Formation of 2,3-Dihydro-1*H*-pyrrolizines as Proline Specific Maillard Products

Roland Tressl,* Dieter Rewicki, Bernd Helak, Heinrich Kamperschröer, and Norbert Martin

During the Maillard reaction proline is transformed into more than 20 2,3-dihydro-1*H*-pyrrolizines. In a series of model experiments, L-proline was heated with glyceraldehyde, erythrose, arabinose, glucose, and rhamnose for 1.5 h at 150 °C and the volatiles were investigated by capillary GC–MS and nitrogen selective detector. Individual components were isolated and enriched by preparative GC or HPLC and identified by MS, IR, and ¹H NMR spectroscopy. Nineteen pyrrolizine derivatives (among them 5-formyl-, 5-acetyl-7-methyl-, 7-acetyl-5-methyl-, 7-formyl-5-methyl-, 5-propionyl-, 6-methyl-5-propionyl-, 5-(3-hydroxypropionyl)-, 5-(4-oxopentanoyl)-, 5-(5-methyl-2-furyl)-, 5-(2-hydroxypropionyl)-6,7-dimethyl-, 7-acetyl-5-ethyl-, and 7-formyl-5,6-dimethyl-2,3-dihydro-1*H*-pyrrolizine) were characterized for the first time as proline specific Maillard products.

INTRODUCTION

In 1975 Shigematsu et al. characterized 5-acetyl-2,3-dihydro-1*H*-pyrrolizine, 5-acetyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine, and 5-formyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine on heating equimolar amounts of L-proline and D-glucose at 200 °C for 5 min. These components, which possess smoky, roasty aromas, were determined in beer and eight pyrrolizine derivatives were identified in proline/sugar model systems (Tressl et al., 1981a,b). Pyrrolidines and piperidines with cereal odor and bitter taste qualities were characterized on heating L-proline with monosaccharides (Tressl et al., 1985). In this paper we report the identification of 22 2,3-dihydro-1*H*-pyrrolizines as proline specific Maillard products and their formation by aldol type reactions.

EXPERIMENTAL SECTION

Sample Preparation. Equimolar amounts of L-proline and monosaccharides (0.056 mol of glyceraldehyde, 0.017 mol of erythrose, 0.033 mol of arabinose, 0.028 mol of glucose, 0.028 mol of rhamnose) dissolved in water were autoclaved for 1.5 h at 150 °C in a stainless steel laboratory autoclave equipped with a magnetic stirrer. After cooling the mixtures to room temperature the pH was adjusted to 3 and the solutions were extracted three times with freshly distilled ether. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL on a 20-cm Vigreux column.

Reaction of 2,3-Butanedione and L-Proline. A mixture of 5.9 g of freshly distilled 2,3-butanedione (0.069 mol) and 8.6 g of L-proline (0.075 mol) in 25 mL of redistilled tetrahydrofuran (THF) was autoclaved for 1 h at 170 °C. The resulting reaction mixture was suspended in 150 mL water and THF was removed with ether by continuous steam distillation–extraction by using a Likens-Nickerson apparature. The aqueous residue then was extracted 4 times with ether (100 mL) and the combined ether extracts were dried over anhydrous Na₂SO₄ and concentrated on a 20-cm Vigreux column. The extract was fractionated on Al₂O₃ and analyzed by GC–MS. In Al₂O₃ fraction F5 compound 18 was found in a yield of 4 mg/g of proline. The pyrrolizine derivative could be isolated directly from fraction F5 without further purification. After removal

of the ether the residue of the evaporated Al₂O₃ fraction F5 was dissolved in CDCl₃ and compound 18 was characterized by IR and ¹H NMR spectroscopy.

Adsorption Chromatography. Separation according to polarity of the components was carried out by liquid-solid chromatography. Aliquot portions of the ether extracts were placed on a water cooled column (200 × 0.9 mm i.d.) filled with 8 g of Al₂O₃ 90 basic (activity II-III, Merck) and the constituents were separated into five fractions with 40 mL of F1 pentane–methylene chloride (P–MC) (9:1), F2 P–MC (3:1), F3 P–ether (9:1), F4 P–ether (1:1), and F5 ether. The different fractions were concentrated to a volume of 1 mL and investigated by GC and capillary GC–MS.

High Performance Liquid Chromatography (HPLC). Compound 12 was isolated from the erythrose–proline model system by HPLC fractionation because of its thermal instability. A Waters HPLC Model 510 equipped with an UV absorbance detector (254 nm) was used with a 30 cm × 3.9 mm i.d. µ Bondpack C₁₈ column. Methanol–water (70:30) was used for the mobile phase. The Al₂O₃ fraction F5 from the erythrose–proline system was dissolved in methanol and repeatedly separated by injection of 25-µL portions. The HPLC fractions were combined and extracted with methylene chloride, dried over Na₂SO₄, and evaporated under reduced pressure. The residue was dissolved either in diethyl ether for GC–MS analysis or in CDCl₃ for IR and ¹H NMR spectroscopy.

