Formation of Furylpyrrolidines and -piperidines on Heating L-Proline with Reducing Sugars and Furancarboxaldehydes

Bernd Helak, Evelyn Kersten, Kurt Spengler, Roland Tressl,* and Dieter Rewicki*

On heating L-proline, furancarboxaldehydes, and monosaccharides to 150 °C for 1.5 h, 2-(2-furyl)-pyrrolidines, -1-pyrrolines, -piperidines, and -3,4,5,6-tetrahydropyridines as well as 3-furfurylidene-1-pyrrolines and 3-furfurylpyrroles were formed. The individual components were characterized and quantified by capillary GC–MS and isolated by liquid–solid chromatography or preparative GC. Their structures were established by MS, IR, and ^{1}H NMR spectroscopy and, in some cases, by separate synthesis. Compounds **1–16** were identified for the first time as proline-specific Maillard products.

During the Maillard reaction of L-proline and reducing sugars more than 120 proline-specific compounds are formed depending on the reaction conditions and the sugars used. Recently, we described the isolation and characterization of 2,3-dihydro-1*H*-pyrrolizines, 2-(1-pyrrolidinyl)-2-cyclopentenones, di- and tetrahydro-1*H*-azepines, several N-substituted pyrrolidines, and 2-acetyl- and 2-furylpiperidines (Tressl et al., 1985a–c). The 2-furylpiperidines were isolated from proline/arabinose and -rhamnose experiments and were synthesized according to La Londe et al. (1977) by reduction of the corresponding tetrahydropyridines with $NaBH_4$. In the present paper we show that some of the closely related 2-furylpyrrolines and -pyrrolidines are main constituents of the same proline/sugar model systems. Furthermore, we describe the formation of these compounds by reaction of arabinose and rhamnose with azetidinic acid as well as their synthesis in analogy to the method of La Londe et al. In view of their way of formation, additional reactions of proline, pyrroline, and pyrrolidine with furancarboxaldehydes were carried out. From these experiments some 3-substituted 1-pyrrolines and pyrroles were isolated and identified, the structures of which are related to known lysine-specific piperidine derivatives.

EXPERIMENTAL SECTION

Sample Preparation. Equimolar amounts of L-proline and the reducing monosaccharide (0.02–0.06 mol of glyceraldehyde, erythrose, arabinose, glucose, and rhamnose, respectively) dissolved in 50 mL of water were autoclaved for 1.5 h at 150 °C in a stainless steel laboratory autoclave equipped with a 100-mL duran glass tube and heated by an electric heater with a magnetic stirrer. After the mixture was cooled to room temperature, it was adjusted to pH 10 with 0.1 N NaOH and the solution extracted three times with 50 mL of freshly distilled diethyl ether. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL on a 20-cm Vigreux column. Aliquot amounts of the extracts were investigated by capillary GC–MS and a nitrogen-selective detector. L-Proline and L-ascorbic acid as well as azetidinic acid and arabinose and rhamnose, respectively, were autoclaved and worked up in the same manner.

Reaction of L-Proline, Pyrrolidine, and 1-Pyrroline with Furfural. L-Proline and furfural and 5-methyl- and 5-(hydroxymethyl)furfural, respectively, were heated and worked up as described above. In the case of pyrrolidine the aqueous reaction mixture was refluxed for 1 h and extracted at the resulting pH (about 11). 1-Pyrroline, prepared according to Quick and Oterson (1976) and used as 10% ethanolic solution, and the aldehydes led to different products depending on the conditions used: The 3-substituted 1-pyrrolines **11–13** were predominantly formed in water at 20 °C during 24 h and extracted by diethyl ether at the resulting pH of 10–11. The 3-pyrroles **15** and **16** were mainly produced at higher temperatures (1.5 h at 160 °C) in absolute ethanol. The yield increased by addition of sodium methylate and calcium oxide for effective removal of the water formed during the reaction. The ethanolic solutions were evaporated under vacuum and the products transferred into diethyl ether.

Synthesis of 2-(2-Furyl)- and 2-(5-Methyl-2-furyl)pyrrolidine and -piperidine. According to La Londe et al. (1977) compounds **1–8** were synthesized by N-acylation of 4-aminobutanoic and 5-aminopentanoic acids with 2-furoyl and 5-methyl-2-furoyl chloride, respectively. The resulting crystalline amido carboxylic acids were decarboxylated by heating with CaO, and by simultaneous cyclization the corresponding 1-pyrrolines and 3,4,5,6-tetrahydropyridines were formed. These compounds were reduced to the pyrrolidines **9** and **10** and piperidines **3** and **4**, respectively, by $NaBH_4$. The synthesized derivatives were characterized by MS, IR, and ^{1}H NMR spectroscopy.

Gas Chromatography (GC)–Mass Spectrometry (MS). The ether extracts prepared by the described experiments were analyzed qualitatively and quantitatively by GC–MS using a 50-m fused silica capillary (0.32-mm i.d.) coated with Carbowax 20M + KOH and coupled with a Finnigan MAT 4500 quadrupole instrument. Conditions were as follows: temperature program, 70–180 °C at 2 °C/min; ionization voltage, 70 eV; resolution, 1000.

Adsorption Chromatography. According to their polarity, the components were separated by liquid–solid chromatography. Aliquot portions of the ether extracts were placed on a water-cooled column (200 × 9 mm) filled with 8 g of Al_2O_3 90 basic (activity II–III, Merck), and the constituents were separated into five fractions with 40 mL of F1 *n*-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 capillary GC–MS. Most of the separated compounds, especially those prepared by synthesis, could be used for IR and ^{1}H NMR without further purification.

