New and Convenient Syntheses of the Important Roasty, Popcorn-like Smelling Food Aroma Compounds 2-Acetyl-1-pyrroline and 2-Acetyltetrahydropyridine from Their Corresponding Cyclic α-Amino Acids

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Novel straightforward syntheses have been developed supplying the important food odorants 2-acetyl-1-pyrroline (AP) and 2-acetyltetrahydropyridine (ATHP) in high yields. The four-step reaction sequence starts from the N-shielded cyclic α-amino acids L-proline and pipecolinic acid, respectively, which, in the first step, are converted into the N-shielded 2-acetyl derivatives. Removing the shielding group with trifluoroacetic acid afforded the 2-acetylpyrrolidine and 2-acetylpiperidine trifluoroacetate, respectively, which, upon increasing the pH of their aqueous solutions to 7.0, are spontaneously oxidized in high yields into AP (43% based on L-proline) or ATHP (35% based on pipecolinic acid), respectively, by air oxygen. The latter step is an important hint at the last step in the yet unclear formation pathways of both odorants in foodstuffs.

Keywords: *Bread flavor; popcorn flavor; Basmati rice flavor; 2-acetyl-1-pyrroline; 2-acetyltetrahydropyridine*

INTRODUCTION

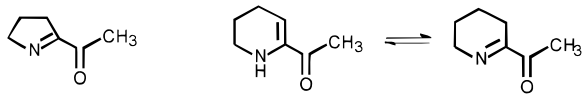
2-Acetyl-1-pyrroline (AP, **1** in Scheme 1) was identified for the first time among the food flavors as a constituent of cooked rice by Buttery and Ling (1982). Application of aroma extract dilution analyses or the odor activity value concept later on established AP as the key odorant in wheat bread crust (Schieberle and Grosch, 1987), popcorn (Schieberle, 1991, 1995), sweet corn (Buttery et al., 1994), and roasted sesame (Schieberle, 1996).

2-Acetyltetrahydropyridine which occurs in a tautomeric equilibrium (ATHP, **2a** and **2b** in Scheme 1) was detected for the first time in a study on wheat bread crust flavor by Hunter et al. (1969) and has recently been established as the key odorant especially in popcorn (Schieberle, 1991, 1995).

Both aroma compounds exhibit a characteristic roasty popcorn-like odor at the extremely low odor thresholds of 0.02 ng/L (AP) or 0.06 ng/L (ATHP) in air, respectively (Schieberle, 1995). Due to their unique flavor properties, both are interesting food flavorings. However, only a limited number of syntheses have been published for either AP (Buttery et al., 1983), ATHP (Büchi and Wuest, 1971), or both (Rewicki et al., 1993; De Kimpe et al., 1993; De Kimpe and Keppens, 1996).

AP and ATHP are known to be relatively unstable when stored, and significant losses may also occur during the preparation and/or purification procedures used in their synthesis. Therefore, there is also a need for a stable intermediate from which both odorants can easily be liberated before use. The purpose of our study was, therefore, to develop a synthetic procedure for the preparation of stable educts yielding straightforward AP and ATHP upon careful treatment.

Scheme 1



1 (Buttery and Ling, 1982)

2a

2b (Hunter et al., 1969)

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially. Triethylamine, *N*-(*tert*-butoxycarbonyl)-L-proline, pipecolinic acid, 2,2′-dipyridyl disulfide, triphenylphosphine, methylmagnesium bromide, and trifuloroacetic acid were from Aldrich (Steinheim, Germany), and 2-[(*tert*-butoxycarbonyl)-oximino]-2-phenylacetonitril and 1,4−dioxane were from Lancaster (Muehlheim, Germany).

Synthesis of 2-Acetyl-1-pyrroline (1). As outlined in Figure 1, **1** was synthesized in a four-step sequence starting from *N*-(*tert*-butoxycarbonyl)-L-proline.