Gas Chromatography (GC)–Mass Spectrometry (MS). The fractions obtained by liquid-adsorption chromatography were investigated by GC–MS by using a 25 × 0.32 mm i.d. glass capillary column coated with Carbowax 2OM + KOH (column A) and a 50 m × 0.32 mm i.d. glass capillary coated with CP Sil 5CB (Chrompack) (column B) coupled with a Finnigan MAT quadrupole instrument. The same columns were used on a Carlo Erba fractovap 2900 gas chromatograph with a nitrogen selective detector (NSD). The temperature of column A was elevated from 70 to 180 °C at a rate of 2 °C/min (column B from 100 to 260 °C at a rate of 4 °C/min). Mass spectra were recorded at an ionization energy of 70 eV and reported in *m/e* with relative intensities in brackets.

Preparative Gas Chromatography. Separations were performed with a Varian Aerograph 2740-1 with two FID's, a linear temperature program, and an effluent splitter (10:1). Column 1:3 m (2 mm i.d.) glass; 15% Carbowax 20M on 80–90 mesh Chromosorb WAW/DMCS; temperature program 60–230 °C, 4 °C/min. Column 2: 3 m (2 mm i.d.) glass; 5% SP 2401 DB on 100–200 mesh Supel-

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Technische Universität Berlin, D-1000 Berlin 65, West Germany (R.T., B.H., H.K., and N.M.), and Freie Universität Berlin, D-1000 Berlin 33, West Germany (D.R.).
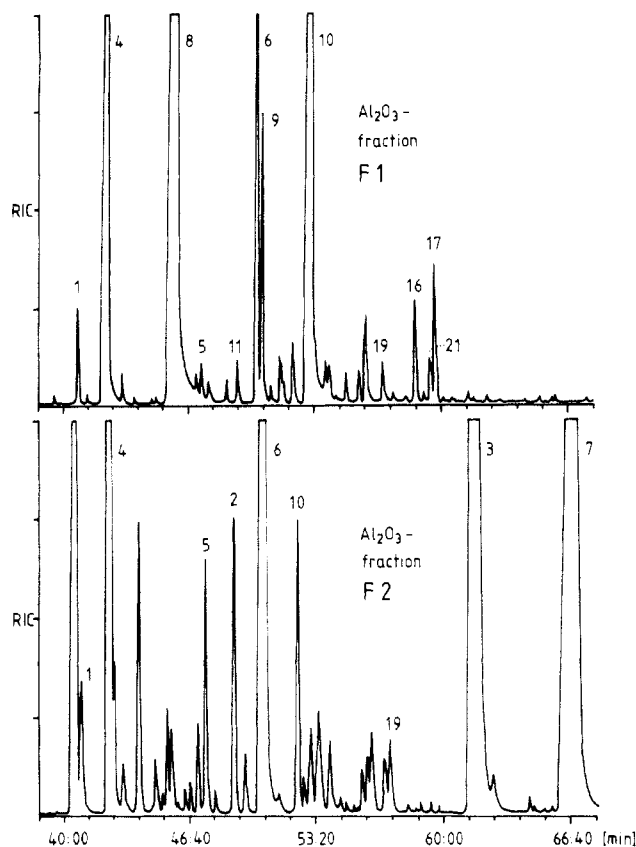
Figure 1. Capillary GC–MS separation of fraction 1 and 2 of the proline/rhamnose model experiment (column A, peak numbers correspond to the component numbers in Table I).

coport; temperature program 100–250 °C, 4 °C/min.

¹H NMR and IR Spectroscopy. ¹H NMR spectra were recorded at 270 MHz on a Bruker WM 270 NMR spectrometer in CDCl₃ solution. Chemical shifts are reported as ppm relative to tetramethylsilane (Me₄Si); coupling constants are in hertz. Infrared spectra were obtained from CDCl₃ or CCl₄ solutions with a Perkin-Elmer Model 357 instrument.

RESULTS AND DISCUSSION

Equimolar amounts of L-proline and monosaccharides were heated for 1.5 h at 150 °C (pH 5–6) in water and the volatiles extracted with ether. The complex mixture of components was separated by liquid–solid chromatography into five fractions according to polarity of the constituents. Each fraction was examined by GC and by capillary GC–mass spectrometry. Figure 1 presents chromatograms of the fractions 1 and 2 from the proline/rhamnose experiment containing most of the pyrrolizine derivatives. Individual components were isolated and enriched by preparative GC and investigated by MS, IR, and ¹H NMR spectroscopy. Table I summarizes the results of this study. Minor constituents were identified by the comparison of MS spectra and GC retentions with isomeric components. The identified 2,3-dihydro-1H-pyrrolizines were quantified by capillary GC–MS as well as nitrogen selective detector. Some of the results are summarized in Table II.

