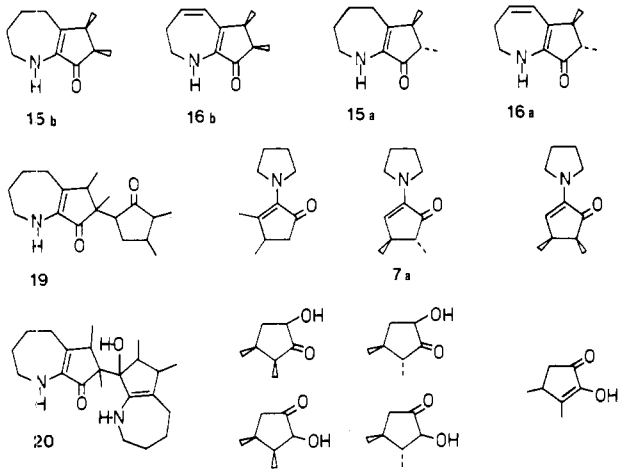

Figure 4. Components characterized in the proline/2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one model experiment.

clotene model experiment was percieved with bitter taste. The isolated compound **3** possessed bitter taste quality and adstringency. The threshold in water was determined at 50 ppm. 7-Methyl-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1H)-one possessed a bitter taste and a threshold at 10 ppm.

The model experiment of proline and 2-hydroxy-3,4-dimethylcyclopenten-1-one produced the most bitter components in this study. Some of the results are shown in Figure 4. As it can be seen, the cyclopent(*b*)azepine derivatives are formed as cis/trans isomers. Compound **15a** was isolated by preparative GC and investigated by ^{1}H NMR spectroscopy. The coupling constant of the protons at C(6) and C(7) exhibited **15a** as the trans isomer. An isomeric compound which possessed the same mass spectrum and a higher retention time was identified as the cis isomer of **15a**. The ratio of trans to cis was determined by GC at 3:1. According to mass spectra and GC retentions, components **16a** and **16b** were characterized as trans/cis derivatives and they were formed in the same ratio. Compounds **19** and **20** were tentatively identified according to mass spectrometric fragmentation.

Cyclopent(*b*)azepine derivatives and 1-pyrrolidinylcyclopentenones are formed under elevated temperatures (pressure cooking) and roasting conditions and may contribute to the bitter taste of roasted foodstuffs. The observation, that they condense by aldol type reaction to higher molecular compounds needs further investigation.

Registry No. **1**, 36287-24-8; **2**, 4933-43-1; **3**, 41357-00-0; **4**, 97826-60-3; **5**, 97826-61-4; **6**, 97826-62-5; **7**, 97826-63-6; **8**, 97826-64-7; **9**, 81978-77-0; **10**, 97826-65-8; **11**, 97826-66-9; **12**, 97826-67-0; **13**, 97826-68-1; **14**, 97826-69-2; **15a**, 97826-70-5; **15b**, 97826-75-0; **16a**, 97826-71-6; **16b**, 97826-76-1; **17**, 97826-72-7; **18**, 97826-73-8; **19**, 97826-77-2; **20**, 97826-74-9; pyrrolidine, 123-75-1; 2-hydroxy-2-cyclopenten-1-one, 10493-98-8; 2-hydroxy-3-methyl-2-cyclopenten-1-one, 80-71-7; 3-ethyl-2-hydroxy-2-cyclopenten-1-one, 21835-01-8; 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one, 21835-00-7; 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one, 21834-98-0; rhamnose, 3615-41-6; glucose, 50-99-7; arabinose, 147-81-9; erythrose, 1758-51-6; glyceraldehyde, 367-47-5.

LITERATURE CITED

Hunter, I. R.; Walden, M. K.; Scherer; J. R.; Lundin, R. E. *Cereal Chem.* **1969**, *46*, 189.
Mills, F. D.; Hodge, J. E. *Carbohydr. Res.* **1976**, *51*, 9.
Pabst, H. M. E.; Ledl, F.; Belitz, H. D. *Z. Lebensm. Unters. Forsch.* **1984**, *178*, 356.
Shigematsu, H.; Shibata, S.; Kurata, T.; Kato, H.; Fujimaki, M. *J. Agric. Food Chem.* **1975**, *23*, 233.
Tressl, R.; Grünewald, K. G.; Helak, B. In "Flavour 81"; Schreier, P., Ed.; Walter de Gruyter: Berlin, New York, 1981; p 397.
Tressl, R.; Helak, B.; Kamperschröer, H.; Rewicki, D. *J. Agric. Food Chem.* **1985**, in press.
Tressl, R.; Helak, B.; Spengler, K.; Rewicki, D.; Schröder, A., in preparation.

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Formation of Pyrroles and Tetrahydroindolizin-6-ones as Hydroxyproline-Specific Maillard Products from Glucose and Rhamnose

Roland Tressl,* Klaus Günter Grünewald, Evelyn Kersten, and Dieter Rewicki

In a series of model experiments hydroxyproline was heated with glucose and rhamnose in water at 100 °C as well as 150 °C. The products were extracted with ether and investigated by capillary GC/MS and preparative GC and identified by mass, IR, and ^{1}H and ^{13}C NMR spectroscopies. More than 30 compounds (among them 1-(1-pyrrolyl)-2-alkanones, (1-pyrrolyl)cyclopentenones, 1-furfurylpyrroles, and 5,6,7,8-tetrahydroindolizinones) were characterized as hydroxyproline-specific Maillard products.

