peak 1 (1a in Figure 2 part C) contains similar subunit compositions as the parent peak and thus represents a smaller size, less aggregated form of Sepharose fraction 1 proteins.

Sepharose 6B fractions 2 and 3 (peaks 2 and 3) from control extract and fractions 2 through 3a from ion exchanged extract contained a typically high concentration of glycinin and β-conglycinin subunits as shown in Figure 2 part A and C. Thus, the appearance of the shoulder region on peak 3 (peak 3a in Figure 2 part C), coupled with the smaller size peak 2 for ion exchanged extract proteins, indicates a shift toward smaller sized protein molecular complexes as a result of the ion exchange treatment. It was also observed that peak 3 from all three extracts contained a higher concentration of β-subunits from β-conglycinin than in peak 2. This observation is consistent with work of Sykes and Gayler (1981), who reported that a β-conglycinin fraction consisting of 3 β-subunits was isolated from the trailing edge region of the glycinin-containing peak separated by Sepharose 6B gel filtration chromatography.

Although Sepharose 6B gel filtration chromatography effectively separates glycinin (11S) and β-conglycinin (7S) from the other soy proteins, it does not separate these two proteins from each other. It is therefore difficult to determine the separate effects of the ion exchange and alkaline pH phytate removal treatments upon each of these proteins. However, based upon the observation that 11S soy protein is essentially phytate free (Brooks and Morr, 1984), it is likely that the phytate removal process treatments would be most effective for altering the β-conglycinin (7S) protein components.

Physicochemical changes in the alkaline pH treated soy extract proteins were more severe than those for ion exchange treated soy extract. Sepharose peak 1 (Figure 2 part B) was not only slightly displaced with respect to control extract peak 1 in terms of elution volume to void volume ratio (Table II), but it was also broader and contained a greater amount of protein. As indicated above, these latter changes, coupled with the reduction in size of peaks 2 and 3, indicate that the alkaline pH treatment (Hartman, 1979) causes a shift toward larger sized protein complexes. Further, SDS-PAGE data (Figure 3) show that alkaline pH treated soy protein components of peak 1 contain significant amounts of glycinin and β-conglycinin subunits, which were absent from peak 1 of the other two extracts. Hartman (1979) reported no apparent denaturation of soy proteins upon holding soy extract at pH 11 for up to 7 h on the basis of disulfide group and lysinoalanine criteria. He concluded that temperatures in the range of ≥50 °C were more damaging to soy proteins than pH, per se. Our results disagree and demonstrate that the highly alkaline pH treatment results in substantial amounts of protein aggregation, which may or may not involve denaturation of the glycinin and β-conglycinin-containing protein components. These differences are probably due to the increased sensitivity of the electrophoretic and gel filtration methods used in this study.

In conclusion, it has been confirmed that the ion exchange process effectively removes phytate from soy protein extracts with a minimum of protein aggregation. Extracts prepared by this procedure should retain their functionality in most food product applications better than those processed by the alkaline pH treatment.

Registry No. Phytic acid, 83-86-3.

LITERATURE CITED

Allen, R. J. L. *Biochem. J.* **1940**, *34*, 858.
Bio-Rad Laboratories. "Technical Bulletin 1051"; Bio-Rad Laboratories: Richmond, CA, 1976.
Brooks, J. R.; Morr, C. V. *J. Food Sci.* **1982**, *47*, 1280.
Brooks, J. R.; Morr, C. V. *J. Agric. Food Chem.* **1984**, *32*, 672.
Chen, B. H.-Y.; Morr, C. V. *J. Food Sci.* **1985**, *50*, 1139.
Cheryan, M. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *13*, 297.
Cosgrove, D. J. *Rev. Pure Appl. Chem.* **1966**, *16*, 209.
Erdman, J. W., Jr. *J. Am. Oil Chem. Soc.* **1979**, *59*, 736.
Hartman, G. H., Jr. *J. Am. Oil Chem. Soc.* **1979**, *59*, 731.
J. T. Baker Chemical Company. "Ion Exchange Resins and Techniques: Nature, Applications, and Evaluation. Product Bulletin 103"; J. T. Baker Chemical Co.: Phillipsburg, NJ, 1958.
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
Maddaiah, V. T.; Kurnick, A. A.; Reid, B. L. *Soc. Exp. Biol. Med.* **1964**, *115*, 391.
Okubo, K.; Waldrop, G. A.; Iacobucci, G. A.; Myers, D. V. *Cereal Chem.* **1975**, *52*, 263.
Ritter, M. A.; Morr, C. V. "Abstracts of Papers", 45th Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June, 1985; Institute of Food Technologists: Chicago, IL, 1985; Abstr. 204.
Sykes, G. E.; Gayler, K. R. *Arch. Biochem. Biophys.* **1981**, *210*, 525.
Wolf, W. J.; Briggs, D. R. *Arch. Biochem. Biophys.* **1959**, *85*, 186.

