Hydroxy-2-butanone **99** was formed upon heating of a mixture of hydroxyacetaldehyde and acetaldehyde, both well-known degradation compounds of carbohydrates, in 29% yield.[111] The model reaction of U-^{13}C-labeled glucose with proline confirmed this pathway since three labeled carbon atoms were present in the propionyl group and one labeled carbon atom was incorporated in the six-membered ring.

From the finding that the reaction of 1-pyrroline and 2-oxobutanal yielded 2-propionyl-1-pyrroline and that the reaction of U-^{13}C-labeled glucose with proline yielded 2-propionyl-1-pyrroline with three carbon labels in the

propionyl group, a reaction mechanism analogous to Scheme 7 can be proposed starting from 1-pyrroline **22** and 1,2-butanedione hydrate **100** as the key intermediates in 2-propionyl-1-pyrroline **13** formation.[111]

Apparently, 2-propionyl-1-pyrroline **13** and 6-propionyl-1,2,3,4-tetrahydropyridine **7**, with an alkyl chain of one carbon atom longer than 2-AP **1** and 6-ATHP **3**, respectively, have similar flavor properties and are formed by analogous reaction pathways (Scheme 16). This cannot be extended to

Scheme 16. Formation of 2-Propionyl-1-pyrroline 13 and 6-Propionyl-1,2,3,4-tetrahydropyridine 7 from 1-Pyrroline 22 and Carbohydrate Fragments



all higher homologues since, for instance, 2-butanoyl-1-pyrroline **14** does not smell roasty at all.[88] Higher homologues have a flavor profile that can be described as 'chemical' and are certainly not useful for food science.

3.5. Formation Pathways of 2-Acetyl-2-thiazoline and 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine

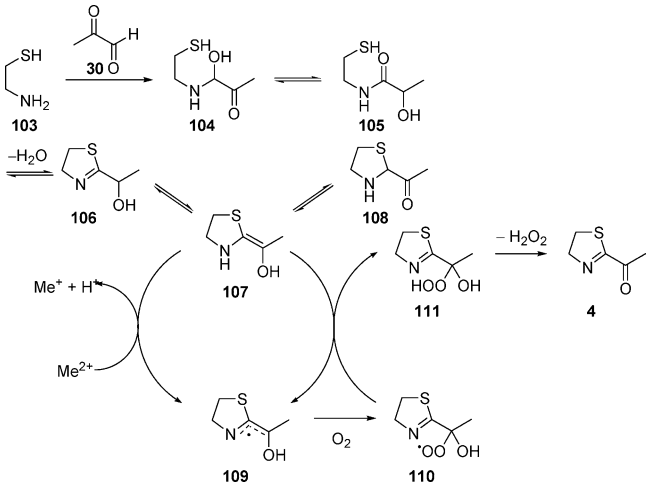
In model reactions of cysteine with ribose, glucose, and rhamnose the roasty flavor compounds 2-acetyl-2-thiazoline (2-AT) **4**, 2-propionyl-2-thiazoline, and 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine (5-ADHT) **5** were identified.[112,113] For 2-acetyl-2-thiazoline **4** and 2-propionyl-2-thiazoline an increase in flavor impact was noted under dry-heating conditions. For 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5**, however, dry heating led to a decrease in flavor significance.[112] Over the years, different model experiments have been performed to elucidate the mechanisms of formation involved.

In 1972, Sakaguchi and Shibamoto first reported on the formation of various thiazolidine derivatives from the reaction of aldehydes with cysteamine, one of the Strecker degradation products of cysteine, including the formation of 2-acetylthiazolidine **108** (2-ATD) from 2-oxopropanal.[114] Researchers from the same group later applied this reaction to the analysis of 2-oxopropanal in food and beverages by the detection of 2-ATD **108** formed from the reaction with cysteamine. Reaction of cysteamine with 2-oxopropanal in a molar ratio of 1:1000 at 25 °C and pH 6 yielded 98% of 2-acetylthiazolidine **108**. 2-Acetyl-2-thiazoline **4** was formed from the corresponding thiazolidine **108** by oxidation at higher reaction temperatures.[115]

In model reactions of cysteine with various α-dicarbonyl compounds, 2-acetyl-2-thiazoline **4** was identified only from the reaction with 2-oxopropanal **30**, together with 2-acetylthiazolidine **108** and 2-acetylthiazole **11**, generating roasted meat and hazelnut tones.[116] Kinetic studies showed that 2-acetyl-2-thiazoline **4** was in turn oxidized to 2-acetylthiazole **11**, which continuously increased in the reaction mixture while 2-acetyl-2-thiazoline **4** was degraded. Higher amounts of 2-AT **4** were formed when cysteine was reacted with 2-oxopropanal at low pH (pH 3.5).[117]

Hofmann and Schieberle later performed a large number of model experiments to gain more detailed insight in the reaction mechanisms and intermediates governing the formation of 2-AT **4**. The intermediates in the reaction path to 2-acetyl-2-thiazoline **4** were identified as the odorless 2-(1-hydroxyethyl)-4,5-dihydrothiazole (HDT) **106** and 2-acetylthiazolidine **108** (2-ATD), which are in tautomeric equilibrium, presumably with 2-(1-hydroxyethylene)thiazolidine **107** as the intermediate compound.[118] Thermal treatment of 2-(1-hydroxyethyl)-4,5-dihydrothiazole **106**, either in aqueous solution or in the gas phase of a chromatographic inlet, was shown to generate significant amounts of 2-acetyl-2-thiazoline **4**, also in the absence of oxidative agents.[118] The yields of 2-AT **4** from boiling 2-ATD **108** in water for 20 min were five times higher than from HDT **106** (34% vs 7%). Since copper(II) ions as well as oxygen significantly increased the oxidation of HDT **106** to 2-AT **4**, the formation route displayed in Scheme 17 was hypothesized, but the proposed

Scheme 17. Hypothesis for the Formation of 2-Acetyl-2-thiazoline 4 from Cysteamine 103 and 2-Oxopropanal 30 via a Metal-Catalyzed Oxidation of Intermediate Enaminol 107[119]



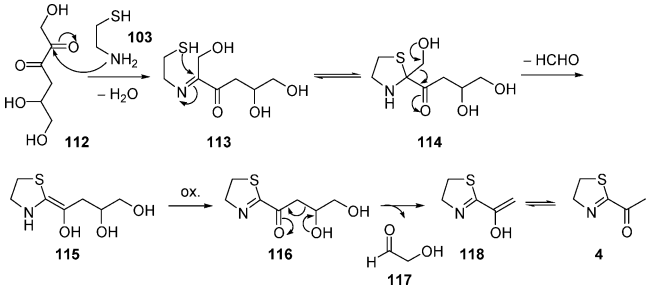
oxidation mechanism has not yet been proven by further experiments. Oxidation of enaminol **107** by atmospheric oxygen occurs in the presence of catalytic amounts of heavy metals to the resonance-stabilized allylic radical **109** in a one-electron reaction. 2-Acetyl-2-thiazoline **4** is then formed via the hydroperoxide **111**, which induces oxidation of the metal ion (M^+), thereby regenerating it for a new cycle.[119]

Three alternative mechanisms for the oxidation of ADT **108** or HDT **106** to 2-AT **4** were suggested by Kerler et al.,[95] starting with the abstraction of a hydrogen radical either from the hydroxyl group of **106** or from the C-2 position of ADT **108**, either with one-electron transfer from the electron-rich double bond of **107** to oxygen. These three alternative mechanisms can equally yield 2-AT **4** via similar oxidation paths.