Compound 1 was synthesized by Shigematsu et al. (1975) and the spectroscopic data of the isolated component were comparable to the published data. In addition, the MS and ¹H NMR spectra of compound 2 were consistent with the published data Shigematsu et al., 1975). Compounds 2 and 3 possessed similar mass spectra and 3 was identified as 7-formyl-5-methyl-2,3-dihydro-1H-pyrrolizine by ¹H NMR spectroscopy. The formyl group at C₇ instead of C₅

shifts the signals of the methylene groups at C₁ to lower (δ 3.10) and C₃ to higher field (δ 3.90). The chemical shift of the olefinic proton (δ 6.40, H-6) is coincident with the C₆-position. The gas chromatographic retention of 3 is much higher than that of 2 (I_K 3 = 2424; I_K 2 = 2157). The mass and NMR spectra of 4 and 6 are slightly different from the published data (Shigematsu et al., 1975) and are in agreement with the proposed structures. The mass spectra of 5, 6, and 7 are very similar. The ¹H NMR signals of 7 (δ 3.08 (H-1), 3.94 (H-3), and 6.40 (H-6)) are in agreement with 7-acetyl-5-methyl-2,3-dihydro-1H-pyrrolizine and the observed changes in chemical shifts comparing compounds 7 and 6 correspond to those found for compounds 3 and 2. The GC retention of 7 (I_K = 2506) is in coincidence with an acetyl group at C₇. Compound 5 possessed the lowest retention (I_K = 2126) and was tentatively identified as 5-acetyl-7-methyl-2,3-dihydro-1H-pyrrolizine. The MS, ¹H NMR, and IR spectra of 8 are consistent with 5-propionyl-2,3-dihydro-1H-pyrrolizine. The parent peak at m/e 163 and the fragments at m/e 134 (base peak, M – C₂H₅) and 57 correspond to a propionyl group. The NMR spectrum indicates a propionyl group at δ 1.18 (t, 3 H, J = 7 Hz) and 2.74 (q, 2 H, J = 7 Hz) and the other signals confirm their position at C₅. The mass spectra of 9 and 10 are very similar. Their parent peaks at m/e 177 and base peaks at m/e 148 suggested isomeric propionyl methyl derivatives. The ¹H NMR spectrum of 10 confirms a propionyl group at C₅ and a methyl group. The signal δ 5.76 (s, 1 H, H-7) in 10 is in coincidence with an olefinic proton at the C₇ position. Therefore, 10 was identified as 6-methyl-5-propionyl-2,3-dihydro-1H-pyrrolizine. The isomeric compound 9 which possessed a lower retention was tentatively identified as 7-methyl-5-propionyl-2,3-dihydro-1H-pyrrolizine. Compound 12 is formed on heating L-proline with erythrose and glucose, respectively. 12 is decomposed during preparative GC and could be isolated and purified by HPLC. The parent peak at m/e 179 and base peak at m/e 134 (M – C₂H₄OH) suggested the presence of a hydroxypropionyl group. The IR spectrum confirmed a hydroxy group (3450 (m) and 1070 cm⁻¹ (m)). The ¹H NMR spectrum is in coincidence with a 5-acyl-2,3-dihydro-1H-pyrrolizine (relative positions of the methylene group signals at C₁ and C₃). The signals at δ 2.96 (t, 2 H, J = 7.0 Hz, COCH₂CH₂), 3.96 (t, 2 H, J = 7.0 Hz, CH₂CH₂OH), and 2.90 (broad s, 1 H, OH) confirm 12 as 5-(3-hydroxypropionyl)-2,3-dihydro-1H-pyrrolizine.

The structure of 13 was determined according to mass spectrometric data and chemical reactions. Compound 13 was formed during heating of 12. The parent peak at m/e 161 and base peak at m/e 134 (M – CH=CH₂) correspond to the proposed structure. In addition compound 13 was transformed into 8 by hydrogenation.

The mass spectrum of compound 14 possessed a parent peak at m/e 205, a base peak at m/e 134 (M – 71), and fragments at m/e 162 (M – 43) and 43. The IR spectrum confirmed the presence of one conjugated and one isolated carbonyl group. The ¹H NMR spectrum is consistent with a 5-acyl-2,3-dihydro-1H-pyrrolizine. The signals at δ 2.23 (s, 3 H, COCH₃) and 2.82, 2.85 (each m, 2 H) confirm 14 as 5-(4-oxopentanoyl)-2,3-dihydro-1H-pyrrolizine. The mass spectrum of 15 (parent peak at m/e 219, base peak at m/e 148 (M – 71) and m/e 176 (M – 43) and 43) is in coincidence with a methyl derivative of 14. According to GC retention 15 was tentatively identified as 6-methyl-5-(4-oxopentanoyl)-2,3-dihydro-1H-pyrrolizine.