*N-(tert-Butoxycarbonyl)-2-[(2-pyridylthio)carbonyl]pyrroline (**4** in Figure 1).* The preparation of the thioester was performed on the basis of procedures published by Endo et al. (1970) and Aoyagi et al. (1993). A mixture of *N*-(*tert*-butoxycarbonyl)-L-proline (10.0 mmol), 2,2′-dipyridyl disulfide (15 mmol), and triphenylphosphine (15 mmol) in dry acetonitrile (35 mL) was refluxed for 1 h. After the mixture was cooled to room temperature, the solvent was distilled off in vacuo (100 mbar/40 °C), and the residue was purified by flash chromatography (35.9 × 1.9 cm; J. T. Baker BV, Deventen, The Netherlands; model 7022-01). The column was filled with a slurry of silica gel (15 cm high, 30−60 μm, Baker analyzed reagent) in *n*-pentane under a slight pressure of nitrogen. After application of an aliquot of the crude reaction mixture (0.5 mL), chromatography was performed using *n*-pentane (A, 150 mL)

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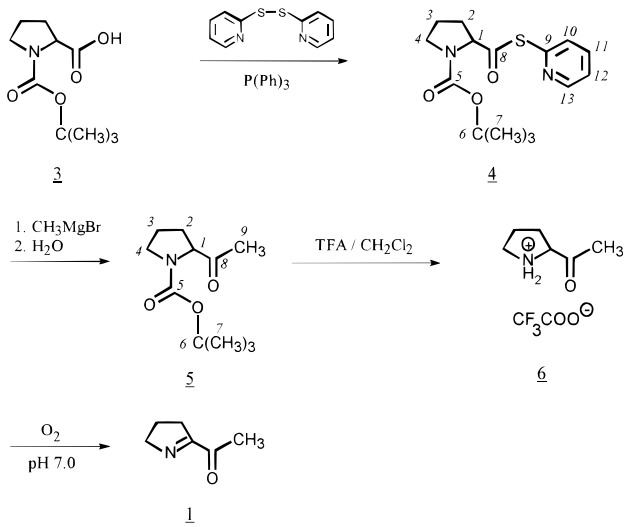


Figure 1. Synthetic route leading to 2-acetyl-1-pyrroline (**1**).

followed by *n*-pentane/diethyl ether mixtures (150 mL each): B (95:5 by volume), C (90:10 by volume), D (80:20 by volume), E (70:30 by volume), and F (50:50 by volume). **4** (9.0 mmol, yield of 90%) which was eluted in fractions E and F was obtained after concentration in vacuo and storage for 1 h at −30 °C as white crystals.

The target compound was characterized by mass spectrometry (D-Cl) and ^{1}H NMR: MS (EI) *m/z* (relative intensity) 168 (100), 170 (80), 253 (80), 253 (80), 309 (55), 198 (18); ^{1}H NMR (360 MHz, $CDCl_3$, numbering of carbon atoms refers to **4** in Figure 1) δ 1.47 (s, 9H, ^{t}Bu), 1.81−2.43 (m, 4H, H at C-2 and C-3), 3.39−3.62 (m, 2H, H at C-4), 4.41 and 4.49 (m, total 1H, H at C-1), 7.21 (br d, 1H, H at C-10), 7.47−7.53 (m, 1H, H at C-11), 7.60−7.65 (m, 1H, H at C-12), 8.55 (br s, 1H, H at C-13).

*2-Acetyl-N-(tert-butoxycarbonyl)pyrrolidine (**5** in Figure 1).* The conversion of the thioester into the 2-alkanone was performed using a method described by Mukaiyama et al. (1973). Methylmagnesium bromide (9.9 mmol or 3.3 mL of a 3.0 M solution in diethyl ether) in dry ethyl ether (6.5 mL) was added dropwise to an ice-cooled, stirred solution of *N*-(*tert*-butoxycarbonyl)-2-[(2-pyridylthio)carbonyl]pyrrolidine (9.0 mmol) in dry diethyl ether (25 mL) maintained under an atmosphere of pure argon. After the cooled mixture was stirred for 1.5 h, water (15 mL) was added and the suspension was extracted three times with diethyl ether (total volume of 100 mL). The organic layer was dried over Na_2SO_4 and then freed from the solvent by evaporation in vacuo (100 mbar/30 °C). The target compound was purified by flash chromatography on silica gel using the solvent mixtures described above. 2-Acetyl-*N*-(*tert*-butoxycarbonyl)pyrrolidine (6.4 mmol, yield of 71%), which was eluted in fractions E and F, was obtained as a pale yellow oil after distilling off the solvent: MS (EI) *m/z* (relative intensity) 70 (100), 57 (88), 114 (47), 41 (27), 43 (21), 170 (12), 96 (11), 140 (8); ^{1}H NMR (360 MHz, $CDCl_3$, numbering of carbon atoms refers to **5** in Figure 1) 1.42 (s, 9H, ^{t}Bu) 1.65−2.29 (m, 4H, H at C-2 and C-3), 2.09 (s, 3H, H at C-9), 3.21−3.45 (m, 2H, H at C-4), 4.15 (m, 1H, H at C-1).