Model reactions of fructose with cysteamine or thiazolidine-2-carboxylic acid (a condensation product of cysteamine

with 2-oxoacetic acid) yielded 2-acetyl-2-thiazoline, 2-propionyl-2-thiazoline, 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine, and 5-propionyl-2,3-dihydro-4*H*-1,4-thiazine.[120] In addition to the pathway starting from 2-oxopropanal **30**, a hypothetical reaction pathway for the formation of 2-acetyl-2-thiazoline **4** was proposed starting from 4-deoxyosone **112** (derived from fructose by the elimination of water) and cysteamine **103** (derived from the reaction of thiazolidine-2-carboxylic acid with α-dicarbonyl compounds) (Scheme 18).[120] This

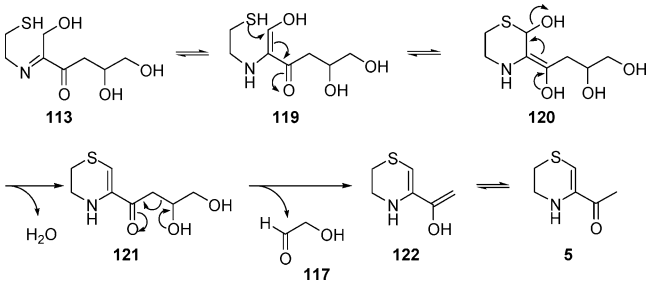
Scheme 18. Hypothetical Reaction Pathway Leading from the 4-Deoxyosone of Fructose 112 and Cysteamine 103 to 2-Acetyl-2-thiazoline 4[120]



pathway is initiated with the nucleophilic addition of the amino group of cysteamine **103** to C2 of 4-deoxyosone **112** and subsequently of the free thiol group to the imine formed. Formaldehyde is eliminated from the resulting thiazolidine by retro-aldol cleavage. An oxidation step is assumed to enable the formation of 2-thiazoline **116**. Elimination of acetaldehyde **117** by retro-aldol cleavage yields 2-acetyl-2-thiazoline **4** after tautomerization of **118**.

Formation of a six-membered ring from intermediate **113** via a comparable pathway leads to the formation of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** from cysteamine **103** and the fructose 4-deoxyosone **112** (Scheme 19).

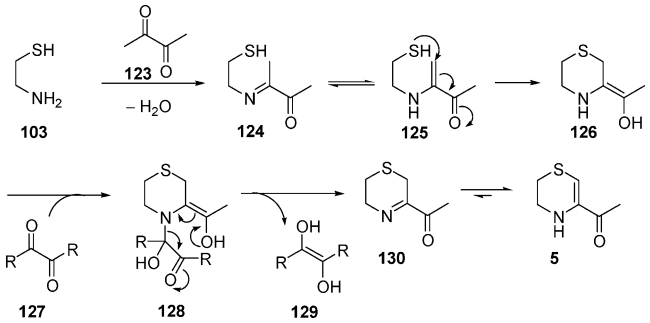
Scheme 19. Hypothetical Reaction Pathway Leading from the Schiff Base 113 of Cysteamine and the 4-Deoxyosone to 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine 5[120]



Model reactions of cysteamine with 2,3-butanedione, analogous to the reaction with 2-oxopropanal for the formation of 2-acetyl-2-thiazoline **4**, yielded relatively low yields (1 mol %) of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5**. Similar model experiments of cysteamine with 2,3-pentanedione and 2,3-hexanedione yielded 5-propionyl-2,3-dihydro-4*H*-1,4-thiazine and 5-butanoyl-2,3-dihydro-4*H*-1,4-thiazine, respectively. Therefore, a hypothetical reaction mechanism, as shown in Scheme 20, was proposed.[98]

Since Umano et al.[121] hypothesized the formation of 2-acetyl-2-methylthiazolidine **131** from the same model reaction of cysteamine **103** with 2,3-butanedione **123**, Huang and co-workers examined the model system 2,3-butanedione/cysteamine in detail.[122] The most important product from the model reaction of cysteamine with 2,3-butanedione was

Scheme 20. Hypothesized Formation Pathway of 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine 5 from Cysteamine 103 and 2,3-Butanedione 123[98]



2-acetyl-2-methylthiazolidine **131**. Besides, 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** and 3-acetyl-2,3,5,6-tetrahydro-4*H*-1,4-thiazine **132** were (tentatively) identified. Performing the reaction in phosphate buffer instead of an aqueous system significantly enhanced the yields of these three compounds (10−20-fold). Via addition of carbamoylazoformamide the authors showed that a redox reaction leads to the conversion of 3-acetyl-2,3,5,6-tetrahydro-4*H*-1,4-thiazine **132** into 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5**, and they proposed a mechanism as shown in Scheme 21. This formation mechanism is similar to Scheme 20, but 5-ADHT **5** is formed through oxidation of 3-acetyl-2,3,5,6-tetrahydro-4*H*-1,4-thiazine **132** via a proton-transfer reaction with one of the various proton acceptors present in the Maillard reaction medium.

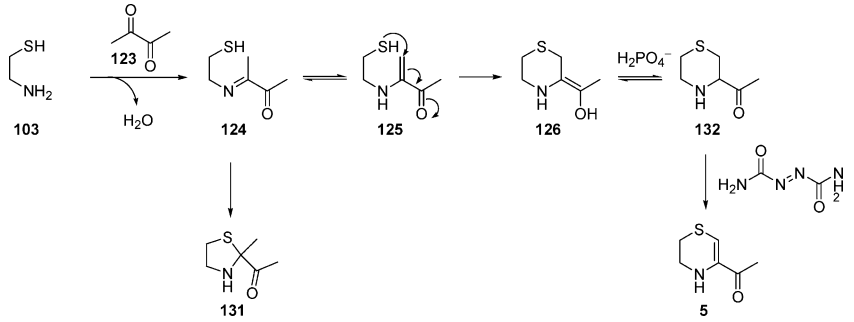
Labeling experiments with 2-^{13}C-fructose revealed that the formation pathways of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** from fructose and cysteamine under dry heating and cooking conditions are completely different, depending on the water content of the system. From these findings and from the position of the ^{13}C-label in the final products, two alternative reaction mechanisms for the formation of 5-ADHT **5** were proposed, as shown in Scheme 22 and Scheme 24.[123] Under dry heating conditions, the label was found almost exclusively at the carbonyl carbon of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5**. The proposed reaction pathway under dry-heating conditions starts from the pyranose (or equally from the furanose) form of fructose **133** by direct elimination of water. Nucleophilic attack of cysteamine **103** leads to the bicyclic structure **137**. Subsequent eliminations of water followed by ring opening yield the 6-substituted 5-acetyl-2,3-dihydro-6*H*-thiazine **141** from which 5-ADHT **5** is formed. Under cooking conditions, on the contrary, the Amadori rearrangement takes place. Subsequent isomerizations, water elimination, and ring closure result in the elimination of acetic acid **149**, which was identified as an important reaction product (Scheme 23). The second reaction product erythrulose **148** is proposed to yield 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** from the reaction with cysteamine **103** as shown in Scheme 24. Separate model experiments of erythrulose **148** and cysteamine **103** were reported to generate large amounts of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5**, confirming this reaction pathway.[123]

4. Biological Origin

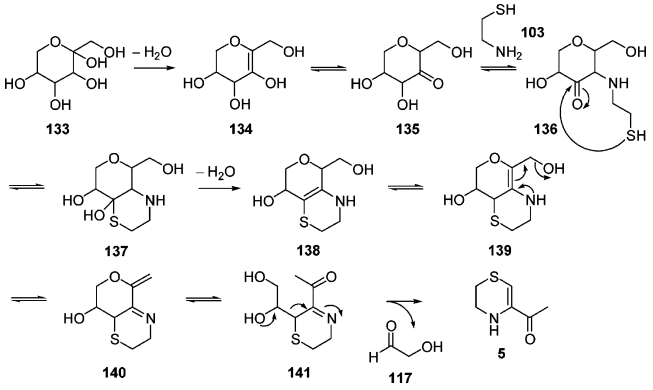
4.1. Biological Formation of 2-Acetyl-1-pyrroline in Rice Plants