Compound 16 was isolated from the L-proline/rhamnose system. Its mass spectrum (parent peak at m/e 187 and

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Table I. 2,3-Dihydro-1*H*-pyrrolizines Characterized in Proline/Sugar Model Experiments

(1) 5-Formyl-2,3-dihydro-1*H*-pyrrolizine: MS 135 (100), 134 (67), 106 (62), 79 (22), 77 (14), 120 (14), 52 (9), 85 (8), 51 (8); I_K CW 20M 1993

(2) 5-Formyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 149 (100), 148 (78), 120 (57), 134 (33), 65 (16), 39 (13), 77 (13), 93 (12), 41 (10), 106 (8), 150 (8); I_K CW 20M 2157

(3) 7-Formyl-5-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 149 (80), 120 (43), 65 (12), 39 (11), 77 (10), 91 (9), 150 (8), 118 (6), 93 (6); ^{1}H NMR (270 MHz, CDCl$_3$), δ 2.30 (s, 3 H), 2.50 (qui, 2 H, J = 7.5 z, H-2), 3.10 (t, 2 H, J = 7.5 Hz, H-1), 3.90 (t, 2 H, J = 7.5 Hz, H-3), 6.40 (s, 1 H, H-6), 9.80 (s, 1 H, CHO); IR cm^{-1} 2660 m, 2860 w, 2720 w, 1675 s, 1650 s, 1555 m, 1525 m, 1445 w, 1410 w, 1290 m, 1150 w, 1090 w; I_K CW 20M 2424

(4) 5-Acetyl-2,3-dihydro-1*H*-pyrrolizine: MS 134 (100), 149 (44), 106 (19), 79 (12), 77 (11), 135 (8), 43 (7), 78 (7), 51 (6); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.38 (s, 3 H, COCH$_3$), 2.52 (qui, 2 H, J = 7.3 Hz, H-2), 2.84 (t, 2 H, J = 7.3 Hz, H-1), 4.30 (t, 2 H, J = 7.3 Hz, H-3), 5.88 (d, 1 H, J = 3.4 Hz, H-7), 6.92 (d, 1 H, J = 3.4 Hz, H-6); I_K CW 20M 2021

(5) 5-Acetyl-7-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 163 (44), 120 (16), 149 (9), 43 (7), 91 (6), 77 (6), 162 (5), 93 (5), 65 (4), 164 (3), 39 (2), 41 (2), 79 (2), 92 (2), 118 (2); I_K CW 20M 2126

(6) 5-Acetyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 163 (38), 120 (13), 149 (10), 65 (8), 77 (7), 91 (7), 43 (6), 39 (5), 93 (5); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.38 (s, 3 H, CH$_3$), 2.39 (s, 3 H, COCH$_3$), 2.45 (qui, 2 H, J = 7.4 Hz, H-2), 2.76 (t, 2 H, J = 7.4 Hz, H-1), 4.26 (t, 2 H, J = 7.4 Hz, H-3), 5.70 (s, 1 H, H-7); I_K CW 20M 2187

(7) 7-Acetyl-5-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 163 (54), 134 (18), 162 (17), 77 (11), 149 (10), 106 (9), 146 (7), 120 (7), 91 (7); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.30 (s, 3 H, CH$_3$), 2.37 (s, 3 H, COCH$_3$), 2.58 (qui, 2 H, J = 7.5 Hz, H-2), 3.08 (t, 2 H, J = 7.5 Hz, H-1), 3.94 (t, 2 H, J = 7.5 Hz, H-3), 6.40 (s, 1 H, H-6); I_K CW 20M 2506

(8) 5-Propionyl-2,3-dihydro-1*H*-pyrrolizine: MS 134 (100), 163 (32), 106 (12), 77 (11), 79 (11), 135 (8), 78 (7), 51 (5); ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.18 (t, 3 H, J = 7 Hz, CH$_2$CH$_3$), 2.50 (qui, 2 H, J = 7.4 Hz, H-2), 2.74 (q, 2 H, J = 7 Hz, COC*H*$_2$CH$_3$), 2.83 (t, 2 H, J = 7.4 Hz, H-1), 4.30 (t, 2 H, J = 7.4 Hz, H-3), 5.88 (d, 1 H, J = 3.5 Hz, H-7), 6.92 (d, 1 H, J = 3.5 Hz, H-6); IR cm^{-1} 2770 m, 2930 s, 2860 m, 1740 m, 1650 s, 1540 w, 1470 m, 1650 s, 1540 w, 1470 s, 1450 m, 1410 m, 1350 w, 1280 m, 1100 m, 1030 w, 960 m; I_K CW 20M 2091

(9) 5-Propionyl-7-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 177 (23), 149 (10), 77 (6), 91 (6), 120 (6), 65 (4), 93 (3), 121 (3), 79 (3), 92 (2), 178 (2), 29 (2), 39 (2), 118 (1); I_K CW 20M 2139