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Formation of 2-(1-Pyrrolidinyl)-2-cyclopentenones and Cyclopent(*b*)azepin-8(1*H*)-ones as Proline Specific Maillard Products

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By means of MS, IR, and ^{1}H NMR spectroscopy eight 2-(1-pyrrolidinyl)-2-cyclopentenones (**1–8**) and eleven cyclopent(*b*)azepin-8(1*H*)-ones (**9–20**) were characterized in proline/monosaccharide and proline/cyclic enolone model experiments. The pyrrolidines possess bitter adstringent taste and the azepine derivates bitter taste qualities. Both classes of compounds are formed by a Strecker-type reaction of proline and cyclic enolones.

It has been demonstrated that the Maillard reaction of L-proline with monosaccharides produce a complex mixture of proline specific compounds with bread, cracker-like, roasty aromas and bitter taste qualities (Hunter et al., 1969; Shigematsu et al., 1975; Mills and Hodge, 1976; Tressl et al., 1981; Pabst et al., 1984). During our investigation of the compounds produced by the reaction of L-proline with D-glucose we isolated a nitrogen containing

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component with a molecular weight of 163 which possessed bitter taste quality. This compound was also detected in roasted malt, wort, and beer. By means of MS, IR, and ^1H and ^{13}C NMR spectroscopy the structure was identified as 7-methyl-2,3,6,7-tetrahydrocyclopent(*b*)azepin-8(1*H*)-one. In addition we characterized ten closely related compounds which are formed in proline/monosaccharide model experiments. Further investigation showed that cyclic 1,2-diketones ⇄ enolones act as precursors and 2-(1-pyrrolidinyl)-2-cyclopentanones with bitter adstringent taste qualities were detected.

In this paper we demonstrate, that the formation of cyclopent(*b*)azepin-(8(1*H*)-ones and 2-(1-pyrrolidinyl)-2-cyclopenten-1-ones is closely correlated. Both classes of compounds result from a Strecker-type reaction of L-proline with cyclic 1,2-diketones ⇄ enolones.

EXPERIMENTAL SECTION

Sample Preparation. Reaction of Pyrrolidine and Cyclic Enolones. Equimolar amounts of pyrrolidine and cyclic enolones (0.02 mol of 2-hydroxy-2-cyclopenten-1-one, 0.02 mol of 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), 0.01 mol of 3-ethyl-2-hydroxy-2-cyclopenten-1-one, 0.01 mol of 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one) dissolved in water were refluxed for 1 h. After the mixtures were cooled to room temperature, the pH was adjusted to 10 with 0.1 N NaOH and the compounds were extracted 3 times with freshly distilled ether. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL on a Vigreux column.

Reaction of L-Proline and Cyclic Enolones. Equimolar amounts of L-proline and cyclic enolones (0.01 mol of 2-hydroxy-2-cyclopenten-1-one, 0.02 mol of 2-hydroxy-3-methyl-2-cyclopenten-1-one, 0.01 mol of 3-ethyl-2-hydroxy-2-cyclopenten-1-one, 0.01 mol of 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one) were dissolved in water and autoclaved for 1.5 h at 150–160 °C in a stainless steel laboratory autoclave (Roth, I Series) equipped with a 100-mL duran glass tube and heated by an electric heater with a magnetic stirrer. After the mixture had cooled to room temperature, the pH was adjusted to 10 and the compounds were extracted three times with freshly distilled diethyl ether. The combined ether extracts were dried over anhydous sodium sulfate and concentrated to a volume of 1 mL. The compounds **1–16** were separated from other products by liquid-solid chromatography on Al_2O_3 (basic, activity III–IV) with pentane/methylene chloride (3:1) and isolated by preparative GC: compound **9**, 20 mg; **10**, 5 mg; **11**, 20 mg; **12**, 5 mg; **13**, 3 mg; **14**, 1 mg; **15**, 5 mg as a 3:1 mixture of trans/cis isomers; **16**, 1 mg as a 3:1 mixture of trans/cis isomers. From **15** 1 mg of the pure trans isomer was separated by preparative GC. The isolated compounds were dissolved in $CDCl_3$ and investigated by IR and ^1H NMR spectroscopy.