Following reports of the formation of 2-AP in the aerial parts of rice plants grown in paddy fields,[53] the biological

Scheme 21. Proposed Formation Mechanism for 2-Acetyl-2-methylthiazolidine 131, 3-Acetyl-2,3,5,6-tetrahydro-4*H*-1,4-thiazine 132, and 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine 5[122]



Scheme 22. Formation of 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine 5 from Fructose 133 and Cysteamine 103 under Dry-Heating Conditions on the basis of Labeling Experiments[123]



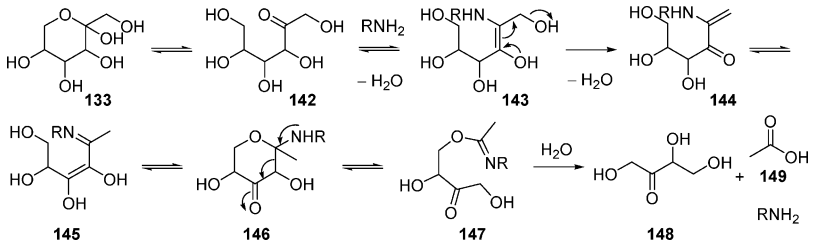
formation of 2-AP was studied in callus and seedlings of aromatic rice.[124] 2-Acetyl-1-pyrroline **1** was formed in aromatic rice at temperatures below that of thermal generation, for instance, in bread baking. Precursor studies indicated an increase in 2-AP concentration when proline (×3), ornithine (×1.5), and glutamic acid (×1.5) were present in the solution. Labeling of proline indicated that proline was the nitrogen source for 2-AP, but the carbon source of the acetyl group of 2-AP was not the carboxyl group of proline. It was proposed that 2-AP is formed by acetylation of 1-pyrroline. This is well in line with the results of the precursor studies since glutamic acid is the common biosynthetic precursor of proline and ornithine and catabolism of ornithine proceeds via 4-aminobutanal, which may cyclize to 1-pyrroline.

Among the volatiles of the rice flavor produced by plant cell cultures of Basmati rice, 2-AP could, however, not be detected.[125] Micropropagation experiments of Indian pandan (*Pandanus amaryllifolius* Roxb.) did not provide the anticipated results since the 2-AP content in the mother plant remained higher than in the tissue-cultured plants.[126]

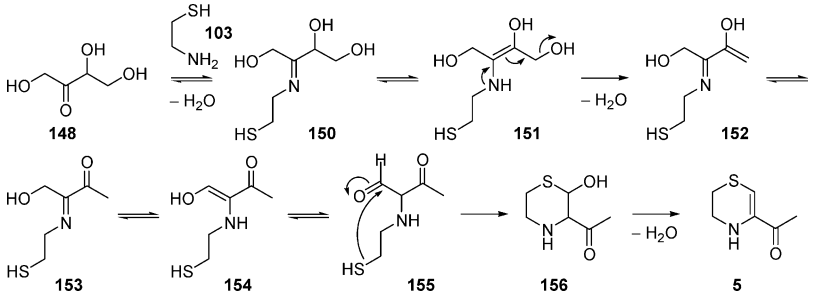
Different studies on the genetic control of the typical aroma of rice have been reported.[127] A recessive gene (*fgr*) on chromosome 8 of rice, largely controlling the level of 2-AP, has been identified in genetic studies. The gene corresponds with the gene that encodes for betaine aldehyde dehydrogenase (BAD), specifically with BAD2 in rice. The accumulation of 2-AP in fragrant rice genotypes may be explained by the presence of mutations resulting in a loss of function of the *fgr* gene product.[128]

The content of 2-acetyl-1-pyrroline in rice is dependent on ecological and cultivation factors. An examination of the 2-acetyl-1-pyrroline content of various rice samples in Thailand showed that the samples from irrigated areas had lower 2-AP contents than from rain-fed areas.[129] Drought conditions during cultivation seemed to have an important contribution to aromatic rice quality. Proline accumulation

Scheme 23. Formation of Erythrulose 148 and Acetic Acid 149 from Fructose under Cooking Conditions[123]



Scheme 24. Formation of 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine 5 from Erythrulose 148 and Cysteamine 103 under Cooking Conditions[123]



is a common metabolic response of higher plants to water deficits[130] and may therefore be responsible for the higher 2-AP production. Elevated fragrance in response to stress corresponds with the biochemistry of betaine aldehyde dehydrogenase discussed above since this enzyme has been linked to stress tolerance in plants.[128]

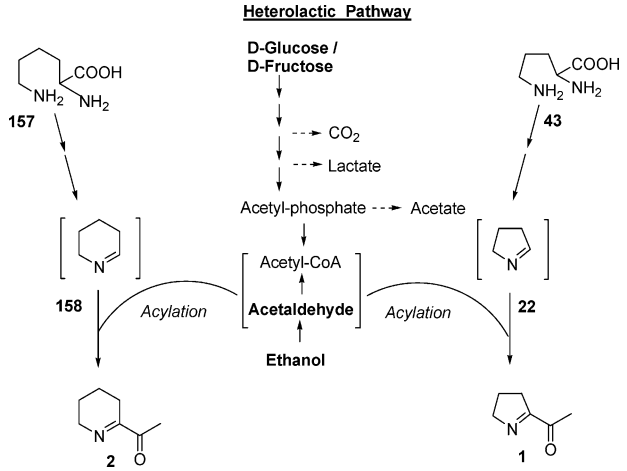
Another study describes the variation of 2-AP concentration in aromatic rice in Japan over a period of 3 years.[131] Most samples showed similar 2-AP concentrations with standard deviations of about 30%. However, a few samples showed extremely high or low 2-AP concentrations as compared to the year average. During grain development, the 2-AP concentration in brown rice reached a maximum at 4 or 5 weeks after heading, decreasing rapidly afterward. The 2-AP concentration was higher in brown rice ripened at lower temperature. On the basis of these results it is recommended to cultivate aromatic rice at cool temperature and high altitude to optimize the scented rice flavor, and it should be harvested earlier than other cultivars. Also, application of nitrogen fertilizer influenced 2-AP concentrations.[131]

4.2. Detection of 2-Acetyl-1-pyrroline and 6-Acetyl-1,2,3,4-tetrahydropyridine as Microbial Metabolites

2-Acetyl-1-pyrroline was shown to be responsible for a 'popcorn, corn chip' aroma formation, which was observed from several *Bacillus cereus* strains isolated from cocoa fermentation boxes in Brazil.[132] Upon further investigations, 2-AP was detected among the volatiles produced by specific strains of *B. cereus*, under specific growth conditions. 2-Acetyl-1-pyrroline was detected from *B. cereus* cultures grown on solid standard plate count agar at a temperature of 35 °C, i.e., well below the temperatures required for its thermal formation. Labeling experiments established proline and glutamic acid as nitrogen sources and glucose as the carbon source required for formation of 2-AP by these *B. cereus* strains. No labeling studies were performed with ornithine. The highest production was noted when *B. cereus* was grown on plate count agar supplemented with 1% of glucose (or amylose) and amounted to 11.5 μg (or 12.8 μg, respectively) 2-AP for 25 g of medium during 2 days. These are very low yields, but due to the low odor threshold, a pleasant flavor is noticeable. The results of these precursor studies are in agreement with the results of Yoshihashi et al.[53] concerning 2-AP formation in rice, indicating a common pathway for the biological formation of 2-AP.