(10) 5-Propionyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 177 (24), 149 (10), 120 (8), 65 (8), 77 (10), 91 (6), 121 (4), 92 (4), 93 (4), 178 (3), 79 (2), 118 (2), 57 (2); ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.18 (t, 3 H, J = 7 Hz, CH$_2$C*H*$_3$), 2.39 (s, 3 H, CH$_3$), 2.45 (qui, 2 H, J = 7.4 Hz, H-2), 2.73 (q, 2 H, J = 7 Hz, COC*H*$_2$CH$_3$), 2.77 (t, 2 H, J = 7.4 Hz, H-1), 4.32 (t, 2 H, J = 7.4 Hz, H-3), 5.76 (s, 1 H, H-7); I_K CW 20M 2246

(11) 5-Butanoyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 177 (24), 149 (10), 120 (8), 65 (8), 77 (6), 91 (6), 121 (4), 92 (4), 93 (4), 39 (4), 178 (3), 79 (2), 118 (2), 41 (2), 66 (2), 57 (1)

(12) 5-(3-Hydroxypropionyl)-2,3-dihydro-1*H*-pyrrolizine: MS 134 (100), 179 (48), 107 (28), 106 (21), 79 (14), 77 (13), 135 (12), 150 (12), 78 (8), 136 (7); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.54 (qui, 2 H, J = 7.4 Hz, H-2), 2.85 (t, 2 H, J = 7.4 Hz, H-1), 2.90 (s, 1 H, OH), 2.96 (t, 2 H, J = 7.0 Hz, COCH$_2$), 3.96 (t, 2 H, J = 7.0 Hz, C*H*$_2$OH), 4.31 (t, 2 H, J = 7.4 Hz, H-3), 5.92 (d, 1 H, J = 3.6 Hz, H-7), 6.97 (d, 1 H, J = 3.6 Hz, H-6)

(13) 5-Acryloyl-2,3-dihydro-1*H*-pyrrolizine: MS 134 (100), 161 (98), 160 (58), 106 (34), 77 (30), 132 (27), 79 (25), 78 (19), 55 (16), 51 (12), 104 (8), 162 (7), 118 (7), 120 (6)

(14) 5-(4-Oxopentanoyl)-2,3-dihydro-1*H*-pyrrolizine: MS 134 (100), 106 (15), 162 (15), 205 (11), 79 (10), 77 (10), 135 (10), 107 (7), 78 (6), 55 (6), 43 (5), 51 (3); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.23 (s, 3 H, COCH$_3$), 2.50 (qui, 2 H, J = 7.5 Hz, H-2), 2.82, 2.85 (m, 4 H, COCH$_2$CH$_2$CO), 3.05 (t, 2 H, J = 7.5 Hz, H-1), 4.27 (t, 2 H, J = 7.5 Hz, H-3), 5.88 (d, 1 H, 4 Hz, H-7), 7.0 (d, 1 H, 4 Hz, H-6); IR cm^{-1} 3450 s, 2980 s, 2930 s, 2880 m, 2830 w, 1710 s, 1640 s, 1550 m, 1515 s, 1430 m, 1370 s, 1300 s, 1130 m, 1075 m, 1010 m, 960 m

(15) 5-(4-Oxopentanoyl)-6-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 148 (100), 45 (18), 176 (17), 120 (15), 43 (11), 89 (10), 219 (8), 41 (7), 55 (6), 65 (4); I_K CW 20M 2820

(16) 5-(5-Methyl-2-furyl)-2,3-dihydro-1*H*-pyrrolizine: MS 187 (100), 186 (66), 144 (41), 43, 117 (8), 89 (7), 77 (7), 172 (6), 188 (6), 94 (4), 159 (4), 115 (4), 130 (4), 143 (4), 158 (3); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.34 (s, 3 H, 5'-CH$_3$), 2.53 (qui, 2 H, J = 7.4 Hz, H-2), 2.87 (t, 2 H, J = 7.4 Hz, H-1), 4 10 (t, 2 H, J = 7.4 Hz, H-3), 5.83 (d, 1 H, J = 3.3 Hz, H-7), 5.98 (d, 1 H, J = 3.5 Hz, H-4'), 6.12 (d, 1 H, J = 3.5 Hz, H-3'), 6.38 (d, 1 H, J = 3.3 Hz, H-6); I_K CW 20M 2367

(17) 5-(5-Methyl-2-furyl)-6-methyl-2,3-dihydro-1*H*-pyrrolizine: MS 201 (100), 200 (62), 158 (37), 186 (22), 101 (14), 130 (12), 77 (10), 43 (10), 202 (9), 65 (7), 156 (6), 131 (5), 89 (4), 103 (4), 117 (4); I_K CW 20M 2389