INTRODUCTION

According to their secondary amine structures proline and hydroxyproline form specific Maillard products (which are not observed with primary α-amino acids) during heating with monosaccharides in water (100 °C). The amounts of these products increase 10–50-fold under pressure-cooking conditions (150 °C). In proline/glucose (rhamnose) model experiments more than 120 proline-specific compounds with bready aroma and bitter taste qualities were characterized (Mills and Hodge, 1976; Doornbos et al., 1981; Pabst et al., 1984; Tressl et al., 1985a). In the analogous hydroxyproline systems only 30–40 specific compounds were formed. Kobayashi and Fujimaki (1965) identified 1-acetonylpyrrole and pyrrole on roasting hydroxyproline and glucose. Fifteen compounds were recently identified in hydroxyproline/arabinose (erythrose) model experiments (Tressl et al., 1985b).

Hydroxyproline and α-dicarbonyls form iminium carboxylate intermediates that are decarboxylated and de-

Technische Universität Berlin, 1000 Berlin 65, West Germany (R.T., K.G.G., E.K.), and Freie Universität Berlin, 1000 Berlin 33, West Germany (D.R.).

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hydrated to the corresponding 1-pyrrolyl 2-oxo compounds. Pyruvaldehyde, 2,3-butandione, 1,2-cyclopentanedione, and furfuraldehyde were transformed into the corresponding derivatives: 1-acetonylpyrrole, 2-(1-pyrrolyl)-3-butanone, 2-(1-pyrrolyl)cyclopentanone, and 1-furfurylpyrrole. They were also characterized in the hydroxyproline/arabinose experiment. 3-Deoxyosones are transformed into the 1-(1-pyrrolyl) 2-oxo derivatives that act as precursors forming most of the hydroxyproline-specific compounds. In 1981 we reported 12 compounds in hydroxyproline/glucose model system, but most of them were only identified by mass spectroscopy.

EXPERIMENTAL SECTION

Sample Preparation. Equimolar amounts of hydroxyproline and monosaccharide (0.05 mol glucose and rhamnose, respectively) dissolved in water were refluxed for 2 h as well as autoclaved for 1.5 h at 150 °C. After cooling to room temperature, the compounds were extracted three times with freshly distilled ether. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 5 mL on a Vigreux column.

Reaction of Hydroxyproline with α-Dicarbonyls ⇄ Enolones. Equimolar amounts of hydroxyproline and 5-methylfurfuraldehyde (5-(hydroxymethyl)furfuraldehyde, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-hydroxy-3-methyl-2-cyclopenten-1-one) were dissolved in water and autoclaved for 1.5 h at 150–160 °C in a stainless-steel laboratory autoclave equipped with a 100-mL duran glass tube. After cooling to room temperature the compounds were extracted with ether. The extract was separated by liquid adsorption chromatography on silica gel. Individual components were isolated by preparative GC and identified by mass, IR, and ^{1}H NMR spectroscopies. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone was a gift of Firmenich (Geneve).

Column Chromatography. The extracts of the hydroxyproline/α-dicarbonyl (monosaccharide) model experiments were separated by liquid adsorption chromatography on silica gel (activity II–III) into five fractions with 9:1 pentane–methylene chloride (F1), 3:1 pentane-methylene chloride (F2), 1:1 pentane–ether (F4), and ether (F5) in 40-mL portions. The eluates were concentrated to 1 mL and analyzed by GC.

Gas Chromatography (GC)/Mass Spectrometry (MS). The extracts of the hydroxyproline/monosaccharide model experiments were analyzed qualitatively and quantitatively by gas chromatography/mass spectrometry (GC/MS) using a 25-m glass capillary (0.32-mm i.d.) coated with CP-Wax Chrompack (column A) and a 50-m glass capillary (0.32-mm i.d.) coated with CP Sil CP (column B) coupled with a Varian MAT CH 5 instrument using on-column injection. Conditions were as follows: column A, temperature program 70–230 °C at 6 °C/min, Carlo Erba fractovap 2101, ionization voltage 70 eV, resolution 2000 (10% valley); column B, temperature program 100–260 °C at 4 °C/min.

Preparative Gas Chromatography. For the isolation of individual compounds from SiO_2 fractions preparative GC was used. On a Varian Aerograph 2700 equipped with a 3 m × 0.25 in. glass column the pyrroles were separated on either 15% carbowax 20M on 80–90 mesh Chromosorb WAW/DMCS or on 5% SP 2401 DB on 100–120 mesh Supelcoport. Nitrogen was used and a glass split to a ratio of 1:10 (FID: split outlet). The column temperature was elevated from 60 to 230 °C at a rate of 4 °C/min. For higher boiling and more polar compounds the column temperature was adapted to the special separation problem. Repeated collections of the effluent fractions in glass capillary tubes were made until sufficient quantities were obtained.

^{1}H NMR and IR Spectroscopies. ^{1}H NMR spectra were recorded at 270 MHz on a Bruker WH 270 NMR spectrometer in $CDCl_3$ solution; chemical shifts are referenced to tetramethylsilane (Me_4Si) as internal standard with coupling constants J in Hertz. Infrared spectra (4000–400 cm^{-1}) were obtained from $CDCl_3$ or CCl_4 solutions.