High-Performance Liquid Chromatography (HPLC). Compound **13** was isolated from the 5-(hydroxymethyl)furfural/1-pyrroline reaction mixture by HPLC because of its high polarity. A Waters HPLC Model 510 equipped with an M-490 UV absorbance detector (254 nm) was used with a 25 cm × 4.6 mm (i.d.) Spherisorb ODS (RP 18) column. Methanol–water (80:20)

Technische Universität Berlin, Seestrasse 13, D-1000 Berlin 65, Germany (B.H., E.K., K.S., R.T.), and Freie Universität Berlin, Takustrasse 3, D-1000 Berlin 33, Germany (D.R.).

0021-8561/89/1437-0405$01.50/0 © 1989 American Chemical Society

Table I. Spectroscopic Data of 2- and 3-Substituted 2-Furyltetrahydropyridines, -piperidines, -3-hydroxypiperidines, -1-pyrrolines, -pyrrolidines, and -pyrroles, Characterized in L-Proline Model Experiments

no.	I_{KI}	component	spectroscopic data: IR, cm^{-1}; MS, m/e (rel intens); ^1H NMR, δ
1	1872	2-(2-furyl)-3,4,5,6-tetrahydropyridine	IR: 3120 (w), 1635 (s), 1485 (s), 1355 (s), 1260 (m), 1160 (s), 1010 (s) MS: 149 (M^+, 100), 148 (20), 134 (8), 122 (73), 120 (14), 108 (4), 107 (3), 106 (3), 94 (21), 93 (96), 91 (5), 81 (38), 79 (4), 77 (5), 65 (21), 55 (13), 53 (10), 51 (10), 41 (21) ^1H NMR: 1.66, 1.78 (each mc, 2 H, 4-H, 5-H), 2.50 (tt, 2 H, J = 6.4 Hz, 2.0 Hz, 3-H), 3.81 (tt, 2 H, J = 5.8 Hz, 2.0 Hz, 6-H), 6.22 (dd, 1 H, J = 3.8 Hz, 1.6 Hz, furan 4-H), 6.69 (d, 1 H, J = 3.8 Hz, furan 3-H), 7.46 (d, 1 H, J = 1.6 Hz, furan 5-H)
2	1984	2-(5-methyl-2-furyl)-3,4,5,6-tetrahydropyridine	IR: 3110 (w), 1635 (s), 1535 (s), 1350 (m), 1205 (m), 1090 (m), 1020 (m), 925 (m) MS: 163 (M^+, 84), 148 (12), 136 (7), 135 (68), 134 (10), 121 (4), 120 (13), 108 (35), 107 (100), 106 (16), 95 (35), 93 (4), 91 (4), 81 (4), 79 (10), 77 (14), 65 (6), 55 (8), 53 (17), 51 (15), 43 (17), 41 (8)
3	1660	2-(2-furyl)piperidine	IR: 3260 (m), 3120 (w), 1635 (s), 1450 (m), 1010 (m) MS: 151 (M^+, 47), 150 (25), 123 (25), 122 (100), 109 (23), 108 (16), 95 (89), 94 (81), 81 (25), 80 (17), 77 (10), 67 (14), 65 (12), 53 (11) ^1H NMR: 1.50, 1.62 (each mc, 2 H, 4-H, 5-H), 1.90 (m, 3 H, 3-H and NH), 2.78 (mc, 1 H, 6-H), 3.15 (mc, 1 H, 6-H), 3.76 (dd, 1 H, J = 10.5 Hz, 2.8 Hz, 2-H), 6.16 (dt, 1 H, J = 3.25 Hz, 0.9 Hz, furan 3-H), 6.31 (dd, 1 H, J = 3.23 Hz, 1.90 Hz, furan 4-H), 7.34 (dd, 1 H, J = 1.9 Hz, 0.9 Hz, furan 5-H)
4	1740	2-(5-methyl-2-furyl)piperidine	IR: 3260 (m), 3120 (m), 1635 (w), 1570 (m), 1440 (m), 1320 (m), 1220 (m), 1110 (m), 1020 (m) MS: 165 (M^+, 52), 164 (23), 150 (30), 148 (18), 136 (60), 123 (20), 122 (61), 109 (100), 108 (57), 95 (25), 94 (20), 81 (13), 53 (14), 43 (21) ^1H NMR: 1.44, 1.57, 1.83 (each mc, 2 H, 4-H, 3-H, 5-H), 2.03 (br s, 1 H, NH), 2.19 (s, 3 H, furan 5-CH_3), 2.70 (dt, 1 H, J = 12 Hz, 2.4 Hz, 6-H), 3.05 br d, 1 H, J = 1i Hz, 6-H), 3.62 (dd, 1 H, J = 12 Hz, 2.8 Hz, 2-H), 5.86, 5.98 d, each 1 H, J = 3 Hz, furan 3-H and 4-H)
5	2112, 2184	2-(2-furyl)-3-hydroxypiperidine (two diastereomers with identical MS)	MS: 167 (M^+, 22), 149 (4), 138 (2), 124 (5), 123 (7), 122 (34), 110 (14), 109 (5), 96 (100), 95 (80), 94 (58), 81 (37), 80 (15), 69 (8), 67 (12), 53 (10), 43 (8)
6	2169, 2242	2-(5-methyl-2-furyl)-3-hydroxy-piperidine (two diastereomers with identical MS)	MS: 181 (M^+, 25), 163 (3), 138 (11), 136 (34), 122 (13), 120 (10), 110 (78), 109 (100), 108 (39), 95 (39), 94 (18), 83 (8), 80 (10), 79 (10), 65 (4), 53 (10), 43 (19)