Microbiologically induced spoilage of wine is characterized by the development of an offensive 'mousy-like' off-flavor. This mousy off-flavor is caused by 6-ethyl-2,3,4,5-tetrahydropyridine, 6-acetyl-1,2,3,4-tetrahydropyridine, and 2-acetyl-1-pyrroline.[61,133] All known type strains of the spoilage yeasts *Brettanomyces* and *Dekkera* and many heterofermentative wine lactic acid bacteria are capable of producing this mousy off-flavor and the mousy *N*-heterocycles.[134] The formation of the mousy heterocycles 2-AP and 6-ATHP by *Lactobacillus hilgardii* was investigated in detail.[135] These studies demonstrated that the biosynthesis of 2-AP and 6-ATHP is simultaneously dependent upon the metabolic pathways involved in the lactic acid fermentation of sugars, the metabolism of ethanol, and of l-ornithine and l-lysine. The catabolism of l-lysine **157** and l-ornithine **43** via the cadaverine and putrescine pathways, respectively, leads to the formation of 2,3,4,5-tetrahydropyridine **158** and 1-pyrroline **22**, respectively. In the presence of a carbohydrate source, such as fructose, and ethanol, acetyl-CoA may accumulate and induce acylation of the 2,3,4,5-tetrahydropyridine **158** and 1-pyrroline **22** intermediates, thus yielding 6-ATHP **3** and 2-AP **1**, respectively (Scheme 25).

Scheme 25. Proposed Pathway for the Formation of 2-Acetyl-1-pyrroline 1 and 6-Acetyl-2,3,4,5-tetrahydropyridine 2 by *Lactobacillus hilgardii* DSM 20176[135]



Another major factor affecting the production of mousy *N*-heterocycles was the presence of metal ions, particularly Fe^{2+}.[135] The reason for this remains unclear.

Microorganisms, such as *Lactobacillus pontis*, can also increase the development of roasty notes in food products by proteolysis, yielding free amino acids such as ornithine as precursors for flavor formation.[136]

2-Acetyl-1-pyrroline has been identified as a characteristic flavor compound in Mediterranean dried sausages covered with mould. Since the surface of the sausages contained higher amounts of 2-AP than the core, it was suggested that the mould growing on the surface of the sausages produced 2-AP. The dominating mould species, *Penicillium nalgiovense*, was grown on media with and without various supplements, but the popcorn odor only developed in media where the sausage product itself was added.[36]

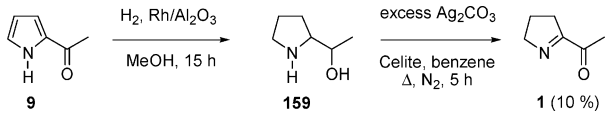
Considering the results of the different studies on the thermal as well as biological origin of 2-acetyl-1-pyrroline, a common mechanism of formation can be presumed since in all cases the acetylation of 1-pyrroline **22** is described as the key step.

5. Synthesis

5.1. Synthetic Procedures Developed for 2-Acetyl-1-pyrroline

Various syntheses of 2-acetyl-1-pyrroline **1** have been described in the literature. The first synthesis (Scheme 26)

Scheme 26[2a]



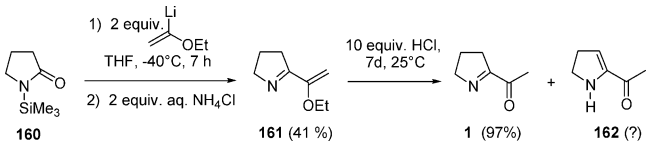
of the flavor compound **1** consisted of an oxidation of 2-(1-hydroxyethyl)pyrrolidine **159** with a large excess of silver carbonate on Celite in benzene.[2a] This procedure was

developed analogous to the first described synthesis of 6-acetyl-1,2,3,4-tetrahydropyridine **3**.[137] For the case of 2-acetyl-1-pyrroline **1**, the reaction mixture consisted of a large number of products, of which only 10% (by GC) appeared to be 2-acetyl-1-pyrroline **1**.[2a] Drawbacks, such as high cost, low yield, and especially an impure final product, disable use of this method on a large scale.

It should be pointed out that 2-acetyl-1-pyrroline **1** occurs exclusively as the imino tautomer. Contrary to 6-acetyl-2,3,4,5-tetrahydropyridine **2**, 2-acetyl-1-pyrroline **1** does not show any tendency to tautomerize to the enamine form **162**. Even deprotonation by base and reprotonation produces the imino tautomer, a phenomenon that is expected from the viewpoint of the stability of unsaturated five-membered ring compounds.

Due to the instability of 2-AP, several synthetic efforts focused on more stable, carbonyl-protected analogues, such as the 1-ethoxyethenyl derivative **161** generated from the addition of 1-ethoxyvinyllithium to 1-(trimethylsilyl)butyrolactam **160** (Scheme 27).[138] Acid hydrolysis under severe

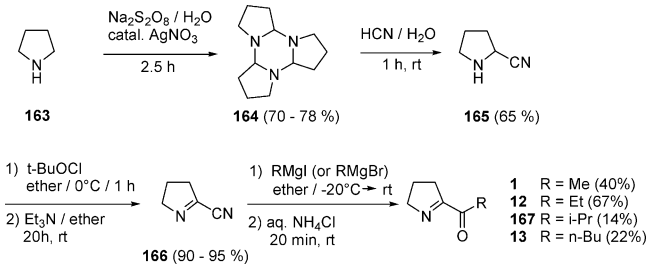
Scheme 27[138]



conditions (100 equiv of HCl during 2 days, or 10 equiv of HCl during 7 days) of enol ether **161** yielded 97% of 2-AP **1**. The presence of the enamino tautomer **162**, however, does not fit any other literature reports and is questionable. In fact, it concerns the isomeric compound **177**, as shown in Scheme 31 (which is discussed below).[140]

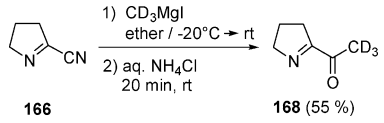
A flexible method for the synthesis of not only 2-acetyl-1-pyrroline **1** but also of other 2-acyl-1-pyrrolines entailed the addition of a Grignard reagent to imidoyl cyanide **165** and subsequent mild hydrolysis (Scheme 28).[139] The imidoyl cyanide **165** was produced by oxidation of pyrrolidine **163** with disodiumperoxydisulfate in the presence of catalytic amounts of silver nitrate. The intermediate 1-pyrroline trimer **164** was subsequently treated with aqueous hydrogen cyanide to provide 2-cyanopyrrolidine **165**, which was oxidized to imidoyl cyanide **166** via *N*-chlorination with *tert*-butyl hypochlorite and following dehydrochlorination with triethylamine (Scheme 28).[139]

Scheme 28[139]



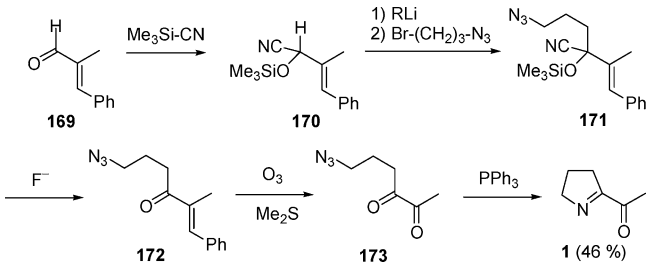
The method described above also allows the synthesis of specifically deuterated 2-acetyl-1-pyrroline **168**, which is in this way available for the quantitative analysis of the rice flavor compound **1** using the stable isotope dilution assay (Scheme 29).[5] Deuterium incorporation was performed by addition of trideuteriomethylmagnesium iodide to imidoyl cyanide **166**.