Reaction of L-Proline and Monosaccharides. Equimolar amounts of L-proline and monosaccharides (0.028 mol of rhamnose, 0.028 mol of glucose, 0.033 mol of arabinose, 0.017 mol of erythrose, and 0.056 mol of glyceraldehyde) dissolved in water were autoclaved and the components extracted as described for proline/cyclic enolones. Aliquote amounts of the extracts were investigated by capillary GC/MS and nitrogen selective detector.

Gas Chromatography (GC)–Mass Spectrometry (MS). Capillary GC–mass spectrometry was carried out by using a 25-m glass capillary column(0.32 mm i.d.) coated with Carbowax 20M + KOH (column A) coupled with a Finnigan MAT 4500 quadrupole instrument and a 50-m glass capillary (0.32 mm i.d.) coated with CP Sil CP (Chrompack) (column B) coupled with a double focussing mass spectrometer CH 5-DF (Varian MAT). Conditions were as follows: column A, temperature program 70–180 °C at 2 °C/min (Carlo Erba fractovap 2101), ionization voltage 70 eV, resolution 1000; column B, on column injection, temperature program 100–260 °C at 4 °C/min, ionization voltage 70 eV, resolution 2000 (10% valley).

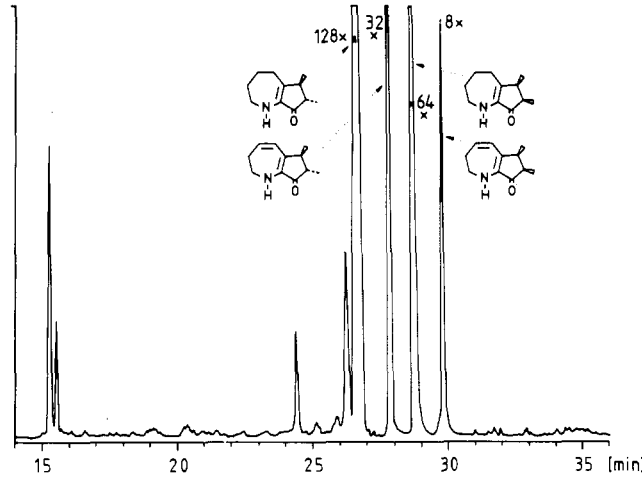


Figure 1. GC separation of cyclopent(*b*)azepin-8(1*H*)-ones formed in the proline/2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one model experiment (column B).

Preparative Gas Chromatography. Investigations were carried out with a Verian Aerograph Column C: 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 D: 3 m (2 mm i.d.) glass 5% SP 2401 BB on 100–200 mesh Supelcoport, temperature program 100–250 °C, 4 °C/min.

^1H NMR and IR Spectroscopy. ^1H 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; coupling constants J are given in Hz. Infrared spectra were obtained from $CDCl_3$ or CCl_4 solutions with a Perkin-Elmer Model 275 instrument.

RESULTS AND DISCUSSION

Cyclic α-dicarbonyls ⇄ enolones are formed during Maillard reaction by aldol-type condensation from glycolaldehyde, pyruvaldehyde, acetol etc, which are produced by retro aldol cleavage of the sugars. In our model experiments the cyclic enolones were heated with L-proline during 1 h at 150 °C as well as with pyrrolidine at 100 °C and the volatiles were extracted with ether, separated according to polarity by liquid adsorption chromatography and investigated by capillary GC/MS. Figure 1 presents a typical gas chromatogram of the reaction products from L-proline with 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one. The components were isolated by preparative GC and investigated by MS, IR, and ^1H NMR spectroscopy. In similar experiments 2-hydroxy-, 2-hydroxy-3-methyl-, 3-ethyl-2-hydroxy-, and 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one were heated with proline and pyrrolidine and the products identified. The results are summarized in Tables I and II.

In Table I the MS, IR, and ^1H NMR spectroscopic data of eight 2-(1-pyrrolidinyl)-2-cyclopenten-1-ones are presented. The spectroscopic data of the characterized azepines are summarized in Table II. The corresponding pyrrolidines, azepines, and vinylogous amides possess similar mass spectra. Pyrrolidine derivatives possess a characteristic fragment at m/e 70.