7	1780	2-(2-furyl)-1-pyrroline	IR: 3120 (w), 1615 (s), 1490 (s), 1320 (s), 1055 (s), 1015 (s), 970 (s) MS: 135 (M^+, 100), 134 (57), 120 (6), 118 (6), 108 (14), 107 (85), 106 (13), 94 (19), 93 (32), 79 (31), 77 (9), 65 (6), 52 (7), 51 (6), 39 (31) ^1H NMR: 1.97 (mc, 2 H, 4-H), 2.85 (mc, 2 H, 3-H), 4.02 (mc 2 H, 5-H), 6.46 (dd, 1 H, J = 3.8 Hz, 1.6 Hz, furan 4-H), 6.80 (d, 1 H, J = 3.8 Hz, furan 3-H), 7.51 (d, 1 H, J = 1.6 Hz, furan 5-H)
8	1865	2-(5-methyl-2-furyl)-1-pyrroline	MS: 149 (M^+, 100), 148 (35), 134 (21), 121 (87), 107 (39), 106 (25), 95 (7), 91 (4), 79 (11), 77 (98), 66 (21), 53 (19), 51 (26), 43 (35), 41 (24)
9	1620	2-(2-furyl)pyrrolidine	IR: 3680 (w), 3350 (s), 3120 (w), 1600 (m), 1505 (m), 1340 (m), 1150 (m), 1000 (s) MS: 137 (M^+, 36), 136 (22), 120 (8), 109 (100), 108 (27), 94 (18), 81 (32), 80 (34), 70 (10), 68 (11), 53 (17), 41 (35) ^1H NMR: 1.7–2.2 (m, 4 H, 3-H, 4-H), 2.49 (s, 1 H, NH), 3.02 (symm mc, 2 H, 5-H), 4.20 (t, 1 H, J = 6.2 Hz, 2-H), 6.16 (d, 1 H, J = 3.2 Hz, furan 3-H), 6.30 (dd, 1 H, J = 1.8 Hz, furan 4-H), 7.36 (d, 1 H, J = 1.8 Hz, furan 5 H)
10	1695	2-(5-methyl-2-furyl)pyrrolidine	MS: 151 (M^+, 71), 150 (31), 136 (38)e, 134 (30), 123 (100), 122 (21), 108 (30), 95 (47), 94 (25), 80 (29), 79 (26), 70 (15), 65 (5), 58 (21), 43 (52), 41 (35)
11	2055, 2065	3-firfurylidene-1-pyrroline (E and Z isomers with identical MS)	IR: 3120 (w), 1640 (w), 1580 (m), 1485 (m), 1359 (m), 1020 (m), 920 (m) MS: 147 (M^+, 100), 146 (30), 130 (3), 119 (38), 118 (63), 117 (5), 104 (5), 91 (70), 80 (9), 78 (11), 65 (28), 63 (17), 59 (8), 51 (24), 41 (35) ^1H NMR: 2.79 (dt, 2 H, J = 5.7 Hz, 2.8 Hz, 4-H), 4.28 (dt, 2 H, J = 5.7 Hz, 2.3 Hz, 5-H), 6.44, 6.48 (AB of ABX, each 1 H, J_{34} = 3.2 Hz, J_{45} = 1.9 Hz, furan 3-H, 4-H), 6.66 (t, 1 H, J = 2.8 Hz, –CH=), 7.47 (d, 1 H, J = 1.9 Hz, furan 5-H), 7.85 (t, 1 H, J = 2.3 Hz, 2-H) (E isomer)
12	2128 (Z), 2135 (E)	3-(5-methylfurfurylidene)-1-pyrroline (E and Z isomers with identical MS)	IR: 3120 (w), 1640 (m), 1575 (m), 1520 (m), 1260 (7), 1020 (s) MS: 161 (M^+, 100), 160 (10), 146 (14), 133 (7), 119 (29), 118 (51), 117 (16), 105 (9), 95 (6), 91 (38), 80 (11), 77 (13), 65 (24), 53 (13), 51 (20), 43 (47) ^1H NMR: 2.33 (s, 3 H, CH_3), 2.75 (mc, 2 H, 4-H), 4.16 (mc, 2 H, 5-H), 6.07 (d, 1 H, J = 3.3 Hz, furan H), 6.34 (d, 1 H, J = 3.3 Hz, furan H), 6.59 (t, 1 H, J = 2.7 Hz, –CH=), 7.83 (t, 1 H, J = 2.1 Hz, 2-H) (E isomer) 2.35 (s, 3 H, CH_3), 2.64 (mc, 2 H, 4-H), 4.02 (mc, 2 H, 5-H), 6.00 (d, 1 H, J = 3.3 Hz, furan H), 6.1 (d, 1 H, J = 3.3 Hz, furan H), 6.27 (t, 1 H, J = 2.7 Hz, –CH=), 8.71 (t, 1 H, J = 2.1 Hz, 2-H) (Z isomer)
13	>2600	3-[5-(hydroxymethyl)furfurylidene]-1-pyrroline (E and Z isomers with identical MS)	IR: 3620 (m), 3200 (s), 1640 (m), 1575 (m), 1520 (m), 1215 (m), 1140 (m), 1020 (m) MS: 177 (M^+, 100), 167 (10), 160 (24), 146 (31), 130 (29), 119 (11), 118 (26), 117 (11), 103 (4), 9 (17), 80 (6), 77 (8), 65 (8), 51 (7) ^1H NMR: 2.75 (mc, 2 H, 4-H), 3.34 (br s, 1 H, OH), 4.22 (mc, 2 H, 5-H), 4.60 (s, 2 H, CH_2OH), 6.36, 6.39 (AB, each 1 H, J = 3.5 Hz, furan 3-H, 4-H), 6.63 (t, 1 H, J = 2.7 Hz, –CH=), 7.76 (t, 1 H, J = 2.1 Hz, 2-H) (E isomer)
14	2045, 2050	3-furfurylidenepyrrolidine (E and Z isomers with identical MS)	IR: 3200 (m), 3120 (w), 1490 (m), 1255 (m), 1015 (s) MS: 149 (M^+, 100), 148 (46), 134 (4), 120 (51), 119 (13), 106 (7), 93 (06), 91 (44), 81 (27), 80 (25), 69 (27), 68 (43), 58 (6), 51 (14)