*2-Acetylpyrrolidine Trifluoroacetate (**6** in Figure 1).* Trifluoroacetic acid was added dropwise to a stirred solution of 2-acetyl-*N*-(*tert*-butoxycarbonyl)pyrrolidine (6.4 mmol) in methylene chloride (3 mL) at room temperature. After the mixture was stirred for 1.5 h, the solvent was blown off at 20 °C under a stream of nitrogen, affording crude 2-acetylpiperidine trifluoroacetate (4.1 mmol, yield of 71%). For mass spectral measurements, the 2-acetylpyrrolidine was freed from its salt by the following procedure. **6** (0.1 mmol) was suspended in methylene chloride under an atmosphere of pure argon. After addition of dicyclohexylamine (0.1 mmol), an aliquot of the solution was immediately analyzed by HRGC/MS using the DB-5 column. The mass spectrum of the free 2-acetylpyrrolidine liberated from its salt is displayed in Figure 2.

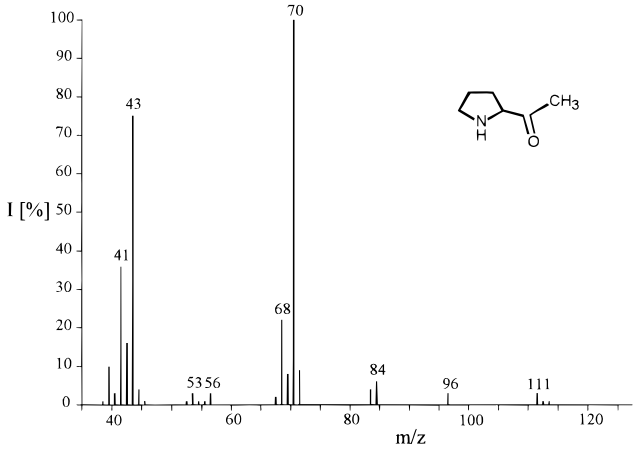


Figure 2. Mass spectrum (MS/EI) of 2-acetylpyrrolidine.

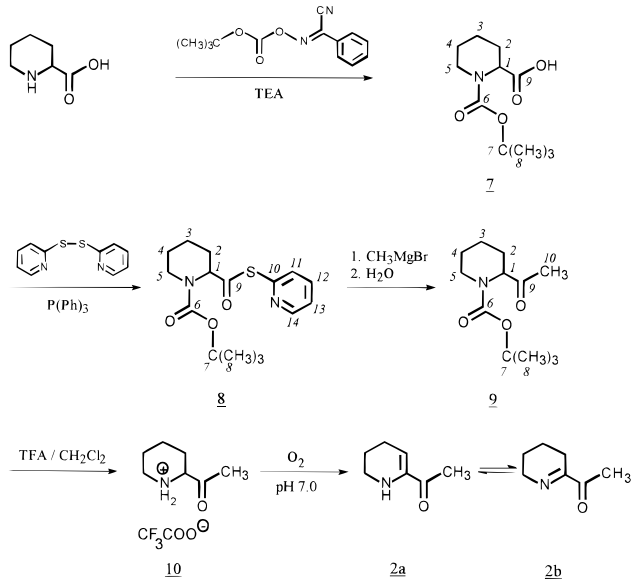


Figure 3. Synthetic route leading to 2-acetyltetrahydropyridine (**2a** and **2b**).