Table I. Mass Spectra, ^{1}H NMR Spectra, and IR Spectra of 2-(1-Pyrrolidinyl)-2-cyclopenten-1-ones[a]

(1) 2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 151 (100), 150 (45), 123 (38), 122 (75), 108 (24), 95 (55), 94 (56), 70 (68), 67 (26), 54 (25); ^{1}H NMR δ 1.85 (mc, 4 H, β'-CH_2), 2.42 (mc, 2 H, H-4), 2.52 (mc, 2 H, H-5), 3.28 (mc, 4 H, α'-CH_2), 5.91 (t, 1 H, J = 3 Hz, H-3); IR 2970 (m), 2930 (w), 2870 (w), 1695 (s), 1610 (s), 1410 (w), 1330 (s), 1300 (m), 1260 (s), 1155 (m), 1020 (m) cm^{-1}

(2) 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 165 (90), 164 (28), 150 (27), 136 (40), 122 (100), 108 (38), 95 (41), 94 (34), 70 (23), 67 (24), 54 (25); ^{1}H NMR δ 1.17 (d, 3 H, J = 7.6 Hz, $CHCH_3$), 1.84 (mc, 4 H, β'-CH_2), 2.10 (dm, $J_{4,4'}$ = 17.7 Hz, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 2.3 Hz, $J_{4,\alpha'}$ = 0.6 Hz, H-4, cis to 5-CH_3), 2.37 (dqui, 1 H, J = 7.6 Hz, J = 2.3 Hz, H-5), 2.76 (ddd qui, 1 H, $J_{4,4'}$ = 17.7 Hz, $J_{4',5}$ = 7 Hz, $J_{3,4'}$ = 3.3 Hz, $J_{4,\alpha'}$ = 0.6 Hz, H-4'), 3.27 (mc, 4 H, α'-CH_2) 5.83 (t, 1 H, J = 3.3 Hz, H-3); IR 2970 (m), 2940 (w), 2880 (w), 2860 (w), 1700 (s), 1610 (s), 1460 (m), 1390 (m), 1160 (m), 980 (m) cm^{-1}

(3) 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 165 (100), 164 (49), 137 (43), 136 (47), 122 (49), 109 (39), 108 (56), 94 (31), 81 (24), 70 (22); ^{1}H NMR δ 1.74 (mc, 4 H, β'-CH_2), 2.08 (s, 3 H, 3-CH_3), 2.29, 2.35 (each mc, 2 H, H-4, H-5), 3.38 (mc, 4 H, α'-CH_2)

(4) 5-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 179 (60), 151 (45), 150 (86), 136 (75), 122 (100), 108 (26), 95 (35), 70 (42), 67 (27), 55 (26), 54 (25), 41 (27); ^{1}H NMR δ 0.95 (t, 3 H, J = 7 Hz, CH_2CH_3), 1.39 (mc, 2 H, $CHHCH_3$), 1.84 (mc, 4 H, β'-CH_2), 2.17 (ddd, 1 H, $J_{4,4'}$ = 18 Hz, $J_{3,4}$ = 3 Hz, $J_{4,\alpha'}$ = 0.5 Hz, H-4, cis to 5-C_2H_5), 2.30 (ddt, 1 H, J_{5,CH_2} = 7 Hz, $J_{4,5'}$ = 6 Hz, $J_{4,5}$ = 2 Hz, H-5), 2.66 (ddd, 1 H, J = 18 Hz, J = 3 Hz, H-4'), 3.27 (mc, 4 H, α'-CH_2) 5.86 (t, 1 H, J = 3 Hz, H-3); relative ^{1}H NMR shift parameters (Eu(dpm)$_3$, $CDCl_3$) 2.80 (H-5), 1.67 (α'-CH_2), 1.60/1.57 (CH_2CH_3), 1.26/1.20 (H-4, H-4'), 1.00 (H-3), 0.56 (CH_2CH_3), 0.38 (β'-CH_2);[b] IR 2970 (m), 2930 (w), 2880 (w), 2860 (w), 1700 (s), 1610 (s), 1490 (w), 1460 (m), 1390 (m), 1350 (m), 1315 (w), 1225 (w) cm^{-1}