Table I (Continued)

no.	I_{KI}	component	spectroscopic data: IR, cm^{-1}; MS, m/e (rel intens); ^1H NMR, δ
15	2287	3-furfurylpyrrole	IR: 3500 (s), 3430 (s), 3120 (w), 2900 (m), 1545 (w), 1505 (w), 1430 (w), 1150 (w), 1070 (m), 1060 (m), 1010 (s), 960 (m), 935 (m), 885 (m), 600 (w) MS: 147 (M^+, 100), 146 (59), 130 (7), 118 (94), 117 (30), 104 (6), 93 (17) 91 (45), 80 (25), 74 (11), 67 (28), 65 (21), 59 (25), 53 (22), 41 (12) ^1H NMR: 3.88 (s, 2 H, CH_2), 6.00 (dd, 1 H, J = 3.3 Hz, 1 H, furan 3-H), 6.12 (m, 1 H, J = 2.5 Hz, 4-H), 6.26 (dd, 1 H, J = 3.3 Hz, 1.8 Hz, furan 4-H), 6.62 (m, 1 H, J = 2.0 Hz, 2-H), 6.72 (m, 1 H, J = 2.5 Hz, 2.0 Hz, 5-H), 7.28 (d, 1 H, J = 1.8 Hz, 1 Hz, furan 5-H), 8.04 (br s, 1 H, NH)
16	2355	3-(5-methylfurfuryl)pyrrole	IR: 3500 (s), 3420 (m), 3120 (w), 2925 (m), 1570 (m), 1220 (m), 1070 (m), 1060 (m), 1020 (s), 970 (w), 950 (w), 890 (w) MS: 161 (M^+, 88), 160 (44), 146 (18), 130 (12), 118 (100), 117 (24), 104 (2), 91 (26), 80 (27), 67 (13), 65 (15), 53 (19), 43 (28) ^1H NMR: 2.28 (s, 3 H, furan 5-CH_3), 3.84 (s, 2 H, CH_2), 5.89, 5.91 (AB, each 1 H, J = 3.7 Hz, furan 3-H and 4-H), 6.18 (m, 1 H, J = 2.5 Hz, 4-H), 6.68 (m, 1 H, J = 2.0 Hz, 2-H), 6.75 (m, 1 H, J = 2.5 Hz, 2.0 Hz, 5-H), 8.10 (br s, 1 H, NH)

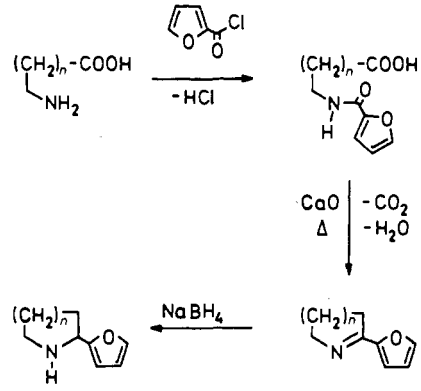


Figure 1. Synthesis of compounds 1/3 and 7/9 starting from ω-amino carboxylic acids and 2-furoyl chloride.

was used for the mobile phase with a flow of 2 mL/min. The HPLC fractions were combined, extracted with chloroform, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was dissolved in $CDCl_3$ for IR and ^1H NMR spectroscopy.

IR and ^1H NMR Spectroscopy. Infrared spectra were obtained from $CDCl_3$ solutions with a Perkin-Elmer Model 357 instrument. ^1H NMR spectra were recorded at 270 MHz on a Bruker WM 270 NMR spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported (ppm) relative to TMS; coupling constants are in hertz.

RESULTS AND DISCUSSION

The volatiles generated by the reaction of L-proline and reducing monosaccharides at 150 °C in aqueous medium were extracted with ether at pH 10 and investigated by capillary GC–mass spectrometry and nitrogen-selective detector. Further separation and purification of individual components from the isolated complex mixtures often was hindered by the strong basic character of the reaction products. Therefore, the identification of the components required straightforward synthetic routes or alternative reactions leading to less complex product spectra as in the case of L-proline/monosaccharide model experiments. Starting from this consideration, compounds 1–4 and 7–10 were synthesized according to La Londe et al. (1977) by N-acylation of 5-aminopentanoic acid (1–4) and 4-aminobutanoic acid (7–10) with 2-furoyl and 5-methyl-2-furoyl chloride, respectively, followed by decarboxylation, cyclization, and reduction with sodium borohydride (Figure 1).