2-Acetyl-1-pyrroline. The crude 2-acetylpyrrolidine trifluoroacetate was suspended in water (20 mL), and the pH of the mixture was adjusted to 7.0. The solution was then stirred for 1 h at 50 °C under an atmosphere of pure oxygen and finally extracted with diethyl ether (4 × 15 mL). The organic layer was dried over Na_2SO_4 and finally concentrated by distilling off the solvent at 35 °C using a Vigreux column and yielding 2-acetyl-1-pyrroline (4.3 mmol, yield of 67%). The MS and ^{1}H NMR data were identical with those reported in the literature (Buttery et al., 1982; De Kimpe and Stevens, 1993).

Synthesis of 2-Acetyltetrahydropyridine (2a and 2b in Scheme 1). The synthesis was performed following the route detailed in Figure 3 starting from pipecolinic acid and *N*-(*tert*-butoxycarbonyl)pipecolinic acid (**7**), respectively.

N-(tert-Butoxycarbonyl)pipecolinic Acid. The target compound was prepared following closely the procedure described by Itho et al. (1975). A mixture of pipecolinic acid (12 mmol), triethylamine (18 mmol), and 2-[(*tert*-butoxycarbonyl)oximino]-2-phenylacetonitrile (13.2 mmol) in 1,4−dioxane/water (15 mL, 1:1 by volume) was stirred for 2 h at room temperature. After the solvent was distilled, water (20 mL) was added and the solution was shaken with ethyl acetate (4 × 20 mL). The aqueous layer was then acidified to pH 3.0 with hydrochloric acid (1 mol/L), and the target compound was isolated by extraction with diethyl ether (four times, total volume of 100 mL). After drying over Na_2SO_4, the solvent was distilled off (100 mbar/30 °C) and the residue was taken up in *n*-pentane/diethyl ether (3 mL, 1:1 by volume). *N*-(*tert*-Butoxycarbonyl)-

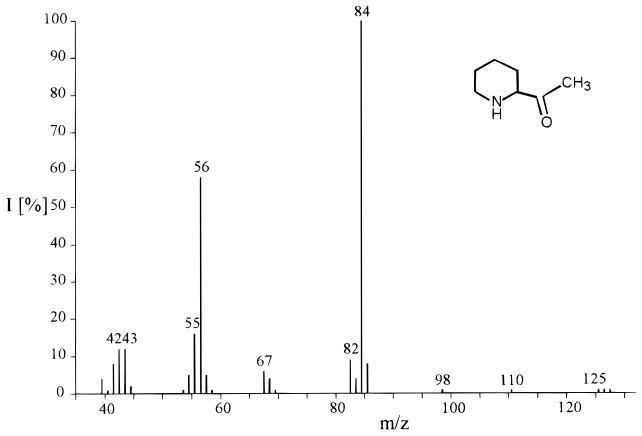


Figure 4. Mass spectrum (MS/EI) of 2-acetylpiperidine.

pipecolinic acid (10.0 mmol, yield of 84%) was obtained by storing the solution at −30 °C as white crystals: MS (D-CI) *m/z* (relative intensity) 175 (100), 230 (90); ^{1}H NMR (360 MHz, $CDCl_3$, numbering of carbon atoms refers to **7** in Figure 3) 1.23−1.73 (m, 5H, H at C-2, C-3, and C-4), 1.49 (s, 9H, ^{t}Bu), 2.27 (br s, 1H, H at C-2), 3.00 (m, 1H, H at C-5), 4.04 (m, 1H, H at C-5), 4.80 and 4.98 (each br s, total 1H, H at C-1), 9.58 (br s, 1H, OH at C-9).

After treatment with 2,2′-dipyridyl disulfide, **7** was then converted into the corresponding thio ester **8** *N*-(*tert*-butoxycarbonyl)-2-[(2-pyridylthio)carbonyl]piperidine in an overall 87% yield: MS (D-CI) *m/z* (relative intensity) 184 (100), 168 (98), 212 (70), 267 (62), 157 (55), 169 (29), 323 (11); ^{1}H NMR (360 MHz, $CDCl_3$, numbering of carbon atoms refers to **8** in Figure 3) δ 1.31−1.80 (m, 5H, H at C-2, C-3, and C-4), 1.44 (s, 9H, ^{t}Bu), 2.27 (br s, H at C-2), 2.97 (m, 1H, H at C-5), 4.06 (m, 1H, H at C-5), 4.84 and 5.05 (each m, total 1H, H at C-1), 7.22 (br d, 1H, H at C-11), 7.48−7.52 (m, 1H, h at C-12), 7.61−7.65 (m, 1H, H at C-13), 8.54 (br s, 1H, H at C-14).