(5) 3-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 179 (63), 178 (18), 164 (72), 150 (23), 136 (100), 122 (30), 108 (40), 95 (17), 94 (31), 70 (17), 55 (17), 41 (21); ^{1}H NMR δ 1.13 (t, 3 H, J = 7 Hz, CH_2CH_3), 1.81 (mc, 4 H, β'-CH_2), 2.34, 2.44 (each mc, 2 H, H-4, H-5), 2.52 (q, 2 H, J = 7 Hz, CH_2CH_3), 3.28 (mc, 4 H, α'-CH_2); IR 2980 (m), 2940 (m), 2880 (m), 1680 (s), 1605 (m), 1460 (m), 1385 (m), 1350 (w), 1210 (w), 1110 (m), 1070 (w) cm^{-1}

(6) 3,4-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 179 (38), 178 (9), 165 (8), 164 (72), 137 (11), 136 (100), 109 (10), 108 (16), 94 (9), 81 (8), 70 (19), 67 (10), 55 (10), 53 (11), 41 (16); ^{1}H NMR δ 1.0 (d, 3 H, J = 6.8 Hz, 4-CH_3), 1.78 (mc, 4 H, β'-CH_2), 1.90 (dd, 1 H, J = 17.8 Hz, H-5), 2.06 (s, 3 H, 3-CH_3), 2.45–2.70 (each m, 2 H, H-4, H-5'), 3.38, 3.46 (each sym mc, 4 H, α'-CH_2)

(7) *trans*-4,5-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 179 (46), 164 (95), 136 (100), 122 (13), 108 (19), 95 (14), 94 (17), 70 (23), 68 (15), 67 (15), 55 (13), 53 (13), 41 (19); ^{1}H NMR δ 1.13, 1.14 (each d, 3 H, J = 7 Hz, 4-CH_3, 5-CH_3), 1.82 (mc, 4 H, β'-CH_2), 1.86 (dq, 1 H, J = 7 Hz, J = 2.5 Hz, H-5), 2.32 (mc, 1 H, H-4), 3.16, 3.25 (sym m, each 2 H, α'-CH_2), 5.70 (d, 1 H, J = 3 Hz, H-3)

(8) 5-methyl-2,5-di-1-pyrrolidinyl-2-cyclopenten-1-one: MS, *m/e* (relative intensity) 234 (2), 208 (12), 165 (48), 137 (13), 136 (33), 122 (35), 111 (100), 110 (30), 109 (19), 98 (10), 96 (10), 83 (52), 70 (19), 68 (21), 55 (14), 54 (11), 42 (29); ^{1}H NMR δ 1.33 (s, 3 H, 5-CH_3), 1.75 (mc, 4 H, β'-CH_2), 1.83 (mc, 4 H, β''-CH_2), 2.14 (dd, 1 H, J = 3.3 Hz, H-4), 2.60 (dd, 1 H, J = 18 Hz, J = 2.2 Hz, H-4'), 2.55, 2.73 (each mc, 2 H, α'-CH_2), 3.25 (mc, 4 H, α''-CH_2), 5.76 (t, 1 H, J = 3 Hz, H-2)

[a] Abbreviations: NMR 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.; IR s = strong, m = medium, w = weak. [b] The NMR shift parameter is defined as $(\nu_i - \nu^\circ_i)/(\nu_{H\text{-}3} - \nu^\circ_{H\text{-}3})$.

Compound **1** was identified as 2-(1-pyrrolidinyl)-2-cyclopenten-1-one. **1** is formed during heating of pyrrolidine with 2-hydroxy-2-cyclopenten-1-one. The NMR spectrum indicates as 1-pyrrolidinyl system (δ 1.85, β'-CH_2, and δ 3.28, α'-CH_2, each mc, 4 H), two methylene groups at δ 2.42, 2.52, and one olefinic proton at δ 5.91. The IR spectrum confirms a conjugated carbonyl group (1695 (s), 1610 cm^{-1} (s)). Therefore all spectroscopic data of **1** are in agreement with the proposed structure.