In a previous paper we discussed the MS, IR, and ^1H NMR spectra of the furylpiperidines 3 and 4 (Tressl et al., 1985b). These data together with the MS, IR, and ^1H NMR data of the isolated new components are summarized in Table I. Because compounds 3 and 4 can be easily prepared from 1 and 2 in an one-step reduction with $NaBH_4$, the latter compounds should represent the corresponding 3,4,5,6-tetrahydropyridines. These structures were verified by spectroscopic data. According to the results of ^1H NMR decoupling experiments, the signals of four methylene groups of compound 1 (δ 1.66, 1.78, 2.50, 3.81) belong to a common coupling system of four adjacent methylene groups in cyclic arrangement, the two low-field signals of which show a homoallylic coupling of 2 Hz via the imino group of the tetrahydropyridine ring system. Furthermore, the spectrum shows the typical ABX pattern of the furan moiety. The ^1H NMR spectrum of compound 2 indicates the same typical pattern of the tetrahydropyridine and furan ring systems.

Starting with 4-aminobutanoicacid, the corresponding 2-(2-furyl)- and 2-(5-methyl-2-furyl)-1-pyrrolines (7 and 8) and -pyrrolidines (9 and 10) were prepared. The ^1H NMR data of compounds 7 and 9 as well as their MS and IR data are in full agreement with the proposed structures. As expected, the ^1H NMR spectrum shows three signals (δ 1.97, 2.85, 4.02) of adjacent ring methylene groups, which was confirmed by decoupling experiments. The 1-pyrroline structure is demonstrated by the observed chemical shifts and, furthermore, by the characteristic changes in the ^1H NMR spectrum caused by the conversion of 7 to 9: The signals of the methylene protons in the 3- and 5-positions are shifted to higher field ($\Delta\delta \sim 1$ ppm) and two further signals [δ 2.49 (NH), 4.20 (CH)] are generated in the expected shift range. The homologous derivatives 8 and 10 were identified by comparing their mass spectra with those of compounds 7 and 9. Compounds 7 and 8 were formed as minor constituents in proline/reducing sugar experiments and as major components in the corresponding azetidinic acid reactions.

The 3-hydroxypiperidines 5 and 6, formed in the L-proline/arabinose and -rhamnose model experiments, respectively, were tentatively identified by mass spectrometric data and GC retention times on Carbowax 20M + KOH. All attempts to isolate these compounds failed because of their high polarity and strong basicity, and therefore, a more extensive investigation by spectroscopic methods was prevented. The most remarkable phenomenon in both the mass spectra of 5 and 6 is the occurrence of two isomers with asbolutely identical fragmentation patterns but very different retention times. The mass spectra of isomers of 5 show a parent peak at m/e 167, base peak at m/e 96 (M – 71), and fragment peaks at m/e 122, 94, and 81. The mass spectra of isomers 6 [parent peak at m/e 181, base peak at m/e 109 (M – 72), and fragment peaks at m/e 136, 110, 108, and 95] are in coincidence with a methyl derivative of 5. In both cases loss of H_2O (M –

18) indicated a hydroxyl group. With respect to the fragmentation pattern and the identity of the mass spectra of the occurring isomers, we assigned the structures of compounds **5** and **6** to 2-(2-furyl)- and 2-(5-methyl-2-furyl)-3-hydroxypiperidine. The postulated structures with two chiral centers easily explain the formation of diastereomeric species observed by capillary GC–mass spectrometry.

From the reaction mixture obtained in the furancarboxaldehyde/1-pyrroline experiments the 3-substituted 1-pyrrolines **11–13** and pyrroles **15** and **16** were separated. Their IR and MS data were not suitable to predict the structures. The ^{1}H NMR spectrum of **11** indicated five olefinic or aromatic protons and two methylene groups. From decoupling experiments the typical ABX system of 2-substituted furans (δ 6.44, 6.48, 7.47) was easily recognized as well as a second spin system involving two neighboring methylene groups (δ 2.79, 4.28) and the two remaining olefinic protons (δ 6.66, 7.85). With regard to the spectroscopic data of analogous 3-substituted piperidine derivatives (Nomura et al., 1983; Miller et al., 1984), which show very similar chemical shifts and coupling constants, we now assign the structure of **11** to 3-furfurylidene-1-pyrroline instead of 2-(2-furyl)-4,5-dihydropyridine (Helak, 1987).