RESULTS AND DISCUSSION

Hydroxyproline and glucose (rhamnose) were heated in water at 100 and 150 °C. The products were extracted with diethyl ether and investigated by capillary GC/MS. Individual compounds were isolated by preparative GC or purified by adsorption chromatography and identified by spectroscopic methods. The results are summarized in Tables I and II. Compounds 1–**15** were identified in the previous study. **16** was identified by MS and ^{1}H NMR spectroscopy as 1-(5-methylfurfuryl)pyrrole. This compound was also formed on heating hydroxyproline with 5-methylfurfuraldehyde. By an analogous reaction **18** was formed from hydroxyproline and 5-(hydroxymethyl)-furfural. The MS and ^{1}H NMR data of **18** were consistent with 1-[5-(hydroxymethyl)furfuryl]pyrrole. According to mass spectrometric fragmentation and chemical reaction **17** was identified as 1-(5-formylfurfuryl)pyrrole. **17** was transformed into **18** by reduction with $NaBH_4$. The mass spectrum of **19** did not permit a structural assignment. **19** was isolated from the hydroxyproline/glucose system by preparative GC. The IR spectrum exhibited an OH group (3400 cm^{-1}) and a conjugated carbonyl group (1710, 1655 cm^{-1}). Both the ^{1}H and ^{13}C NMR data clearly show a N-substituted pyrrole system and a methyl group bound to a tertiary C atom. The increased chemical shift difference of the pyrrole protons (1.02 ppm instead of the usually observed 0.5–0.6 ppm) as well as their deshielding points to an unsaturated electronegative N-substituent (Pretsch et al., 1981). This substituent must be integrated into a carbocyclic ring system, as results from the molecular formula, if the two quarternary olefinic carbons established by ^{13}C NMR (135.3, 138.5 ppm) and the identified functional groups (C=O, 183.5 ppm; OH 6.58 ppm, exchangeable by D_2O) are taken into account. ^{1}H homonuclear decoupling experiments confirmed a $CH_2CH(CH)_3$ fragment as part of a five-membered ring system showing the expected magnitudes of geminal and vicinal coupling constants ($J_{4,4'}$ = 15.6, $J_{4,5}^{cis}$ = 6.5, $J_{4,5}^{trans}$ = 1.8 Hz). Finally, the substituent pattern at the cyclopentenone system was determined by measuring the NMR shift parameters using tris(dipivaloylmethanato)europium as shift reagent. These parameters (cf. Table I) clearly show the neighboring position of the $CH(CH_3)$ group to the enolone function, where the coordination of the shift reagent will occur. The 2-position of the OH group and the 3-position of the pyrrolyl group, respectively, follow from the small ^{13}C chemical shift difference between the olefinic carbons: if these substituents would be interchanged, this difference has to be about 40–60 ppm. **20** was isolated from the rhamnose/hydroxyproline system by preparative GC. The mass spectrum (parent peak m/e 179, major fragments at m/e 136 (M − 43) and 99 (M − 80), and base peak 80) suggested a 1-pyrrolyl 2-oxo component. **20** was transformed into a compound with a parent peak at m/e 183 and a base peak at m/e 81 by reduction with $NaBH_4$, indicating two carbonyl groups in **20**. The ^{1}H NMR data exhibited a 1-pyrrolyl system (δ 6.24, 6.66 (α′-H and β′-H)), a $>NCH_2CO$ group at δ 4.72 (s, 2 H), a $COCH_3$ group at δ 2.17 (s, 3 H), and two methylene groups at δ 2.52, 2.76

Table I. Mass and ^1H NMR Spectra of Pyrroles, Characterized in Hydroxyproline Model Experiments[a]

1-(5-methylfurfuryl)pyrrole (**16**): MS *m/e* 161 (27), 117 (2), 96 (19), 95 (100), 94 (18), 67 (7), 53 (6), 43 (27), 41 (9); ^1H NMR δ 2.24 (s, 3 H, 5′-CH_3), 4.98 (s, 2 H, >NCH_2), 5.92 (mc, 1 H, H-4′), 6.14 (d, 1 H, *J* = 3.2 Hz, H-3′), 6.16 (mc, 2 H, H-3, H-4), 6.72 (mc, 2 H, H-2, H-5).

1-(5-formylfurfuryl)pyrrole (**17**): MS *m/e* 175 (62), 147 (10), 146 (35), 109 (100), 80 (5), 67 (2), 53 (39).

1-[5-(hydroxymethyl)furfuryl]pyrrole (**18**): MS *m/e* 177 (40), 146 (10), 117 (8), 111 (100), 83 (49), 81 (14), 80 (15), 67 (10), 65 (23), 55 (40), 53 (34), 51 (25), 43 (19), 41 (23), 39 (44), 31 (50); ^1H NMR δ 1.75 (br s, 1 H, OH), 4.55 (s, 2 H, OCH_2), 5.00 (s, 2 H, NCH_2), 6.15 (mc, 2 H, H-3′, H-4′), 6.18 (AB, *J* = 3.3 Hz, 1 H, H-4), 6.23 (AB, *J* = 3.3 Hz, 1 H, H-3), 6.71 (mc, 2 H, H-2, H-5).