Components **2** and **3** were formed from cyclotene and pyrrolidine in a ratio of 1:1. The IR spectra indicate a conjugated carbonyl group and the ^{1}H NMR spectra confirm a 1-pyrrolidinyl system in both components. The signals at δ 1.17 and 2.37 clearly show a >$CHCH_3$ group in **2**. Decoupling experiments confirmed the H-3 and the nonequivalent methylene protons H-4 and H-4', which show a more complex pattern by several vicinal and long range couplings. Therefore, **2** was identified as 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one. All spectroscopic data of **3** are in agreement with 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one, the methyl signal is shifted by 1 ppm to lower field because of the proximity of the double bond and an olefinic proton cannot be observed. Compounds **4** and **5** were isolated from the pyrrolidine/3-ethyl-2-hydroxy-2-cyclopenten-1-one model system. The isomeric products possessed a conjugated carbonyl system **4** (1700 (s), 1610 cm^{-1} (s)) and **5** (1680 (s), 1605 cm^{-1} (m)), respectively. The ^{1}H NMR spectrum of **4** is in full agreement with the proposed structure. Especially it shows one diastereotopic methylene group and one olefinic proton. The position of the pyrrolidinyl substituent was in this case established by measuring the NMR shift parameters with tris(dipivalomethanato)europium as shift reagent. The observed lanthanid induced shifts (cf. Table I) are largest for H-5, the diastereotopic methylene protons of the ethyl group, and the α-CH_2 protons of the pyrrolidine group and much lower for the olefinic H-3. In the case of the isomeric 3-yrrolidinyl compound a large induced shift is predicted for the olefinic H-2 independent from the position where the lanthanide shift reagent is coordinated by the difunctional substrate molecule. As expected, the ^{1}H NMR spectrum of **5** is less complex, showing neither diastereotopic methylene group nor an olefinic proton, and is in coincidence with 3-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one.

Compounds **6** and **7** were enriched from the 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one/pyrrolidine experiment. The NMR spectrum of **6** showed two methyl groups. The other spectroscopic data are in agreement with 3,4-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one. Compound **7** possessed two >$CHCH_3$ groups (δ 1.13, 1.14) and one olefinic proton at δ 5.70. Decoupling experiments confirmed **7** as *trans*-4,5-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one.

On heating maltol and pyrrolidine as already described (Tressl et al., 1985) a further pyrrolidinyl compound (**8**) was formed, which was identified during this work. The mass spectrum showed a parent peak at *m/e* 234 and the typical fragment *m/e* 70 of pyrrolidines suggesting two pyrrolidinyl groups. The NMR spectrum confirmed two

Table II. Mass Spectra and ^{1}H NMR Spectra of Cyclopent(*b*)azepin-8(1*H*)-ones

(9) 2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 151 (99), 150 (39), 136 (6), 123 (45), 122 (44), 108 (30), 95 (100), 94 (44), 80 (25), 67 (47), 55 (26), 53 (31); ^{1}H NMR δ 1.63 (mc, 2 H, H-C(4)), 1.72 (mc, 2 H, H-C(3)), 2.25–2.55 (m, 6 H, H-C(5), H-C(6), H-C(7)), 2.99 (mc, 2 H, H-C(2)), 4.10 (br s, 1 H, H-N(1))

(10) 2,3,6,7-tetrahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 149 (81), 148 (100), 134 (18), 121 (5), 120 (17), 106 (22), 93 (17), 91 (14), 80 (12), 79 (16), 77 (16), 66 (12), 65 (17), 52 (13); ^{1}H NMR δ 2.42, 2.53 (mc, each 2 H, H-C(6), H-C(7)), 2.63 (dt, J = 4.2, 3.8 Hz, 2 H, H-C(3)), 3.29 (dt, J = 4.2, 3.8 Hz, 2 H, H-C(2)), 4.51 (br s, 1 H, H-N(1)), 6.03 (mc, J = 10.6 Hz, 2 H, H-C(4), H-C(5))

(11) 7-methyl-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 165 (100), 164 (32), 150 (20), 137 (33), 136 (43), 122 (83), 109 (51), 108 (62), 95 (70), 94 (46), 81 (29), 80 (28), 67 (59), 55 (24), 54 (24), 53 (34); ^{1}H NMR δ 1.07 (d, J = 7.5 Hz, 3 H, CH_3-C(7)), 1.55 (mc, 2 H, H-C(4)), 1.65 (mc, 2 H, H-C(3)), 1.96 (ddt, J = 16.9, 1.7, 1 Hz, 1 H, H-C(6)), 2.22–2.37 (m, 3 H, H-C(5) and H-C(7)), 2.61 (ddt, J = 16.9, 6.4, 1 Hz, 1 H, H-C(6)), 2.92 (mc, 2 H, H-C(2)), 4.04 (br s, 1 H, H-N(1))