Scheme 29[139]



Three methods entailing the cyclization of 6-amino- or 6-azidohexane-2,3-diones to form 2-acetyl-1-pyrroline **1** have been published. Rewicki et al.[101] created access to different monocyclic and bicyclic α-acylenamines by intramolecular aza-Wittig reaction from the corresponding azidodiketone (Scheme 30). The yields obtained are lowered by thermal

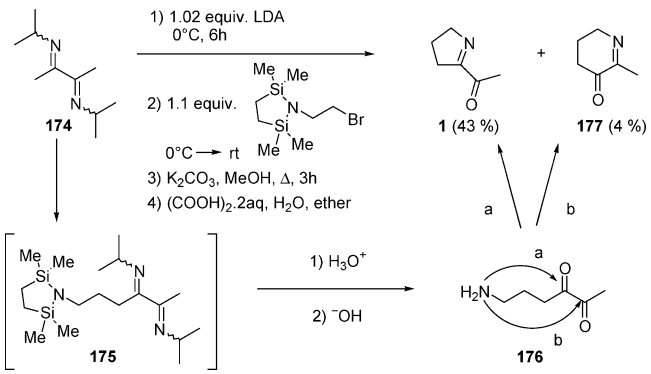
Scheme 30[101]



decomposition during the isolation procedure (the yield of 2-AP from **173** is 46%). The γ-azidodiketone **173** was formed by a sequence of reactions involving addition of trimethylsilylcyanide to α,β-unsaturated aldehyde **169**, α-deprotonation of the nitrile **170** and alkylation with 3-bromopropyl azide, desilylation, and ozonolysis.

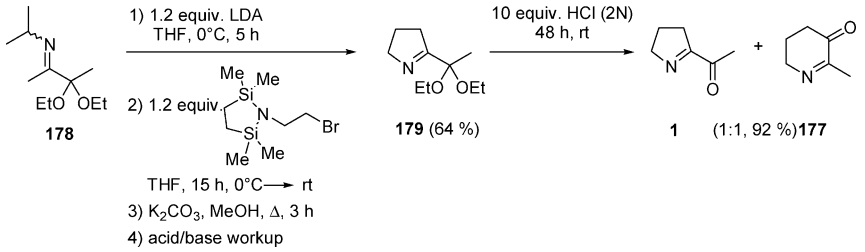
A short synthetic strategy was developed depending on an amino-protected functionalized vicinal diimine **175** as the key intermediate (Scheme 31).[140] This labile key intermediate

Scheme 31[140]



was produced by α-deprotonation of α-diimine **174** (generated from butane-2,3-dione and isopropylamine in the presence of titanium(IV) chloride) and subsequent alkylation with 'stabase'-protected 2-bromoethylamine. Isolation of this α-diimine **175** proved to be very difficult, and therefore, it was used in situ. After deprotection and hydrolysis of the α-diimine into the α-dione **176**, spontaneous cyclization led to the desired azaheterocycle (2-AP **1** in 43% yield). The extent of the formation of structural isomer **177** depended on the excess of acid applied. It is proposed that it concerns this isomer **177**, formed by acid hydrolysis of compound **161** (Scheme 27),[138] instead of the stated enamine tautomer of 2-acetyl-1-pyrroline **162**, which has not been identified elsewhere.[140] The same sequence of reactions as in Scheme 31 could be used for the preparation of the carbonyl-protected 2-AP analogue **179**, starting from α,α-diethoxyketimine

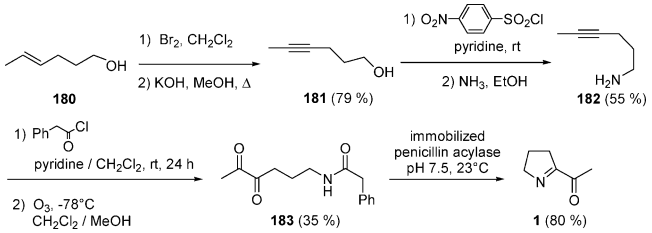
Scheme 32[140]



178.[140] Using the procedure shown in Scheme 32, the diethyl acetal **179** was obtained in pure form after column chromatography. These types of more stable compounds show great potential for use as flavorant in food products, since gradual hydrolysis yields 2-AP **1** and ethanol.

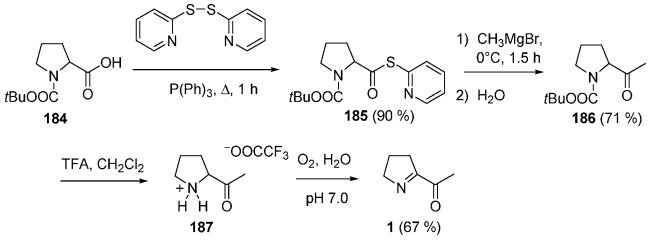
Favino et al.[141] developed a synthesis of the *N*-protected acyclic 1-amino-4,5-diketone **183**. This α-dione **183** was synthesized from (*E*)-4-hexen-1-ol **180**, which was oxidized into the corresponding alkyne **181** by bromination/double dehydrobromination. This alkynol **181** was sulfonylated, phenylacetylated, and ozonolyzed to afford the *N*-phenylacetylated aminodione **183**. This compound **183** was enzymatically hydrolyzed using immobilized penicillin acylase into 1-amino-4,5-hexanedione **176**, which spontaneously ring closed to 2-AP **1** (overall yield 80%) (Scheme 33).

Scheme 33[141]



The finding that 2-acetylpyrrolidine **26** is oxidized spontaneously in aqueous medium at neutral pH under the influence of oxygen (air) to afford 2-acetyl-1-pyrroline **1** is of fundamental importance.[103] One of the most recent synthetic procedures developed for 2-AP, starting from *N*-Boc-protected proline **184**, is based on this oxidation step and was developed to prove this oxidation (Scheme 34). The

Scheme 34[103]



thioester **185** underwent addition of one equivalent of methylmagnesium iodide while the protective Boc-group was cleaved with trifluoroacetic acid to give the trifluoroacetammonium compound **187**. 2-Acetylpyrrolidine **26**, obtained after basification of the aqueous solution to pH 7, was spontaneously oxidized by air oxygen (overall yield of 2-AP **1** 43% from *N*-Boc-proline **180**).

Recently, another synthetic procedure for the preparation of 2-acetyl-1-pyrroline **1** from proline **31** was published in the patent literature.[142] The procedure starts with the esterification of proline **31** via the acid chloride prepared with thionyl chloride (Scheme 35). The resulting methyl ester of proline **188** is then subjected to an oxidation and a Grignard reaction in a procedure identical to what was described earlier for the preparation of 2-acetyl-1-pyrroline **1** via 2-cyanopyrrolidine **166**.[139] The described process claims to provide access to 2-acetyl-1-pyrroline **1** in gram scale and 60% overall yield, starting from proline **31**, in four steps.[142] However, monoaddition of the Grignard reagent to ester **189** is questionable.[139] An alternative approach, which avoids the risk of overaddition of the Grignard reagent by using a nitrile instead of an ester function, can be found in Scheme 28.

In a very recent synthesis, 2-acetyl-1-pyrroline **1** was prepared from 2-pyrrolidone in 10% overall yield.[143] This synthetic procedure gave better results for the bread flavor compound 6-acetyl-1,2,3,4-tetrahydropyridine **3**.

5.2. Synthetic Procedures Developed for 6-Acetyl-1,2,3,4-tetrahydropyridine

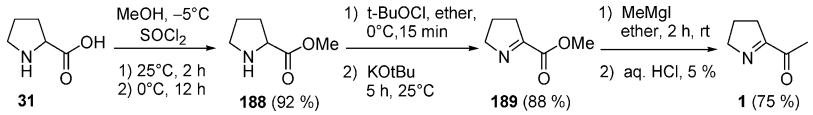
Many synthetic strategies developed for 2-AP **1** can be applied to 6-ATHP **3** and vice versa, although with varying yields.