2-hydroxy-5-methyl-3-(1-pyrrolyl)-2-cyclopenten-1-one (**19**): MS *m/e* 177 (100), 162 (1), 148 (6), 120 (7), 107 (3), 106 (3), 94 (1), 93 (6), 92 (1), 81 (1), 79 (5), 78 (1), 77 (2), 67 (4), 66 (1), 65 (1), 53 (3), 52 (2); ^1H NMR δ 1.31 (d, 3 H, *J* = 7.6 Hz, 5-CH_3), 2.49 (dd, 1 H, *J* = 15.6, 1.8 Hz, H-4), 2.65 (dqui, 1 H, *J* = 7, 1.8 Hz, H-5), 3.16 (dd, 1 H, *J* = 15.6, 6.5 Hz, H-4′), 6.35 and 7.37 (each mc, 2 H, α′-H and β′-H), 6.58 (s, 1 H, OH); relative ^1H NMR shift parameters[b] ($Eu(dpm)_3$, $CDCl_3$) 1.72 (H-5), 1.00 (5-CH_3), 0.64 (H-4), 0.53 (H′-4), 0.16 (α′-H), 0.08 (β′-H); ^{13}C NMR δ 16.7 (q, CH_3), 31.8 (t, CH_2), 36.2 (d, $CHCH_3$), 111.8 (d, β′-C), 120.0 (d, α′-C), 135.3 and 138.5 (s, >C=C<), 183.5 (s, C=O); IR 3450 (m), 3300 (m), 2970 (m), 2930 (m), 1720 (s), 1710 (s), 1655 (s), 1640 (s), 1485 (s), 1415 (s), 1370 (s), 1260 cm^{-1} (s).

1-(1-pyrrolyl)-2,5-hexandione (**20**): MS *m/e* 179 (27), 136 (6), 99 (53), 81 (19), 80 (100), 78 (13), 71 (16), 57 (4), 55 (5), 53 (25), 43 (33); ^1H NMR δ 2.17 (s, 3 H, $COCH_3$), 2.52 and 2.76 (each mc, 2 H, A_2B_2 system of CH_2CH_2), 4.72 (s, 2 H, >NCH_2CO), 6.24 (mc, 2 H, β′-H), 6.66 (mc, 2 H, α′-H).

2,5-dimethyl-4-(1-pyrrolyl)-3(2*H*)-furanone (**21**): MS *m/e* 177 (100), 162 (2), 148 (6), 134 (14), 120 (5), 106 (54), 79 (17), 78 (13), 67 (6), 43 (33); ^1H NMR δ 1.55 (d, 3 H, *J* = 7.4 Hz, 2-CH_3), 2.30 (d, 3 H, *J* = 0.8 Hz, 5-CH_3), 4.62 (qq, 1 H, *J* = 7.4, 0.8 Hz, H-2), 6.25 (mc, 2 H, β′-H), 6.68 (mc, 2 H, α′-H).

6-hydroxy-1-(1-pyrrolyl)-2,5-hexandione (**22**): MS *m/e* 195 (33), 164 (7), 136 (8), 115 (26), 81 (38), 80 (100), 67 (8), 59 (7), 55 (11), 53 (16), 31 (16).

5-hydroxy-1-(1-pyrrolyl)-3-hexen-2-one (**23**): MS *m/e* 179 (11), 135 (14), 134 (30), 106 (9), 99 (12), 81 (27), 80 (100), 78 (8), 71 (9), 67 (8), 53 (28), 45 (5), 43 (41).

8-(1-hydroxyethyl)-5,6,7,8-tetrahydroindolizin-6-one (**24**): MS *m/e* 179 (32), 135 (55), 134 (100), 118 (20), 106 (50), 93 (9), 79 (12), 78 (9), 77 (8), 53 (8), 45 (10), 43 (15).

5-methyl-2-(1-pyrrolyl)-2-cyclopenten-1-one (**25**): MS *m/e* 161 (100), 146 (7), 132 (39), 118 (43), 117 (18), 105 (7), 104 (7), 91 (31), 79 (17), 67 (17), 65 (13), 64 (16); ^1H NMR δ 1.27 (d, 3 H, *J* = 7.3 Hz, 5-CH_3), 2.27 (ddd, 1 H, *J* = 19.6, 3.2, 2.6 Hz, H-4, cis to 5-CH_3), 2.59 (dqui, 1 H, *J* = 7, 2.6 Hz, H-5), 2.92 (ddd, 1 H, *J* = 19.6, 7, 3.2 Hz, H-4′), 6.21 (mc, 2 H, β′-H), 7.17 (t, 1 H, *J* = 3.1 Hz, H-3), 7.25 (mc, 2 H, α′-H).

3-methyl-2-(1-pyrrolyl)-2-cyclopenten-1-one (**26**): MS *m/e* 161 (100), 160 (20), 146 (4), 132 (43), 119 (10), 118 (50), 117 (24), 105 (22), 104 (24), 66 (18), 65 (17), 51 (17), 43 (22); ^1H NMR δ 2.20 (s, 3 H, 3-CH_3), 2.58 and 2.64 (each m, 2 H, AA′BB′ system, H-4 and H-5), 6.26 (mc, 2 H, β′H), 6.80 (mc, 2 H, α′-H).

2-methyl-5-(1-pyrrolyl)-2-cyclopenten-1-one (**27**): MS *m/e* 161 (100), 132 (39), 118 (10), 117 (10), 95 (36), 93 (41), 68 (6), 67 (28), 66 (11), 65 (7), 53 (4), 51 (6); ^1H NMR δ 1.86 (dt, 3 H, *J* = 1.8, 2.0 Hz, 2-CH_3), 2.78 (dddq, 1 H, *J* = 18.5, 5.6, 2.6, 1.8 Hz, H-4), 3.20 (dm, 1 H, *J* = 18.5 Hz, H-4′), 4.59 (dd, 1 H, *J* = 7.5, 3.3 Hz, H-5), 6.16 (mc, 2 H, β′-H), 6.59 (mc, 2 H, α′-H), 7.34 (m, 1 H, H-3).