The homologous methyl- and hydroxymethyl derivatives **12** and **13** showed very similar proton spectra with a methyl signal (δ 2.33) and the signals of the hydroxymethyl group (δ 3.34, 4.60) instead of the furan proton signal at δ 7.47. In the capillary GC–mass spectrometry of **11–13**, two isomeric species appeared, showing identical mass spectra and different retention indices. In the case of **12** the isomer ratio was determined as 3:1 by ^{1}H NMR; in the other cases only one of the isomers could be separated. The minor isomer of **12** was identified by the remarkable downfield shift of the olefinic 1-pyrroline proton ($\Delta\delta \sim$ 0.9 ppm), which is induced by the anisotropic effect of the furan moiety only in the *Z* configuration. Therefore, the major isomer of **12** as well as the isolated isomers of **11** and **13** are to be assigned the *E* configuration according to the observed chemical shift of H-2. On the basis of these assignments the isomers of **11** and **13** observed in the GC–MS separation could be characterized as *E* and *Z* diastereomers according to their different retention indices. Compound **14** was prepared from **11** by reduction with $NaBH_4$. As expected for the resulting 3-furfurylidenepyrrolidine, the IR spectrum of **14** showed a broad NH absorption at 3200 cm^{-1}. The GC–MS investigation again indicated both *E* and *Z* diastereomers of **14**.

When furfural and 5-methylfurfural were heated with 1-pyrroline, further compounds (**15** and **16**) were isolated. These compounds possessed very similar mass spectra compared to **11** and **12**, but the IR data indicated either an OH or NH group (3500, 3430 cm^{-1}). The ^{1}H NMR spectrum of **15** showed an isolated methylene group (δ 3.88), six olefinic or aromatic protons, and a broad singlet (δ 8.04) characteristic for NH protons of pyrroles. By decoupling experiments starting at the NH signal, the signals at δ 6.12, 6.62, and 6.72 could be determined as part of a pyrrole moiety. The remaining signals at δ 6.00, 6.26, and 7.28 were assigned to a furan system. Both heterocyclic systems are connected via a methylene bridge in 3- and 2-position, respectively, to give 3-furfurylpyrrole (**15**). The ^{1}H NMR spectrum of the corresponding methyl homologue **16** is in full coincidence with the proposed structure of 3-(5-methylfurfuryl)pyrrole.

Formation of 2-(2-Furyl)piperidine Derivatives in L-Proline/Sugar Model Systems. In earlier papers (Tressl et al., 1981, 1985) we discussed the formation of 2-substituted tetrahydropyridines by ring-enlargement reaction of an iminium intermediate generated from the 3-deoxyosone of the initial monosaccharide and L-proline. As demonstrated in Figures 2 and 3, the corresponding reaction of arabinose and rhamnose lead to iminium ions, which are plausible precursors for compounds **1**, **3**, and **5**

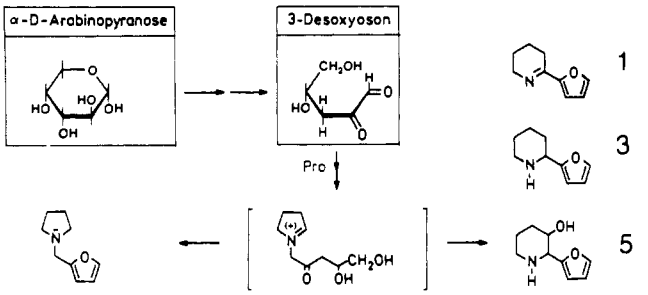


Figure 2. Iminium intermediate from the 3-deoxyosone of arabinose as the precursor for compounds **1**, **3**, and **5**.

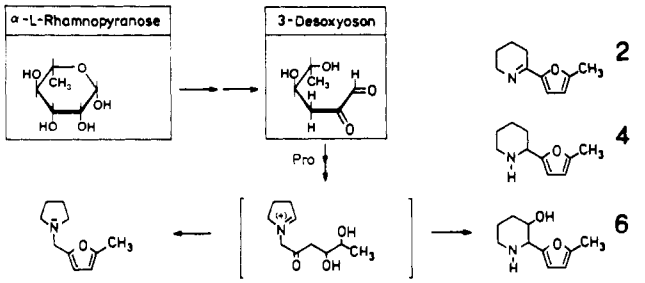
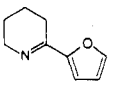
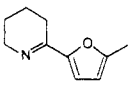
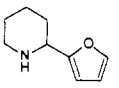
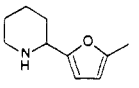
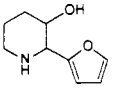
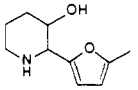
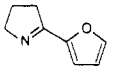
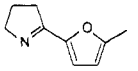
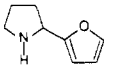
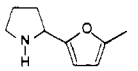


Figure 3. Iminium intermediate from the 3-deoxyosone of rhamnose as the precursor for compounds **2**, **4**, and **6**.

Table II. 2-(2-Furyl)piperidine and -pyrrolidine Derivatives (1–10) from Proline Model Systems with Monosaccharides and Ascorbic Acid (Concentration, ppm)

		Gly	Ery	Ara	Glu	Rham
1		2	3	210	10	
2						160
3		15	1	150	2	15
4			1			240
5				100	5	
6						300
7						
8						
9						
10						