In the classical "Hunter experiment", the neat reaction of proline with 1,3-dihydroxyacetone in the presence of bisulfite, low yields of 6-ATHP were obtained together with some 2,3-dihydro-1*H*-pyrrolizine side products.[64,106]

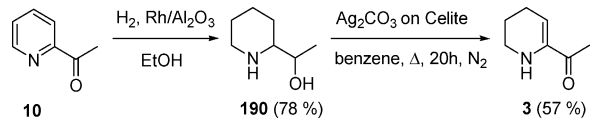
The first rational synthesis of 6-ATHP **3** was developed in 1971 to confirm the structure determination and evaluate the sensorial properties of this bread flavor compound and included the oxidation of 2-(1-hydroxyethyl)piperidine **190** with a large excess of silver carbonate.[137] It concerns a well-established procedure starting from the relatively inexpensive 2-acetylpyridine **10** with an overall yield of 44% (Scheme 36). On the lab scale, this procedure is still widely used, although it is less attractive for industrial purposes because of the use of a large excess of silver reagent.

The addition of Grignard reagents to 6-cyano-2,3,4,5-tetrahydropyridine **194** opened the door to the bread flavor component **3** (44% yield) (Scheme 37) as well as the higher analogues 6-propionyl-1,2,3,4-tetrahydropyridine **7** and 2-propionyl-3,4,5,6-tetrahydropyridine **98**,[144] which show the same popcorn-like odor note and a similar low odor threshold as the 6-ATHP tautomers **2** and **3**.[111] The synthesis of 6-cyano-2,3,4,5-tetrahydropyridine **194** proceeded via tripiperideine **192**, which was prepared by *N*-chlorination of piperidine **191** using *tert*-butyl hypochlorite with subsequent dehydrochlorination of the resulting *N*-chloropiperidine with sodium methoxide. The resulting 1-piperideine trimerized immediately, and tripiperideine **192** was isolated and treated directly with aqueous hydrogen cyanide. 2-Cyanopiperidine **193** was then oxidized to imidoyl cyanide **194**, and Grignard additions yielded 6-acetyl-1,2,3,4-tetrahydropyridine **3** in 44% yield (purity ≥ 98%). This process provided access to large-scale production of the flavor compound.

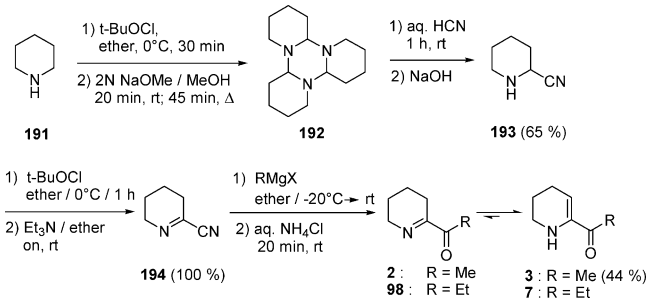
Scheme 35[142]



Scheme 36[137]



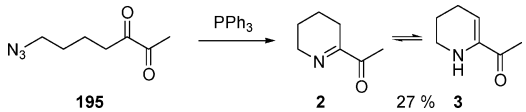
Scheme 37[144]



Using this synthetic procedure, the freshly prepared bread flavor compound occurred as a 4:1 mixture of the imino form **3** and the enamine form **2**, as shown by ^{1}H NMR ($CDCl_3$). On standing, however, this ratio gradually changed to a ratio in favor of the enamine form (up to 1:2). Both tautomers can be isolated, but GC analysis of imino tautomer **2** already shows an equilibration to the enamine tautomer **3**. Gas chromatographic separation of both tautomers proceeds better on a nonpolar stationary phase than on a polar phase.[145] A polar stationary phase enhances tautomerism, and the interchange between two forms occurs to an appreciable extent during chromatography. In the literature, many controversial results have been published on the GC elution order of both tautomers.[67,102,106,111] On a nonpolar stationary phase the imino tautomer **2** elutes before the enamine tautomer **3**. This elution order is supported by isolation by preparative gas chromatography, small differences in the EI mass spectra of both tautomers, and the higher concentration of the imine tautomer. On a polar poly(ethylene glycol) stationary phase, an elution order enamine−imino (**3**−**2**) has been published,[102,133] although here as well the enamine structure is expected to have a higher retention time due to hydrogen bonding.

Rewicki et al. accomplished a synthesis for 6-ATHP **3** via aza-Wittig reaction of δ-azidodiketone **195**, in analogy with the 2-acetyl-1-pyrroline pathway described in Scheme 30. 6-ATHP was obtained in 27% yield (Scheme 38).[101]

Scheme 38[101]



A comparable synthetic approach toward 6-ATHP and some of its more stable acetal and enol ether derivatives was accomplished by elaboration and ring closure of appropriately functionalized imines. Aza-Wittig type cyclization of the functionalized δ-azido ketone **198**, carrying an acetal function in the α'-position, proved to be the most successful route, as shown in Scheme 39.[146] The resulting stabilized acetal **199** can be hydrolyzed under mild reaction conditions to the desired flavor compound **3** (yield 6-ATHP **3** from **198** 47%). The functionalized imine **196** originates from the monoacetal of butane-2,3-dione after imination and alkylation with 1-bromo-3-chloropropane.[146]

A very recent adaptation of the latter procedure involved the microwave-assisted aza-Wittig reaction of alkyl azides.[147] Following the above-described synthetic procedure,[146] 6-ATHP could be synthesized in a one-pot approach from the corresponding δ-chloro-α',α'-dimethoxyketone **197**, triphenylphosphine, and sodium azide by flash heating with microwave irradiation. The α,α-dimethoxyimine **199** was thus obtained from **197** in one step and 98% yield after 18 min of microwave irradiation.

Cyclization of the appropriate amino-protected functionalized vicinal diimine, similar to the synthesis of 2-AP described in Scheme 31, gave good results for the synthesis of 6-ATHP **3** (65% yield) (Scheme 40).[140]

Another synthetic pathway is based on the oxidation of 2-acetylpiperidine, which is prepared from the cyclic α-amino acid pipecolic acid **203** (Scheme 41). 2-Acetylpiperidine is liberated from its salt **207** after basification of the aqueous solution (pH 7) and oxidizes spontaneously with oxygen to provide 6-ATHP **3** in 35% yield (based on pipecolic acid **203**).[103]

The most recent synthetic pathway involves the synthesis of 6-acetyl-1,2,3,4-tetrahydropyridine **3** in three steps from 2-piperidone with an overall yield of 56%.[143] In this case, the flavor compound was released from a carbamate-acetal function under basic conditions, thus avoiding losses due to acidic conditions in the final step.

5.3. Synthesis of 2-Acetyl-2-thiazoline

The chemical preparation of 2-thiazolines is generally carried out by the condensation reaction of cysteamine with a nitrile, a carboxylic acid, or an ester.[148]

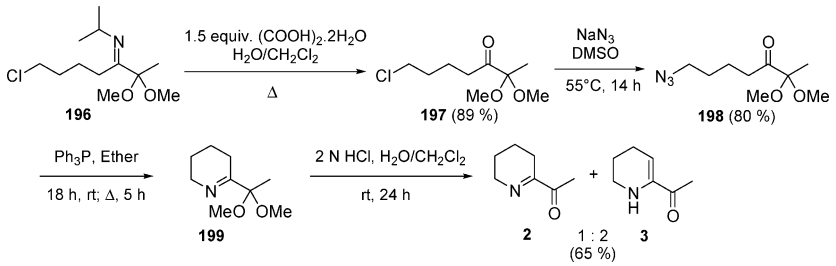
Condensation of cysteamine **103** with 2,2-diethoxypropionitrile **208** yields the diethyl acetal **209**, derived from 2-acetyl-2-thiazoline **4**. Both 2-acetyl- and 2-propionyl-2-thiazoline were thus prepared as the corresponding diethyl acetal, which can be hydrolyzed to the respective 2-acyl-2-thiazoline.[149] In this way, 2-acetyl-2-thiazoline **4** was obtained in 90% yield (Scheme 42).