2-methyl-5-(1-pyrrolyl)cyclopentan-1-one (**28**): MS *m/e* 163 (56), 120 (4), 107 (6), 96 (4), 94 (8), 93 (100), 92 (4), 68 (4), 67 (10), 66 (12), 65 (4), 53 (4), 51 (5), 41 (16), 39 (13); ^1H NMR δ 1.20, 1.24 (each d, *J* = 7 Hz, 3 H, 2-CH_3), 1.8–2.7 (m, 5 H, H-2, H-3, H-4), 4.30, 4.48 (each dd, *J* = 12.2 and 8.1, 10.2 and 8.8 Hz, 2:1, 1 H, H-5), 6.20 (m, 2 H, β′-H), 6.62, 6.65 (each m, 2 H, α′-H) (mixture of cis/trans isomers); IR 2970 (m), 2940 (m), 2875 (m), 1745 (s), 1695 (m), 1480 (m), 1450 (m), 1275 cm^{-1} (m).

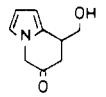
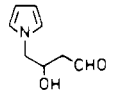
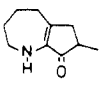
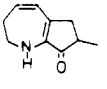
5-methylene-2-(1-pyrrolyl)cyclopenten-1-one (**29**): MS *m/e* 159 (100), 158 (68), 131 (34), 130 (96), 118 (40), 117 (23), 104 (21), 103 (16), 93 (13), 77 (20), 66 (25).

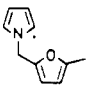
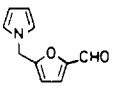
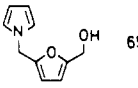
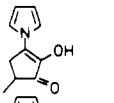
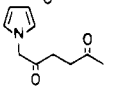
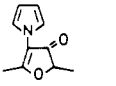
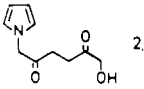
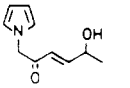
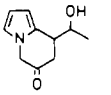
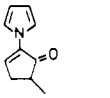
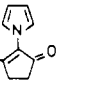
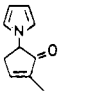
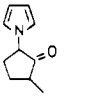
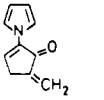
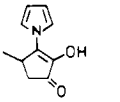
2-hydroxy-4-methyl-3-(1-pyrrolyl)-2-cyclopenten-1-one (**30**): MS *m/e* 177 (100), 162 (12), 148 (7), 134 (11), 132 (15), 120 (4), 106 (12), 94 (4), 80 (6), 79 (6), 67 (12), 53 (6).

[a] Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, qui = quintet, m = multiplet, mc = center of a multiplet, br s = broad singlet, dd = doublet of doublets, dt = doublet of triplets, etc. [b] The NMR shift parameter is defined as $(\nu_i - \nu_i^0)/(\nu_{CH_3} - \nu_{CH_3}^0)$.

(each mc; 2 H). Therefore, all spectroscopic data are consistent with 1-(1-pyrrolyl)-2,5-hexandione. **21** exhibiting a parent peak at *m/e* 177 and major fragments at *m/e* 106 and 43 was isolated from the same model system as **20**. The ^1H NMR spectrum again indicates a 1-pyrrolyl system, a $CHCH_3$ group, and one methyl group at δ 2.30 (d, 3 H), showing long-range coupling (0.8 Hz) to the H-2. These data are in full agreement with 2,5-dimethyl-4-(1-pyrrolyl)-3(2*H*)-furanone. In addition, **21** was formed on heating 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone with hydroxyproline. **22** was identified according to mass spectrometric fragmentation and chemical reactions as 6-hydroxy-1-(1-pyrrolyl)-2,5-hexandione. **22** showed an analogous fragmentation as the 6-deoxy compound **20** (parent peak at *m/e* 195, major fragments at *m/e* 164 (M − 31) and 115 (M − 80), and base peak at *m/e* 80). In addition **22** was transformed into a derivative with parent peak at *m/e* 199 and base peak at *m/e* 81 by reduction with $NaBH_4$. The mass spectrum of **23** (parent peak *m/e* 134 (M − 45), and base peak at *m/e* 80) suggested a derivative of **20**. By reduction with $NaBH_4$ **20** and **23** were transformed into 2,5-dihydroxy-1-(1-pyrrolyl)hexane (MS: *m/e* 183 (23), 165 (1), 150 (3), 138 (2), 120 (2), 110 (2), 106 (4), 103 (3), 95 (4), 85 (18), 81 (100), 80 (36), 68 (26), 67 (15), 57 (8), 53 (10), 45 (9), 43 (15), 41 (9)]. Therefore, **23** was identified as 5-hydroxy-1-(1-pyrrolyl)-3-hexen-2-one. **24** was separated by capillary GC into two components with similar mass spectra suggesting diastereoisomeric compounds. According to mass spectrometric fragmentation **24** was characterized as an 8-hydroxyethyl derivative of 5,6,7,8-tetrahydroindolizin-6-one, which was identified in the hydroxyproline/erythrose model system. **24** showed a parent peak at *m/e* 179, a major fragment at *m/e* 135 (M − 44), a base peak at *m/e* = 134 (M − 45), and fragments at *m/e* 118 and 106 (M − 45 + 28)). The position of the hydroxyethyl group (*m/e* 45) at C-8 corresponds to the observed fragmentation. The 7-hydroxyethyl derivative should form an intensive fragment at *m/e* 93 as detected in 5,6,7,8-tetrahydroindolizine-6-one. In addition **24** was reduced with $NaBH_4$ to a derivative with a parent peak at *m/e* 181 and major fragments at *m/e* 137 (59), 136 (100), 118 (44), 108 (9), 106 (7), 95 (23), 93 (7), and 45 (44), comparable to **7** and **14**. Compounds **25–27** exhibited similar mass spectrometric fragmentations to compounds **12** and **13** with parent peaks at *m/e* 161 and major fragments at M − 29, M − 43, and M − 56 suggesting methyl(1-pyrrolyl)cyclopentenones. They were isolated from the hydroxyproline/rhamnose model experiment and identified by ^1H NMR spectroscopy. The ^1H NMR spectra confirm a 1-pyrrolyl system in all three components. The ^1H NMR spectrum of **25** clearly shows a $CHCH_3$ group, the methine proton of which give a double quintet at 2.59 ppm, resulting from additional vicinal couplings (*J* = 7 bzw, 2.6 Hz) to the protons at 2.27 and 2.92 ppm. These signals correspond to the neighboring cis and trans methylene protons, which give a more complex pattern by geminal coupling (*J* = 19.6 Hz) as well as by coupling (*J* = 3.2 Hz) to the olefinic proton at 7.17 ppm. By the