Treatment of **8** with CH_3MgBr then yielded 2-acetyl-*N*-(*tert*-butoxycarbonyl)piperidine (**9** in Figure 3): MS(EI) *m/z* (relative intensity) 84 (100), 57 (83), 128 (58), 184 (13), 155 (7), 110 (6), 227 (1); ^{1}H NMR (360 MHz, $CDCl_3$, numbering of carbon atoms refers to **9** in Figure 3) δ 1.19−1.70 (m, 5H, H at C-2, C-3, and C-4), 1.46 (s, 9H, ^{t}Bu), 2.14 (s, 3H, H at C-10), 2.17 (m, 1H, H at C-2), 2.85 (m, 1H, H at C-5), 3.97 (m, 1H, H at C-5), 4.65 (m, 1H, H at C-1).

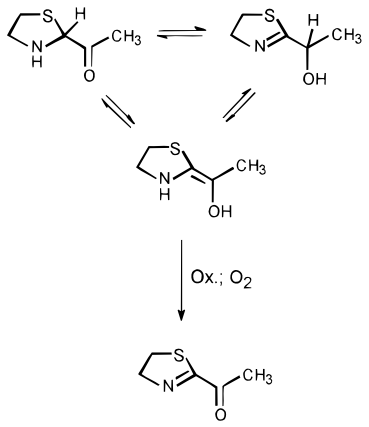
After treatment of **9** with trifluoroacetic acid, 2-acetylpiperidine trifluoroacetate (**10**) was obtained in 67% yield. The mass spectrum of the free 2-acetylpiperidine liberated from its salt at pH 7.0 is shown in Figure 4. By MS/CI, the expected molecular weight of 127 could by established. Oxidation of **10** finally yielded **2a** and **2b** (61% yield) which were characterized by their mass spectra, in good agreement with data published earlier (Schieberle, 1995).

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). High-resolution gas chromatography/mass spectrometry (HRGC/MS) was performed using a DB-5 capillary column (30 m × 0.32 mm; J + W Scientific, Fisons Instruments, Mainz, Germany) which was installed in a type 5300 gas chromatograph (Fisons) coupled to a type 8230 mass spectrometer (Finigan, Bremen, Germany). The mass spectrometer was operated either in the electron impact mode (MS/EI) at 70 eV or in the chemical ionization mode (MS/CI) at 115 eV with isobutane as the reagent gas. The samples were applied by the cold on column injection technique at 40 °C. After 2 min, the oven temperature was raised by 40 °C/min to 50 °C, held for 2 min isothermally, then raised at 6 °C/min to 230 °C, and held for 5 min isothermally.

Direct inlet mass spectra (D-CI) were recorded with the type 8230 mass spectrometer (Finnigan) running in the chemical ionization mode with isobutane as the reagent gas.

NMR Spectroscopy. ^{1}H NMR spectra were recorded in $CDCl_3$ (MSD isotopes, Montreal, Canada) with an AM 360 spectrometer (Bruker, Karlsruhe, Germany). The signals were assigned using tetramethylsilane as the internal standard.