(12) 7-methyl-2,3,6,7-tetrahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 163 (78), 162 (100), 148 (16), 134 (10), 121 (3), 120 (18), 106 (16), 93 (15), 91 (15), 80 (10), 79 (17), 77 (18), 66 (11), 65 (17), 53 (11), 52 (12), 51 (11); ^{1}H NMR δ 1.20 (d, J = 7.5 Hz, 3 H, CH_3-C(7)), 2.27 (dd, J = 16.5, 2, 1 Hz, 1 H, H-C(6)), 2.47 (d qui, J = 7.5, 2 Hz, 1 H, H-C(7)), 2.66 (dt, J = 4.2, 3.8 Hz, 2 H, H-C(3)), 2.82 (dd, J = 16.5, 7.5 Hz, 1 H, H-C(6)), 3.32 (mc, 2 H, H-C(2)), 4.55 (br s, 1 H, H-N(1)), 6.05 (AB, J = 10.6 Hz, 1 H, H-C(5)), 6.10 (AB, J = 10.6 Hz, 1 H, H-C(4))

(13) 7-ethyl-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 179 (100), 178 (97), 178 (24), 164 (14), 151 (43), 150 (54), 136 (55), 123 (36), 122 (100), 108 (39), 95 (45), 94 (31), 81 (22), 80 (23), 77 (17), 67 (34), 65 (15), 55 (21), 53 (26); ^{1}H NMR δ 0.96 (t, J = 7.5 Hz, 3 H, CH_3), 1.38, 1.85 (each mc, signals of the diastereotopic methylene protons, 1 H), 1.64 (mc, 2 H, H-C(4)), 1.73 (mc, 2 H, H-C(3)), 2.14 (dd, J = 16.5, 1.5 Hz, 1 H, H-C(6)), 2.32 (mc, 1 H, H-C(7)), 2.40 (mc, 2 H, H-C(5)), 2.62 (dd, J = 16.5, 6.4 Hz, 1 H, H-C(6)), 3.02 (mc, 2 H, H-C(2)), 4.12 (br s, 1 H, H-N(1))

(14) 7-ethyl-2,3,6,7-tetrahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 177 (78), 176 (100), 162 (7), 149 (11), 148 (10), 134 (8), 121 (5), 106 (14), 93 (11), 91 (17), 79 (14), 77 (21), 66 (6), 65 (13), 52 (5), 51 (6); ^{1}H NMR δ 0.98 (t, J = 7.5 Hz, 3 H, CH_3), 1.40, 1.86 (each mc, signals of the diastereotopic methylene protons, 1 H), 2.24 (dd, J = 16.5, 1.6 Hz, 1 H, H-C(6)), 2.38 (mc, 1 H, H-C(7)), 2.66 (mc, 2 H, H-C(3)), 2.74 (dd, J = 16.5, 6.4 Hz, 1 H, H-C(6)), 3.33 (mc, 2 H, H-C(2)), 4.50 (br s, 1 H, H-N(1)), 6.06–6.12 (m, 2 H, H-C(4), H-C(5))

(15) 6,7-dimethyl-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 179 (37), 164 (100), 151 (3), 150 (4), 136 (42), 122 (9), 109 (4), 108 (13), 95 (16), 94 (13), 91 (9), 80 (9), 79 (9), 77 (9), 67 (17), 55 (10), 53 (13); ^{1}H NMR δ 1.13 (d, J = 7.5 Hz, 6 H, CH_3), 1.45–1.65 (m, H-C(4)), 1.65–1.80 (m, 2 H, H-C(3)), 1.90, 2.18 (each dq, J = 7.5, 1.5 Hz, 1 H, H-C(6), H-C(7)), 2.30 (mc, 2 H, H-C(5)), 2.94 (mc, 2 H, H-C(2)), 4.07 (br s, 1 H, H-N(1)) (trans isomer)

(16) 6,7-dimethyl-2,3,6,7-tetrahydrocyclopent(*b*)azepin-8(1*H*)-one: MS, *m/e* (relative intensity) 177 (57), 176 (22), 162 (100), 148 (4), 134 (20), 121 (2), 120 (5), 106 (6), 105 (50), 93 (9), 91 (18), 79 (16), 77 (18), 65 (14), 53 (10)

(17/18) 7-methyl-7-[3(or 5)-methyl-2-oxocyclopentyl]-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one:[b] MS, *m/e* (relative intensity) 261 (26), 246 (4), 164 (40), 163 (100), 148 (10), 138 (10), 137 (7), 120 (5), 108 (13), 106 (6), 93 (7), 92 (7), 90 (6), 81 (6), 79 (9), 77 (8), 67 (14), 65 (5), 55 (10), 53 (8), 41 (16), 39 (6)