(18) 5-(2-Hydroxypropionyl)-6,7-dimethyl-2,3-dihydro-1*H*-pyrrolizine: MS 162 (100), 207 (12), 134 (5), 91 (3), 77 (3), 65 (3), 170 (1), 119 (1), 192 (1), 190 (1), 53 (1), 51 (1); ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.38 (d, 3 H, J = 7 Hz, CH(OH)C*H*$_3$), 2.11 (s, 3 H, 7-CH$_3$), 2.26 (s, 3 H, 6-CH$_3$), 2.55 (qui, 2 H, J = 7.5 Hz, H-2), 3.08 (t, 2 H, J = 7.5 Hz, H-1), 3.87 (dt, 2 H, J = 7.5 Hz, J = 3 Hz, H-3), 4.13 (s, 1 H, OH), 4.70 (q, 1 H, J = 7 Hz, C*H*(OH)CH$_3$); IR cm^{-1} 3450 s, 2980 s, 2930 s, 2880 m, 2830 w, 1710 s, 1640 s, 1550 m, 1515 s, 1430 m, 1370 s, 1300 s, 1130 m, 1090 w, 1075 m, 1035 s, 1010 m, 960 m; I_K CP-Sil 1966

(19) 5-Acetyl-6,7-dimethyl-2,3-dihydro-1*H*-pyrrolizine: MS 162 (100), 177 (42), 77 (15), 43 (13), 176 (11), 134 (10), 79 (10), 91 (9), 93 (6), 120 (5), 118 (5), 107 (5), 150 (3), 105 (3), 65 (3); I_K CW 20M 2340

(20) 7-Acetyl-5,6-dimethyl-2,3-dihydro-1*H*-pyrrolizine: MS 162 (100), 177 (38), 134 (22), 91 (10), 176 (9), 77 (9), 67 (6), 43 (5), 119 (3), 118 (3), 81 (3), 148 (1), 106 (1); I_K CW 20M 2640

(21) 7-Acetyl-5-ethyl-2,3-dihydro-1*H*-pyrrolizine: MS 162 (100), 177 (36), 134 (18), 77 (7), 119 (6), 106 (6), 132 (5), 120 (4), 118 (4), 117 (4), 79 (4), 43 (4), 148 (2); ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.18 (t, 3 H, J = 7 Hz, CH$_2$C*H*$_3$), 2.35 (s, 3 H, COCH$_3$), 2.54 (qui, 2 H, J = 7 Hz, H-2), 2.78 (q, 2 H, C*H*$_2$CH$_3$), 3.11 (t, 2 H, J = 7 Hz, H-1), 3.95 (t, 2 H, J = 7 Hz, H-3), 6.41 (s, 1 H, H-6); I_K CW 20M 2580

(22) 7-Formyl-5,6-dimethyl-2,3-dihydro-1*H*-pyrrolizine: MS 162 (100), 163 (90), 134 (62), 65 (18), 91 (12), 148 (10), 39 (10), 77 (10), 119 (8), 118 (8), 135 (8), 132 (8), 59 (7), 117 (7), 51 (6), 120 (5), 94 (5), 107 (4), 79 (4), 106 (4); ^{1}H NMR (270 MHz, CDCl$_3$) δ 2.1 (s, 3 H, CH$_3$), 2.13 (s, 3 H, CH$_3$), 2.54 (qui, 2 H, J = 7 Hz, H-2), 3.08 (t, 2 H, J = 7 Hz, H-1), 3.85 (t, 2 H, J = 7 Hz, H-3), 9.76 (s, 1 H, CHO); IR cm^{-1} 2950 m, 2920 s, 2860 s, 2710 w, 1710 m, 1655 s, 1575 w, 1530 s, 1450 w, 1430 m, 1375 m, 1300 m, 1260 m, 1210 m, 1075 m, 1000 m, 975 m; I_K CW-20M 2596

major fragments at m/e 186 (M – H) and 144 (M – 43)) was not suitable to predict a structure. The ^{1}H NMR spectrum of **16** showed a 2,3-dihydro-1*H*-pyrrolizine with a substituent at C$_5$. The signals at δ 5.98 (d, 1 H, J = 3.5 Hz, furan-H4), 6.12 (d, 1 H, J = 3.5 Hz, furan-H3), and 2.34 (s, 3 H, CH$_3$) confirm **16** as 5-(5-methyl-2-furyl)-2,3-dihydro-1*H*-pyrrolizine. According to mass spectrometric

fragmentation and GC retention **17** was tentatively identified as 5-(5-methyl-2-furyl)-6-methyl-2,3-dihydro-1*H*-pyrrolizine.