Scheme 2



(according to Hofmann and Schieberle, 1995)

RESULTS AND DISCUSSION

In a previous investigation, we observed that 2-(1-hydroxyethyl)-4,5-dihydrothiazol is easily oxidized into 2-acetylthiazoline simply when heated in water for 10 min in the presence of oxygen (Hofmann and Schieberle, 1995). It might be concluded that the oxidation runs via the tautomeric forms shown in Scheme 2. This result gave us the idea that probably 2-acetylpyrrolidine and 2-acetylpiperidine, having the same structural element of a cyclic α-aminoketone, may also be key intermediates in the generation of the food flavorants 2-acetyl-1-pyrroline (AP) and 2-acetyltetrahydropyridine (ATHP). A literature search, however, revealed that neither the 2-acetylpyrrolidine nor the 2-acetylpiperidine had yet been characterized by analytical data. Therefore, we have developed a concept for the synthesis of cyclic α-aminoketones starting from the *N*-*tert*-butoxycarbonyl-shielded L-proline and pipecolinic acid. The synthetic concept is discussed below for the synthesis of 2-acetyl-1-pyrroline. In the first step, the carboxy function of the *N*-(*tert*-butoxycarbonyl)-L-proline (**3** in Figure 1) is converted into the pyridyl thioester in nearly 90% yield by treatment with 2,2′-dipyridyl disulfide and triphenylphosphine. The thioester **4** obtained was then exposed to methylmagnesium bromide, yielding 2-acetyl-*N*-(*tert*-butoxycarbonyl)pyrrolidine **5** after hydrolysis with water. The structure of **5** was established by the disappearance of the aromatic protons in the ^{1}H NMR spectra and the new signal of the methyl group at δ 2.09 (C-9 in Figure 1). The yield of this Grignard reaction was relatively high (71%). Deprotection of **5** with trifluoroacetic acid then yielded 2-acetylpyrrolidine trifluoroacetate **6**.

In the first experiment, we tried to liberate 2-acetylpyrrolidine from an aliquot of **6** by treatment with a weak base. However, only the oxidized homologue 2-acetyl-1-pyrroline was detectable after HRGC/MS. To establish that we really had the 2-acetylpyrrolidine at hand, an aliquot of **6** was suspended in dichloromethane in a closed vessel under an atmosphere of pure helium. After addition of an aquimolar amount of a weak base (dicyclohexylamine), the 2-acetylpyrrolidine formed was immediately analyzed by HRGC/MS, yielding one main product showing the spectrum displayed in Figure 2. The molecular weight ($M^+ + 1 = 114$) obtained by

Table 1. Time Course of the Generation of 2-Acetyl-1-pyrroline (AP) from 2-Acetylpyrrolidine (APD)[a]

reaction time (min)	AP (μg)	conversion of APD (%)
5	12.0	27
30	32.5	72
60	42.1	94
5[b]	2.0	4

[a] 2-Acetylpyrrolidine trifluoroacetate (0.4 μmol, 45 μg) was dissolved in tap water (1 mL) which had been saturated with oxygen and contained dicyclohexylamine (0.5 μmol) and was allowed to stand at 25 °C in a brown glass vial. [b] The oxygen had been removed by flushing with helium.

running a chemical ionization spectrum confirmed the structure of the 2-acetylpyrrolidine.

In a further experiment, the helium was substituted by pure oxygen while adding the base, which led to a spontaneous oxidation of the 2-acetylpyrrolidine into the 2-acetyl-1-pyrroline in a nearly quantitative yield after 60 min (Table 1).

The same concept was then successfully used in the synthesis of the homologous 2-acetyltetrahydropyridine, indicating 2-acetylpiperidine as an important educt for yielding ATHP simply by treatment of the piperidine with air oxygen.

These results corroborate our previous findings on the formation of the homologuos 2-acetylthiazoline from 2-(1-hydroxyethyl)-4,5-dihydrothiazol or 2-acetylthiazolidine by air oxidation and indicated that obviously α-cycloalkylaminoketones are very susceptible to oxidation.

CONCLUSIONS

Straightforward syntheses of 2-acetylpyrrolidine and 2-acetylpiperidine are presented which allow the general preparation of α-cycloalkylaminoketones from their corresponding cyclic α-amino acids. As their salts, the cyclic amines are attractive stable intermediates for quickly and quantitatively generating the corresponding cyclic imines 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine simply by air oxidation of the free base in either an aqueous or an organic medium. 2-Acetylpyrrolidine and 2-acetylpiperidine may also be formed during the Maillard reaction of carbohydrates and amino acids. Both can, therefore, also be regarded as possible intermediates in the formation of AP and ATHP during food processing. Further studies on this type of oxidation will soon be published in more detail.

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