Ruthenium-catalyzed oxidation of 2-acetylthiazolidine **108**, obtained from the condensation of cysteamine **103** with 2-oxopropanal **30** under slightly basic conditions, yielded 2-acetyl-2-thiazoline **4** in 70% isolated yield (Scheme 43).[148]

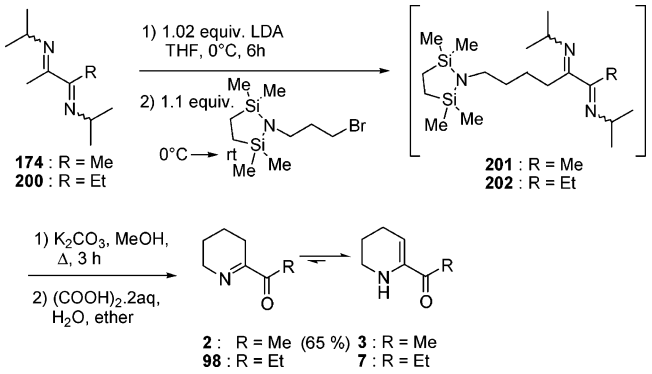
5.4. Synthesis of 5-Acetyl-2,3-dihydro-4*H*-1,4-thiazine

Preparation of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** from the model reaction of 2,3-butanedione with cysteamine at 145 °C for 20 min in an autoclave yielded only 1% of the flavor compound.[98] Two more efficient synthetic routes for the preparation of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** have been described.[150] In the first route 1-bromo-3,3-dimethoxy-2-butanone **210** was condensed with a small

Scheme 39[146]



Scheme 40[140]



excess of cysteamine hydrochloride. The reaction mechanism proceeds via nucleophilic substitution, followed by ring closure by imine formation and successive hydrolysis of the acetal function, yielding 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** after basic workup. To improve the yield, a second route was developed using *tert*-butyl *N*-(2-mercaptoethyl)carbamate as the nucleophilic reagent. The carbamate **211**, obtained in 86% yield, was cleaved with an excess of trifluoroacetic acid in dichloromethane and afforded pure 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** after basic workup in 88% yield (Scheme 44).

The synthesis of starting compound **210** from 2,3-butanedione **123** is displayed in Scheme 45 and includes subsequent monoacetalization of 2,3-butanedione, imination in the presence of titanium(IV) chloride, bromination with *N*-bromosuccinimide, and hydrolysis of the α-bromoimine formed.

In contrast to the analogous 6-acetyl-2,3,4,5-tetrahydropyridine **2**, the imino tautomer of 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine **5** was never detected. This different behavior can be explained by a more extended conjugation of the thiazine due to the sulfur atom.[150]

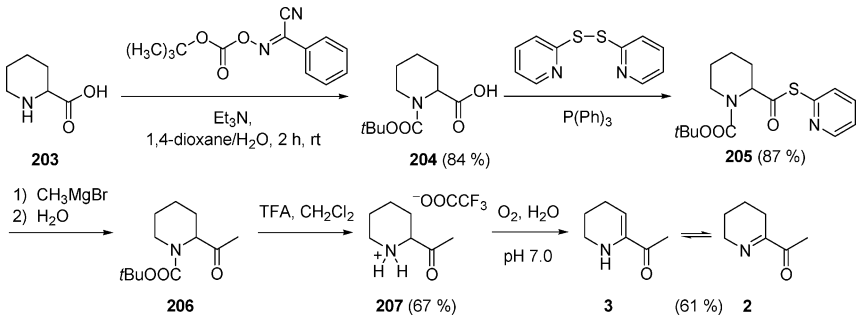
For the preparation of deuterium-labeled 5-ADHT **219** for the application of aroma extract dilution analysis, Engel and Schieberle reacted [1,1,2,2-^{2}H]cysteamine with 1-bromo-2,3-butanedione, achieving a yield of about 50% and a purity of 90% (by GC).[120] Deuterium-labeled [1,1,2,2-^{2}H]cysteamine was prepared as outlined in Scheme 46 from [1,1,2,2-^{2}H]-dibromoethane **215**. Nucleophilic substitution with sodium diformylamide and potassium thioacetate in the next step is followed by immediate hydrolysis of the thioester **217**.

6. Stability and Applications

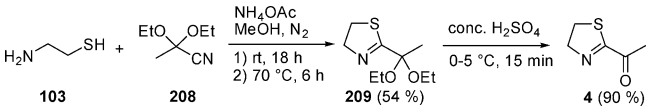
The maintenance of their particular flavor characteristics is of crucial importance in the marketing of fragrant rice varieties. However, during storage the flavor of rice can deteriorate as a result of different mechanisms: breakdown, diffusion into the environment, and generation of undesirable volatiles. To investigate the loss of 2-AP **1** in rice, rice was stored at 30 °C in three different stages, namely, as paddy, brown (dehulled), and white (milled) rice, and in two different conditions, namely, in air and under reduced pressure (at 84% relative humidity).[151] In all cases, the 2-AP content of the rice diminished by 40−50% after 3 months. Neither reduced oxygen tension nor the way of storage could preserve the flavor compound as a contributor to the desirable characteristics of stored fragrant rice. Another study compared the effect of different drying methods and storage time on losses of 2-AP from aromatic rice.[52] The average 2-AP concentration of the rice samples subjected to the different drying methods had decreased more than four times after 10 months of storage as compared to after 1 month of storage, the highest rates of decrease occurring in the beginning of storage. In general, slightly higher concentrations of 2-AP were found from the drying methods that employed lower temperatures.

2-Acetyl-1-pyrroline **1** and 6-acetyl-1,2,3,4-tetrahydropyridine **3** are among the most important flavor compounds of freshly popped corn. From the observation that the flavor of popcorn is not stable, the stability of four of the most important popcorn flavor compounds was investigated, namely, 6-ATHP (437 μg/kg popcorn), 2-AP (24 μg/kg), 2-propionyl-1-pyrroline (17 μg/kg), and acetylpyrazine (8 μg/kg).[50] Storage in polyethylene bags at room temperature led to a significant decrease of the presence of the flavor compounds. After 1 week of storage, only 25−30 % of the

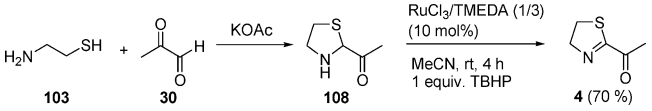
Scheme 41[103]



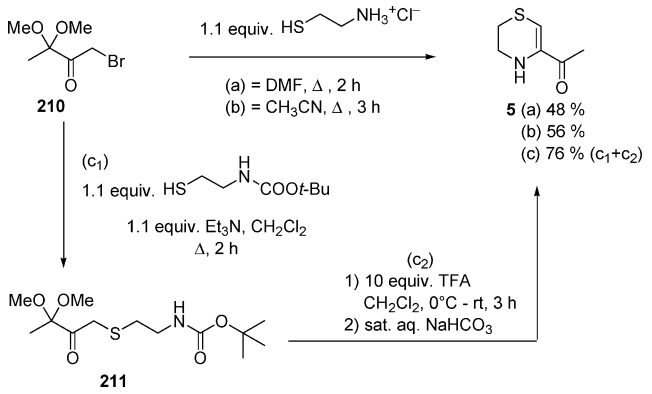
Scheme 42[149]