Table II. Pyrroles Characterized in Hydroxyproline/Monosaccharide Model Experiments ((a) 2 h, 100 °C; (b) 1.5 h, 150 °C; Figures Represent Concentrations in ppm)

		GLU		RHA	
		a	b	a	b
1		4	70	20	90
2		80	640	180	520
3		+	20	+	50
4		2	15	510	1340
5		-	+	-	-
6		50	70	-	-
7		3	6	-	-
8		-	+	-	-
9		-	5	-	-
10		1	65	-	-
11		-	7	-	-
12		-	15	-	-
13		-	+	-	-
14		-	10	-	-
15		-	5	-	-
		-	10	-	-
		-	+	-	-

		GLU		RHA	
		a	b	a	b
16		+	15	80	4970
17		0.5	15	-	-
18		65	2800	-	-
19		24.5	1070	-	-
20		0.5	25	1370	1880
21		-	-	20	50
22		2.5	175	-	-
23		-	-	180	860
24		-	-	15 30	85 115
25		0.5	5	10	65
26		1	5	40	75
27		2	15	940	750
28		-	+	-	-
29		+	20	-	-
30		+	35	-	-

observed couplings, established by decoupling experiments, and by the low-field shift of the olefinic proton, which is only compatible with a β-position to the carbonyl group, **25** was identified as 5-methyl-2-(1-pyrrolyl)-2-cyclopenten-1-one. In contrast **26** shows no signal in the olefinic range. The methyl group, shifted to lower field (2.20 ppm) as expected for substitution at a sp^2 carbon, now gives a singlet. The two methylene groups form an AA'BB' system. Thus, all ^1H NMR data are consistent with 3-methyl-2-(1-pyrrolyl)-2-cyclopenten-1-one (**26**). Finally, in **27** an olefinic proton is observed, which has to occupy a β-position relative to the carbonyl function by chemical shift arguments. Furthermore, the chemical shift and the pattern of the methyl signal clearly show that the methyl group is bound to the C-2 of the 2-cyclopenten-1-one. The further spectroscopic details, especially the chemical shift

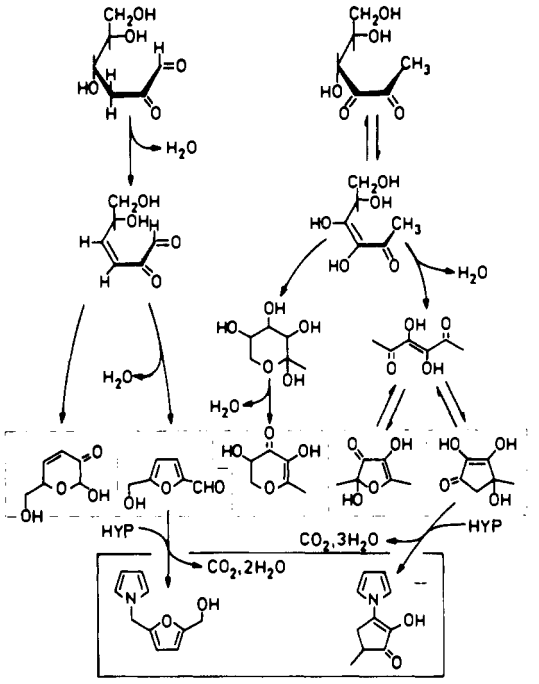


Figure 1. Formation of **18** and **19** via 3- and 1-deoxyosone routes respectively.

(4.59 ppm, >NCHC=O) and the coupling pattern (J = 7.5 and 3.3 Hz, respectively) of the H-5, are in full agreement with the proposed structure of 2-methyl-5-(1-pyrrolyl)-2-cyclopenten-1-one. Compound **28** was formed on heating hydroxyproline with cyclotene and identified by ^{1}H NMR spectroscopy as 2-methyl-5-(1-pyrrolyl)cyclopentanone (cis:trans ratio 1:2). **29** was tentatively identified as 5-methylene-2-(1-pyrrolyl)-2-cyclopenten-1-one and **30** as 2-hydroxy-4-methyl-3-(1-pyrrolyl)-2-cyclopenten-1-one according to mass spectrometric fragmentations.