Compound **18** is formed as a major product by the reaction of L-proline with 2,3-butandione and as a minor component in the other model experiments. **18** was isolated from the proline/2,3-butandione model system. The

Table II. 2,3-Dihydro-1H-pyrrolines Characterized in Proline/Monosaccharide Model Experiments[a]

		GLY	ERY	ARA	GLU	RHAM
1		20	40	5	10	30
2		22	2	15	15	50
3		60	10	15	15	380
4		350	30	150	150	290
5		55	2	–	5	30
6		480	10	40	20	260
7		12	9	4	1	300
8		–	30	9	15	300
9		–	5	–	1	20
10		2	5	–	5	200
11		–	–	–	–	5
12		–	50	–	80	–
13		–	5	–	5	–
14		–	–	–	2	80
15		–	–	–	10	60
16		–	–	–	–	8
17		–	–	–	–	10
18						
19		8	2	2	1	7
20		–	+	–	+	50
21		+	+	–	+	60
22		+	+	+	4	100

[a] Figures represent concentrations in mg/kg (proline + sugar).

parent peak at m/e 207 and a base peak at m/e 162 (M – C_2H_4OH) suggested a hydroxypropionyl dimethyl derivative. The IR spectrum confirmed a hydroxy group and a conjugated carbonyl group. The NMR spectrum showed two methyl groups (δ 2.11 and 2.26) attached to the pyrrolizine system. The signals at δ 1.38 (d, 3 H, J = 7 Hz, CH(OH)CH_3), 4.13 (s, 1 H, OH), and 4.70 (q, 1 H, J = 7 Hz, CH(OH)CH$_3$) are in coincidence with the proposed structure.

Components 19–21 possess similar mass spectra with parent peaks at m/e 177, base peaks at m/e 162 (M – CH_3), and fragments at m/e 134 (M – 43) and 43. These results correspond to acetyldimethyl- or acetylethyl-2,3-dihydro-1H-pyrrolizines. Compound 21 was isolated by preparative GC and investigated by ^{1}H NMR spectroscopy. The spectroscopic data are in coincidence with a 7-acetyl derivative and the signals at δ 1.18 (t, 3 H, 7 Hz) and 2.78 (q, 2 H, 7 Hz) confirm an ethyl substituent. The olefinic proton δ 6.41 (s, 1 H) corresponds to C_6. Therefore, 21 was identified as 7-acetyl-5-ethyl-2,3-dihydro-1H-pyrrolizine. The mass spectra of components 19 and 20 possess a fragment at m/e 176 (M – H) which is not formed from 21. This fragmentation is typical for dimethyl derivatives. According to GC retention compound 19 was tentatively identified as 5-acetyl-6,7-dimethyl- and 20 as 7-acetyl-5,6-dimethyl-2,3-dihydro-1H-pyrrolizine. The mass spectrum of compound 22 (parent peak at m/e 163, a base peak at m/e 162, and a major fragment at m/e 134 (M – 29) suggested a formyldimethyl or formylethyl derivative. The IR spectrum confirmed a conjugated formyl group. The ^{1}H NMR spectrum of 22 showed two methyl groups and a formyl group (δ 9.76 (s, 1 H)), and the other signals are consistent with the proposed structure.

Formation of 2,3-Dihydro-1H-pyrrolizines in Proline/Sugar Model Experiments. Among more than 120 proline specific Maillard products, which were characterized in proline/monosaccharide model experiments, pyrrolizine derivatives are formed as major constituents. As demonstrated in Table II they are produced in different amounts depending on the reducing sugar. Compounds 1–6 are main constituents in the proline/glyceraldehyde and proline/rhamnose model systems. Rhamnose is the best precursor for the compounds possessing propionyl groups, for 7-acetyl-(7-formyl)-5-methyl derivatives, and for compounds 14–17. The formation of components 1–7 from glyceraldehyde demonstrates that aldol type reactions may be operative. During the Maillard reaction proline acts as a catalyst, bringing about the 1,2- and 2,3-enolizations of the sugars, leading to reactive 3- and 1-deoxyosones depending on the pH of the medium. 3-Deoxyosones undergo dehydrations forming furans and 1-deoxyosones are transformed into furanones and γ-pyranones. In addition the osones (and sugars) undergo retroaldol cleavage forming reactive α-dicarbonyls which react with proline by Strecker degradation.

During this reaction proline and α-dicarbonyls form iminium carboxylate intermediates which are decarboxylated to reactive ylides or iminium ions which act as key compounds for proline specific components. As demonstrated, proline and glyceraldehyde (pyruvaldehyde) form 1-pyrroline and acetol, pyrrolidine and pyruvaldehyde, and 1-acetonylpyrrolidine as well as 2-acetyl-1,4,5,6- and 2-acetyl-3,4,5,6-tetrahydropyridines (Tressl et al., 1981a,b) from these reactive intermediates. As outlined in Figure 2 the formation of 2,3-dihydro-1H-pyrrolizines is also possible in this reaction sequence.