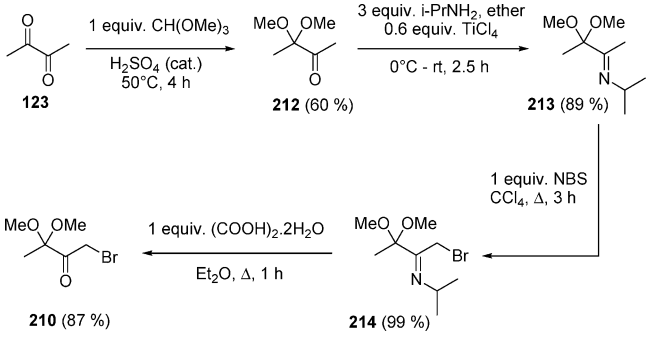
Scheme 43[148]



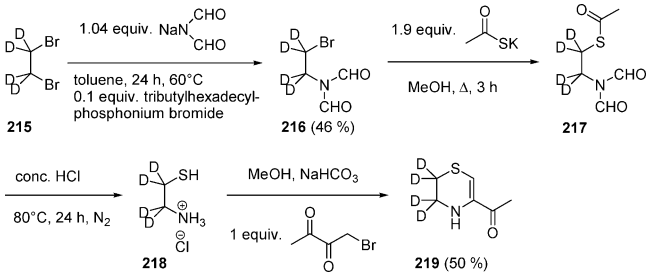
Scheme 44[150]



Scheme 45[150]



Scheme 46[120]



amounts of the flavor compounds remained, except for acetylpyrazine, which remained present in constant amounts. The presence of an aromatic ring system seems to increase the stability of the flavor compound but also involves weaker flavor characteristics, as indicated by significantly higher odor thresholds (cf. Table 3).

The results of these different investigations lead to the conclusion that in order to apply these flavor compounds in food products, their stability has to be increased.

In the flavor industry encapsulation is a popular modern technique for converting a volatile aroma concentrate into a stable powder form. Maltodextrin and gum acacia were tested as wall materials for microencapsulation of 2-AP by spray drying.[152] Whereas the concentration of 2-AP in basic solution decreased by 63% after 7 days and by 30% after 35 days in acidic solution (formation of salts), microencapsulation with 70:30 acacia−maltodextrin resulted in only 28% loss after 72 days of storage. Favino et al.[141] enhanced the stability of 2-AP in the presence of β-cyclodextrin due to the formation of inclusion complexes formed in aqueous solution and in the gas phase, as proven by NMR and mass spectrometry experiments. The same applied to 6-ATHP. Duby and Huynh-Ba synthesized a stable precursor of 2-AP (Scheme 27) and encapsulated 2-AP immediately after hydrolytic conversion on carbohydrate matrixes. With 1% β-cyclodextrin, the flavoring agent remained stable during a period of 110 days at −20 °C.[138] Similar encapsulation procedures can be applied to other carbonyl-protected derivatives of 2-AP that have been developed.[140] Variations of this microencapsulation process have been described for the stabilization of 2-acetyl-1-pyrroline in an easily dispersible powder form.[153] Starch, or gum acacia, was dissolved in water containing an emulsifier, after which a solution of 2-acetyl-1-pyrroline in ethanol was added and homogenized. Subsequently, the mixture was dried by vacuum shelf drying or spray drying to obtain the flavor compound in a dry powder form useful for the flavoring of rice and other food products. Sensory analysis of sweetened basmati rice revealed that the rice with the added flavor was preferred to natural basmati rice.[153]

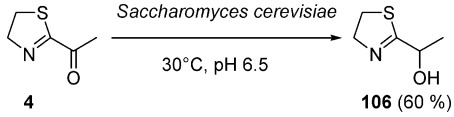
The use of 2-AP **1** as a food flavoring is patented, and the authors describe the preparation of stable salts by physiologically acceptable acids such as citric acid.[154] Also, 6-ATHP **3**, as such or as its bisulfite complex or its salts, was claimed to be useful for flavoring bread and other bakery products.[155,156]

Many applications of 2-acetyl-1-pyrroline **1** as a food flavoring have been patented: for instance, starch foods containing 2-acetyl-1-pyrroline, useful in low-protein diets,[157] a food coating composition manufactured from scented rice, containing at least 40 ppb 2-acetyl-1-pyrroline,[158] distilled alcoholic beverages containing 0.2−200 ppb of 2-acetyl-1-pyrroline,[159] and a 2-acetyl-1-pyrroline-containing flavor composition for tea beverages.[160] Only very recently 2-acetyl-1-pyrroline has been included in GRAS 22 (the 22nd publication by the Expert Panel of FEMA on recent progress in the consideration of flavoring ingredients generally recognized as safe under the Food Additives Amendment), and average usual and maximum use levels in different food categories have been listed.[161]

2-Acetyl-2-thiazoline **4** was shown to be unstable during heat treatment in the presence of water, but a significant stabilization was observed in oil. Fat-containing food matrixes might, therefore, enhance the stability of 2-acetyl-2-thiazoline.[118] The biogeneration and stability of the 2-acetyl-2-thiazoline precursor 2-(1-hydroxyethyl)-4,5-dihydrothiazole **106** was investigated by Bel Rhlid and co-workers.[162] Fermentation of cysteamine, ethyl-L-lactate and D-glucose with baker's yeast yielded a flavor mixture containing both 2-acetyl-2-thiazoline and 2-(1-hydroxyethyl)-4,5-dihydrothiazole. Microbial reduction of 2-acetyl-2-thiazoline **4** with baker's yeast resulted in the formation of 2-(1-hydroxyethyl)-4,5-dihydrothiazole **106** (Scheme 47). Addition of this compound to pizza dough increased the roasted, toasted, and popcorn-like flavor notes of the food product after baking.

Use of 2-acetyl-2-thiazoline **4** has been described in various process flavorings for enhancing the roasty notes of

Scheme 47. Microbial Reduction of 2-Acetyl-2-thiazoline 4 to 2-(1-Hydroxyethyl)-4,5-dihydrothiazole 106[162]



bakery products,[163] for improving the meaty flavor of foodstuffs,[164] for tea beverages,[165] in a coffee flavor formulation,[166] and in various roasted, baked, or fried food products.[149]

7. Summary and Outlook

Since its discovery as the most important flavor compound of cooked rice, 2-acetyl-1-pyrroline **1** has not ceased to reveal its presence in a vast variety of food products, always being one of the most flavor-significant compounds present. 6-Acetyl-1,2,3,4-tetrahydropyridine **3** is the six-membered ring analogue and is often detected in cereal products. Both potent flavor compounds are closely related in formation and occurrence. The sulfur-containing analogues 2-acetyl-2-thiazoline and 5-acetyl-2,3-dihydro-4*H*-1,4-thiazine, possessing the same α-iminoketone unit, demonstrate similar popcorn-like flavor properties. Understanding the mechanisms of formation of these flavor compounds, however, is a difficult task due to the low yields and the high reactivity of intermediates and end products, which hinder isolation and identification. Due to the instability and volatility of these flavor compounds, the search for efficient synthetic strategies and development of stable precursors remain a great challenge for many organic chemists. The extremely low odor thresholds and pleasant cracker-like flavor properties create a large interest for these compounds in the food industry, and many attempts have been undertaken to facilitate their application, for instance, by encapsulation.

A lot of research has been conducted to unravel the chemistry of these extraordinary Maillard flavor compounds, but many questions still have to be answered before their formation can be controlled and their exceptional flavor properties can be optimally applied to enhance the flavor of food products. It is amazing to discover that such simple compounds, which occur in daily foodstuffs, have such a remarkable and fascinating chemical rearrangement behavior in terms of their formation.

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