As shown in Table II 30 components were characterized in the hydroxyproline/glucose experiment. **1–15** were formed as typical compounds in the corresponding erythrose and arabinose systems. 1-[5-(Hydroxymethyl)-furfuryl]pyrrole and **19** ("pyrrole reductone" comparable to the piperidino hexose reductone characterized by Mills et al. (1970)) were identified as main products. Figure 1 presents a scheme to explain their formation via the 3- and 1-deoxyosone route, respectively. The degradation of the Amadori rearrangement products via 1,2- or 2,3-enolization as outlined by Hodge (1953) and Anet (1964) form 3- and 1-deoxyosones as reactive intermediates. 3-Deoxyhexosone is further transformed into 5-(hydroxymethyl)furfural by dehydration or into a β-pyranone as demonstrated by Ledl et al. (1983). 1-[5-(Hydroxymethyl)furfuryl]pyrrole is obviously formed by this reaction sequence. 1-Deoxyhexosone is transformed into 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, 2,4-dihydroxy-2,5-dimethyl-3-furanone (Mills and Hodge, 1976), and methyl reductinic acid (Ledl and Severin, 1982). In the proline/glucose system we characterized the corresponding 1-[5-(hydroxymethyl)furfuryl]pyrrolidine and 5-methyl-2,3-bis(1-pyrrolidinyl)cyclopenten-1-one (Tressl et al., 1985). In the hydroxyproline experiment the Strecker-active **19** was not transformed into the corresponding dipyrrolyl derivative. In addition **19** was not detected in the rhamnose experiment. Figure 2 may explain the formatin of hydroxyproline-specific compounds from rhamnose via 3-deoxyosone route. **A** may be formed as an intermediate from 3-deoxyosone and act as precursor forming **23** and **20** which are further transformed into **16** and **26**, respectively. In addition, the iminium intermediate may form

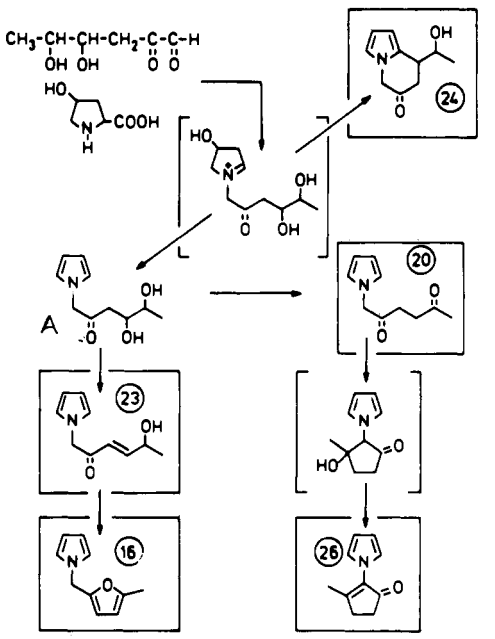


Figure 2. Formation of pyrroles characterized in the hydroxyproline/rhamnose model experiments.

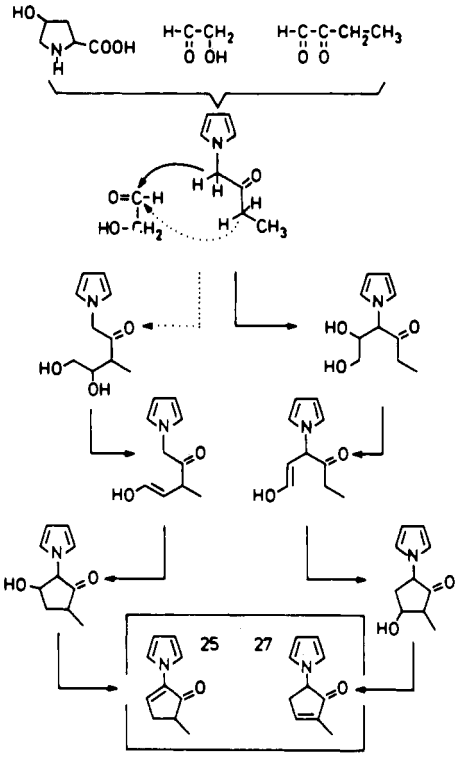
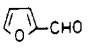
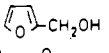
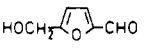
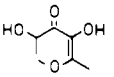
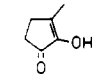
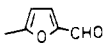


Figure 3. Formation of (1-pyrrolyl)cyclopentenones in the hydroxyproline/rhamnose model experiments.

24 after dehydration and Michael addition. **21** can be explained via 1-deoxyosone route and is also formed on heating hydroxyproline with 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone. Three isomeric methyl(1-pyrrolyl)cyclopentenones were characterized as major compounds in the rhamnose experiment. The corresponding 1-pyrrolidinyl derivatives of **25** and **26** were also identified in the proline/glucose and proline/cyclotene model systems, respectively. When hydroxyproline is heated with cyclotene, only **28** is formed (cis:trans ratio 1:2) and **25** or **26** were not detected. On the other hand no cyclopent[*b*]azepine derivatives were formed from hydroxyproline which were characterized as title compounds in the corresponding proline experiment. As outlined in Figure 3 **25** and **27** may

Table III. Furans, Furanones, and Pyranones Characterized in Hydroxyproline/Monosaccharide Model Experiments (1.5 h, 150 °C; Figures Represent Concentrations in ppm)