α-Dicarbonyls and α-hydroxy carbonyls (which are common products from Strecker degradation with proline)

Proline Specific Maillard Products

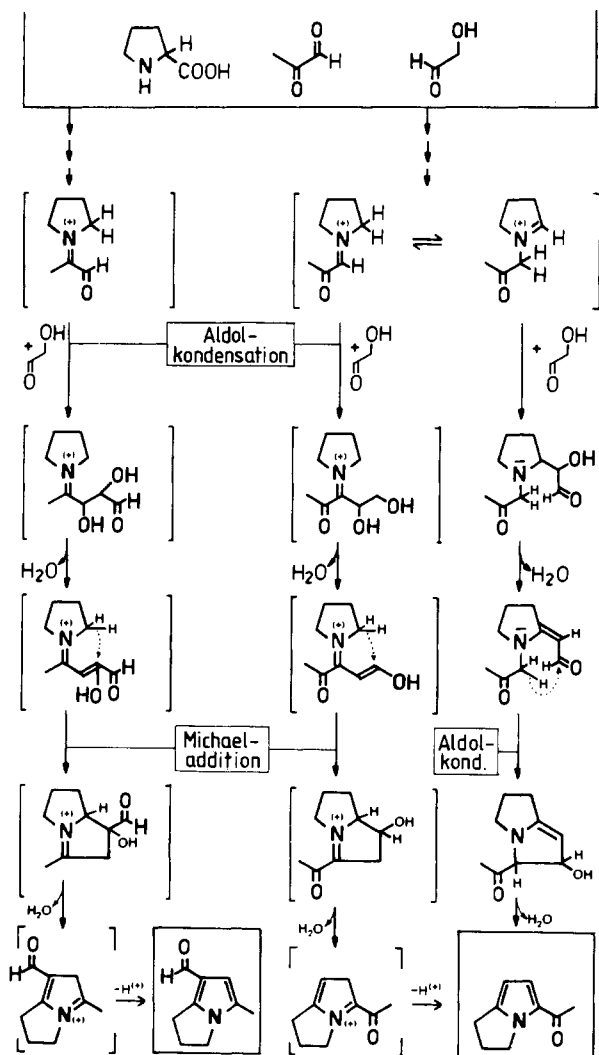*J. Agric. Food Chem.*, Vol. 33, No. 5, 1985 **923**

Figure 2. Scheme to explain the formation of 2,3-dihydro-1*H*-pyrrolizines in proline monosaccharide model experiments.

may form intermediates which are transformed by Michael addition or aldol condensation and dehydration into the characterized 2,3-dihydro-1*H*-pyrrolines. Vinylogous amides were also identified during the Maillard reaction of proline. Compound 12 is only formed from erythrose and glucose. As shown in Figure 3 compound 12 may be formed from the 3-deoxyosone of erythrose. The reactive iminium ion intermediate and glycolaldehyde may form a six carbon derivative which is transformed by Michael addition and dehydration into 5-(3-hydroxypropionyl)-2,3-dihydro-1*H*-pyrrolizine. In addition the iminium ion intermediate is transformed by ring elongation, dehydration, and Michael addition into the tetrahydropyridine derivative. Both components were characterized as main constituents from erythrose and glucose. Components 14–17 may be explained by analogous reactions from the 3-deoxyosone of rhamnose, glycolaldehyde, and pyruvaldehyde, respectively.
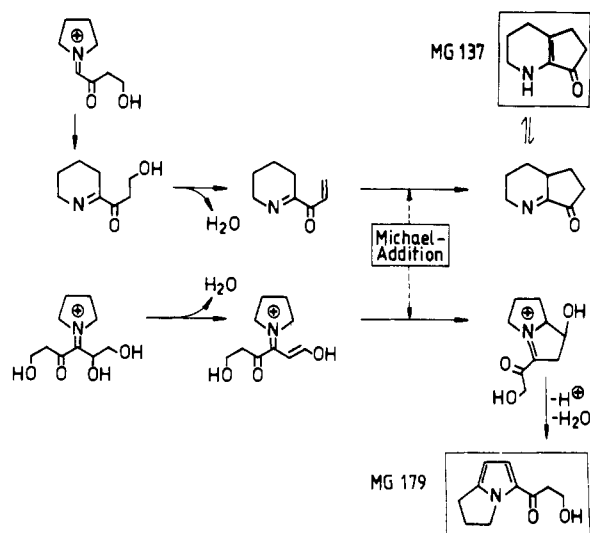

Figure 3. Reaction scheme to explain the formation of 5-(3-hydroxypropionyl)-2,3-dihydro-1*H*-pyrrolizine and 1,2,3,4,5,6-hexahydrocyclopenta(*b*)pyridine-7(1*H*)-one in the proline/erythrose model experiment.

Compound 18 and 5,6-dimethyl-2,3-dihydro-1*H*-pyrrolizine are formed on heating L-proline with 2,3-butandione. Therefore, 2,3-butandione may act as precursor forming compounds 19, 20, and 21 in the proline/rhamnose model experiments. Amino acids with primary amino groups form α-amino carbonyls during Strecker degradation which are further transformed into pyrazines. Hydroxyproline and α-dicarbonyls form pyrroles by this reaction. Both components were not detected in the proline model experiments. Therefore, 2,3-dihydro-1*H*-pyrrolizines may be seen as proline specific Maillard products.

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