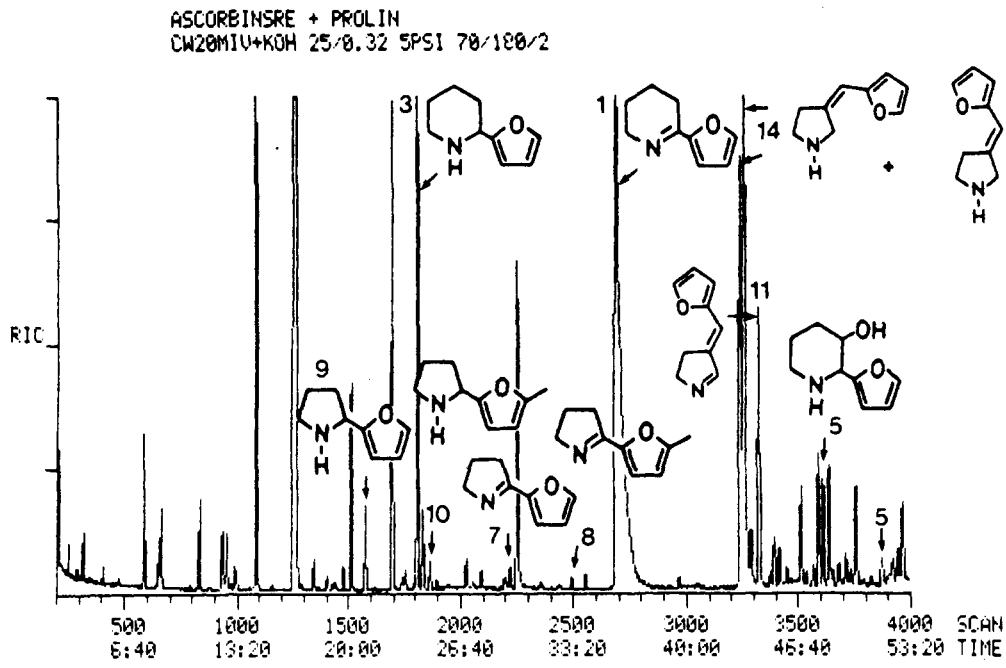
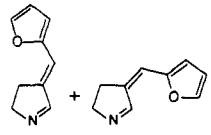
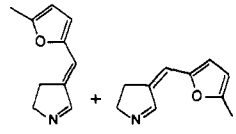
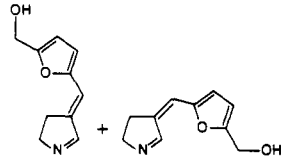
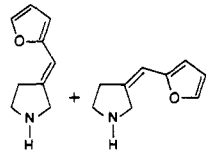
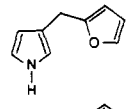
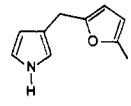


Figure 4. GC separation of piperidine and pyrrolidine derivatives from a pyroline/ascorbic acid model experiment.

Table III. 3-Substituted 2-Furylpyrrolidine Derivatives (11–16) Formed in the Model Experiments of 2-Formylfurans and Ascorbic Acid with L-Proline (Concentration, ppm) and 1-Pyrroline (Percentage in Parentheses)

		Furf	5-Me-furf	HMF	Ascocid
11		20 (12)			50
12			30 (15)	20	
13				1 (10)	
14					100
15		(10)			5
16			(10)		

and for **2**, **4**, and **6**, respectively. As primary products, the 3-hydroxypiperidines **5** and **6** may be dehydrated to **1** and **2** and further reduced to **3** and **4**, respectively. Table II represents the concentrations of **1–6** (ppm) depending on the parent material used. Additionally, the corresponding pyrrolines and pyrrolidines **7–10**, which were found to be minor compounds in the reaction of L-proline with ascorbic and glucoronic acid, were taken up in Table II. The 2-substituted 1-pyrroline structures are related to the recently characterized rice impact compound 2-acetyl-1-pyrroline (Buttery et al., 1983), which we also found in our proline/sugar experiments (Tressl et al., 1985d).

Formation of 3-Substituted Furyl-1-pyrrolines, -pyrrolidines, and -pyrroles. In the proline/ascorbic acid experiments furyl derivatives **11**, **14**, and **15** were formed as shown in the capillary GC in Figure 4. As outlined in Table III these derivatives and homologues are proline-specific products, with furancarboxaldehydes occurring in the ppm range. The reaction of 1-pyrroline with furancarboxaldehydes, however, is much more effective,

leading to 11–13 in 10–15% yield. This aldol-like condensation of the activated methylene group of 1-pyrroline with the furancarboxaldehydes is in analogy to the formation of 3-substituted tetrahydropyridines from piperidine and furancarboxaldehydes as demonstrated by Miller et al. (1984) for the lysine/glucose system. As will be shown in a future paper, reactions of arginine and different sugars also lead to compounds 11–13 as well as to different substituted cyclopent[*b*]azepinones. These results will demonstrate a closely related pool of reactive intermediates in both the proline and the arginine experiments. Reductive media such as proline/ascorbic acid easily transform 1-pyrroline 11 into the corresponding pyrrolidine 14, a main reaction product of this experiment. Compounds 15 and 16 derive from compounds 11 and 12, respectively, at elevated temperatures by isomerization to the more stable pyrrole systems.

Registry No. 1, 104704-36-1; 2, 104704-35-0; 3, 97073-24-0; 4, 97073-18-2; 5 (diastereomer 1), 118248-22-9; 5 (diastereomer 2), 118248-23-0; 6 (diastereomer 1), 118248-24-1; 6 (diastereomer 2), 118248-25-2; 7, 104704-31-6; 8, 118248-26-3; 9, 90086-89-8; 10, 118248-27-4; (*E*)-11, 118248-28-5; (*Z*)-11, 118248-29-6; (*E*)-12, 118248-30-9; (*Z*)-12, 118248-31-0; (*E*)-13, 118248-32-1; (*Z*)-13, 118248-33-2; (*E*)-14, 118248-34-3; (*Z*)-14, 118248-35-4.