	Δ150	ERY	ARA	GLU	RHA
1	CH_3-COOH	1060	700	480	30
2	CH_3-C(=O)-CH_2OH	30	125	140	70
3		+	150	5	-
4		-	125	10	-
5		-	1240	-	-
6		-	-	180	-
7		-	-	380	-
8		-	-	7	-
9		40	10	10	•
10		-	-	5	860
11		-	-	15	2980

be formed from **4** by aldol condensation with glycolaldehyde and **26** by cyclization of **20**. During the Maillard reaction of hydroxyproline we observed no pyrazines. The amino acid catalyzes the 1,2-/2,3-enolizations of the sugars forming furans and furanones (γ-pyranones) as summarized in Table III. The reactive α-dicarbonyls are further transformed into 1-pyrrolyl derivatives and tetrahydroindolizine-6-ones. Most of the hydroxyproline-specific compounds are methylene active and condense to colored products. The compounds **2–14**, which have been identified in hydroxyproline/arabinose (erythrose) model experiments, are formed by analogous reaction sequences (Tressl et al., 1985b). The results show that most of the hydroxyproline-specific compounds result via 3-deoxyosone route. Only **19** and **21** are formed via 1-deoxyosone from glucose and rhamnose, respectively. In addition, we observed no 3-hydroxypyrrolidine derivatives. Obviously the amino acid is decarboxylated and dehydrated to 1-pyrrolyl derivatives at the stage of 1,2-enolization. 3-Hydroxypyrrolidine derivatives were characterized on heating 3-hydroxypyrrolidine with monosaccharides and cyclic enolones.

In contrast to proline the compounds formed from hydroxyproline possessed no bready or bitter taste qualities. Compounds **2** and **4** had mushroomlike aromas, and **3** was percieved with a fruity note. The tresholds of **2** and **3** were determined in water solution by using established procedures (Guadagni and Buttery, 1978) at 100 ppb. **6** had a geranic-like aroma and **10** and **16** possessed green notes and at higher concentrations mushroomlike odor qualities. Their tresholds were determined at 100 and 10 ppb, respectively. **21** and the 1-pyrrolylcyclopentenons possessed cookie-caramel-like aromas.

Registry No. 1, 109-97-7; **2**, 4805-24-7; **3**, 98612-18-1; **4**, 98612-19-2; **5**, 98612-20-5; **6**, 98612-21-6; **7**, 98612-22-7; **8**, 98612-23-8; **9**, 98612-24-9; **10**, 1438-94-4; 11, 98612-25-0; **12**, 98612-26-1; **13**, 98612-27-2; **14**, 98612-28-3; **15**, 98612-29-4; **16**, 13678-52-9; **17**, 98612-30-7; **18**, 98612-31-8; **19**, 91999-32-5; **20**, 98612-32-9; **21**, 81453-53-4; **22**, 98612-33-0; **23**, 98612-34-1; **24** (isomer 1), 98612-35-2; **24** (isomer 2), 98612-36-3; **25**, 81453-50-1; **26**, 81453-52-3; **27**, 81453-51-2; *cis*-**28**, 98612-37-4; *trans*-**28**, 98612-38-5; **29**, 98612-39-6; **30**, 98612-40-9; AcOH, 64-19-7; $AcCH_2OH$, 116-09-6; 2-furancarboxaldehyde, 98-01-1; 2-furanmethanol, 98-00-0; 4-hydroxy-5-methyl-3(2*H*)-furanone, 19322-27-1; 5-(hydroxymethyl)-2-furancarboxaldehyde, 67-47-0; 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, 28564-83-2; 2,3-dihydro-3-hydroxy-6-methyl-4*H*-pyran-4-one, 98612-41-0; 2-hydroxy-3-methyl-2-cyclopenten-1-one, 80-71-7; 5-methyl-2-furancarboxaldehyde, 620-02-0; 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3658-77-3; D-glucose, 50-99-7; rhamnose, 3615-41-6; hydroxyproline, 51-35-4; 7-methyl-1,2,3,4,5,6,7,8-octahydrocyclopent[*b*]azepin-8-one, 97826-66-9; 7-methyl-1,2,3,6,7,8-hexahydrocyclopent[*b*]azepin-8-one, 97826-67-0.

LITERATURE CITED

Anet, E. F. L. *J. Adv. Carbohydr. Chem.* **1964**, *19*, 181.

Doornbos, T.; van den Ouweland, G. A. M.; Tjan, S. B. *Prog. Food Nutr. Sci.* **1981**, *5*, 57.

Guadagni, D. G.; Buttery, R. G. *J. Food Sci.* **1978**, *43*, 1346.

Hodge, J. E. *J. Agric. Food Chem.* **1953**, *1*, 929.

Kobayashi, N.; Fujimaki, M. *Agric. Biol. Chem.* **1965**, *29*, 1059.

Ledl, F.; Hiebl, J.; Severin, T. *Z. Lebensm. Unters. Forsch.* **1983**, *177*, 353.

Ledl, F.; Severin, T. *Z. Lebens. Unters. Forsch.* **1982**, *175*, 262.

Mills, F. D.; Baker, B. G.; Hodge, J. E. *Carbohydr. Res.* **1970**, *15*, 205.

Mills, F. D.; Hodge, J. E. *Carbohydr. Res.* **1976**, *51*, 9.

Pabst, H. M. E.; Ledl, F.; Belitz, H.-D. *Z. Lebensm. Unters. Forsch.* **1984**, *178*, 356.

Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. "Tabellen zur Strukturaufklärung Organischer Verbindungen mit spektroskopischen Methoden", 2nd ed.; Springer: Berlin, Heidelberg, New York, 1981; p H295.

Tressl, R.; Grünewald, K. G.; Helak, B. In "Flavour 81"; Schreier, P., Ed.; Walter de Gruyter: Berlin, New York, 1981; p 397.

Tressel, R.; Helak, B.; Kamperschröer, H.; Rewicki, D. *J. Agric. Food Chem.*, in press.

Tressl, R.; Grünewald, K. G.; Kersten, E.; Rewicki, D. *J. Agric. Food Chem.*, in press.

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