LITERATURE CITED

Buttery, A. G.; Ling, L. C.; Teranishi, R.; Mon, T. R. Cooked Rice Aroma and 2-Acetyl-1-pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823–826.

Helak, B. Modellumsetzungen von sekundären Aminen und Aminosäuren mit cyclischen Enolonen als Beitrag zur Kenntnis der Maillard-Reaktion. Dissertation TU Berlin, 1987; p 147.

La Londe, R. T.; Muhammad, N.; Wong, C. F. A Stereocontrolled Synthesis of (±)-Anhydronupharamine. The ^1H and ^{13}C Nuclear Magnetic Resonance of Piperidine Nuphar Alkaloids. *J. Org. Chem.* **1977**, *42*, 2113–2118.

Miller, R.; Olsson, K.; Pernemalm, P.-A. Formation of Aromatic Compounds from Carboxylates. IX. Reaction of D-Glucose and L-Lysine in Slightly Acidic, Aqueous Solution. *Acta Chem. Scand.* **1984**, *B38*, 689–694.

Nomura, Y.; Bando, T.; Takeuchi, Y.; Tomoda, S. Synthesis of Cyclic Imines Having Conjugated Exocyclic Double Bond. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3199–3200.

Quick, J.; Oterson, R. A Convenient Synthesis of Pelletierine (2-Piperidylpropanone). *Synthesis* **1976**, 745–746.

Tressl, R.; Grünewald, K. G.; Helak, B. Formation of Flavour Components from Proline and Hydroxyproline with Glucose and Maltose and Their Importance to Food Flavour. In *Flavor 81*; Schreier, P., Ed.; Walter de Gruyter: Berlin, New York, 1981; pp 397–416.

Tressl, R.; Rewicki, D.; Helak, B.; Kamperschröer, H.; Martin, N. Formation of 2,3-Dihydro-1*H*-pyrrolizines as Proline-Specific Maillard Products. *J. Agric. Food Chem.* **1985a**, *33*, 919–923.

Tressl, R.; Rewicki, D.; Helak, B.; Kamperschröer, H. Formation of Pyrrolidines and Piperidines on Heating L-Proline with Reducing Sugars. *J. Agric. Food Chem.* **1985b**, *33*, 924–928.

Tressl, R.; Helak, B.; Köppler, H.; Rewicki, D. Formation of 2-(1-Pyrrolidines and Cyclopent[*b*]azepin-8(1*H*)-ones as Proline-Specific Maillard Products. *J. Agric. Food Chem.* **1985c**, *33*, 1132–1137.

Tressl, R.; Helak, B.; Martin, N. Bildung von Prolin-spezifischen Aroma- und Geschmacksstoffen. *EBC Proc.*, *20* **1985d**, 355–362.

Received for review December 29, 1987. Accepted June 23, 1988.

Assignment of Bitter Almond Oil to Natural and Synthetic Sources by Stable Isotope Ratio Analysis

Maria Butzenlechner, A. Rossmann, and H.-L. Schmidt*

Benzaldehyde from natural sources showed a mean $\delta(^{13}C)$ value −29.7 ± 0.5‰ and a mean $\delta(^{2}H)$ value −125 ± 14‰. Synthetic benzaldehyde had $\delta(^{13}C)$ and $\delta(^{2}H)$ values that depended on the manufacturing process. Products synthesized from benzal chloride had a mean $\delta(^{13}C)$ value −28.7 ± 1.5‰ and a mean $\delta(^{2}H)$ value −40 ± 21‰ and those derived from catalytic oxidation of toluene −26.8 ± 0.4‰ and +777 ± 20‰, respectively. Taking into account that also degradation of benzaldehyde by air exposure can proceed with ^{2}H enrichment, the $\delta(^{2}H)$ determination is a nonadulterable method for the origin assignment of benzaldehyde.

Benzaldehyde is the main component of the essential oils from the seeds and kernels of bitter almonds, apricots, peaches, plums, cherries, and cherry laurel. It is an important flavor additive for many food products. Because of the limited supply and high price of the natural product, synthetic benzaldehyde is often (legally) used as a substitute. However, the application of the less expensive artificial flavor has to be indicated, and it is suspected that this is not always done. Thus, analytical methods for determining the origin of this flavoring compound are needed.

The distinction of natural and synthetic flavoring compounds is commonly only possible by the determination of their ^{14}C content (which can be adulterated) or by stable isotope ratio analysis. A number of corresponding applications have been reported in the last few years. Well-known examples are the distinction of natural vanillin (from vanilla sp., a CAM plant) from semisynthetic vanillin (base lignin, eugenol, or guajacol from C_3 plants) (Hoffman and Salb, 1979) and of lemon grass (C_4 plant) citral from synthetic citral (Bricout and Koziet, 1976).

While it is generally possible to identify the source of aromatic compounds originating from C_4 plants, CAM plants, or some synthetic sources on the basis of $\delta(^{13}C)$ values, a corresponding distinction among products from different C_3 plants or between C_3 plants and many synthetic products is not possible. More conclusive results are obtained from the D/H ratios. Thus, the $\delta(^{2}H)$ values of natural isoprenoids like citral, anethol, linalool, menthol, and carvone are significantly more negative than those of their synthetic counterparts. In general, most natural

Lehrstuhl für Allgemeine Chemie und Biochemie der Technischen Universität München, D-8050 Freising-Weihenstephan, FRG.

0021-8561/89/1437-0410$01.50/0 © 1989 American Chemical Society