(19) 6,7-dimethyl-7-[3,4(or 4,5)-dimethyl-2-oxocyclopentyl]-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one:[b] MS, *m/e* (relative intensity) 289 (30), 274 (11), 221 (4), 191 (11), 178 (43), 177 (100), 176 (19), 163 (11), 162 (56), 152 (8), 149 (9), 136 (13), 134 (11), 133 (11), 122 (10), 120 (8), 110 (9), 108 (11), 105 (8), 97 (6), 96 (20), 91 (10), 81 (11), 79 (13), 77 (11), 73 (27), 69 (13), 67 (20), 55 (21), 53 (12)

(20) 6,7-dimethyl-8-[6,7-dimethyl-8-oxo-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-7-yl]-2,3,4,4,5,6,7-hexahydrocyclopent(*b*)azepin-8-ol:[b] MS, *m/e* (relative intensity) 358 (9), 341 (3), 330 (16), 274 (9), 273 (10), 267 (12), 246 (10), 221 (5), 205 (14), 191 (13), 190 (10), 181 (22), 180 (100), 1 9 (41), 178 (19), 164 (11), 151 (7), 148 (8), 163 (9), 133 (14), 123 (7), 122 (7), 120 (6), 110 (7), 108 (7), 96 (24), 95 (18), 81 (12), 79 (11), 73 (32), 67 (24), 55 (26)

[a] Abbreviations: see Table I. [b] Tentatively assigned.

pyrrolidinyl groups, one methyl group, and one olefinic proton. Decoupling experiments confirmed the structure of 8 as 5-methyl-2,5-bis(1-pyrrolidinyl)-2-cyclopenten-1-one.

The MS and ^{1}H NMR spectroscopic data of the cyclopent(*b*)azepine derivatives characterized in this study are summarized in Table II. As far as we know, azepine derivatives were reported for the first time as Maillard products. The elucidation of the structures was carried out by high resolution MS, IR, and ^{1}H and ^{13}C NMR spectroscopy as well as by 2D NMR spectroscopy. These results will be published in detail (Tressl et al., in preparation).

Cyclopent(*b*)azepines and 2-(1-pyrrolidinyl)-2-cyclopenten-1-ones are formed in the ppm range in the proline/monosaccharide model experiments. Some results are presented in Table III. Their concentrations increased 1000-fold in the proline (pyrrolidine)/cyclic enolone experiments as shown in Table IV.

These results demonstrate, that cyclic α-dicarbonyls ⇄ enolones may act as precursors for both classes of compounds. The observation, that 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one is not transformed into a corresponding azepine derivate shows that a methylene group in an α-position to a carbonyl group of the cyclic α-dicarbonyl is essential. Aliphatic α-dicarbonyls like methylglyoxal (and homologous components) are transformed into tetrahydropyridines and 2,3-dihydro-1*H*-pyrrolizines and 2,3-butandione (and homologous components) into 2,3-dihydro-(1*H*)-pyrrolizines and 5,6-dihydroindolizines (Tressl et al., 1981). Figure 2 may explain the closely correlated formation of pyrrolidinylcyclopentenones and cyclopent(*b*)azepine derivatives. Cyclic enolones and proline form an iminium carboxylate intermediate, which is decarboxylated via a reactive iminium ion and further degraded into pyrrolidine and a cyclic enolone or into 1-pyrroline and a cyclic α-hydroxy ketone. The formation of 1-pyrrolidinylcyclopentenones in the proline/monosaccharide model experiments may be explained by this route. In addition, pyrrolidine and cyclic enolones are also converted into 1-pyrrolidinylcyclopentenones. The formation of azepine derivatives may be explained by an

Table III. Cyclopent(*b*)azepine Derivatives Characterized in Proline/Monosaccharide Model Systems (Figures Correspond to Concentrations in ppm)

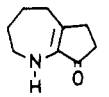
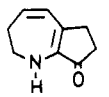
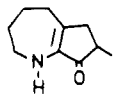
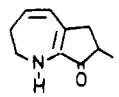
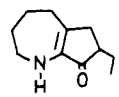
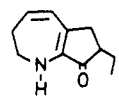
	GLY	ERY	ARA	GLU	RHAM
	7	5	30	5	5
	8	5	60	20	10
	21	10	1	15	30
	77	3	10	25	20
	1	1	-	5	2
	6	5	-	2	1

Table IV. Formation of Pyrrolidinylcyclopentenones and Cyclopent(*b*)azepin-8(1*H*)-ones in Proline (Pro) and Pyrrolidine (Pyrr) Model Experiments[a]

	A			B		
		PYRR	PRO		PYRR	PRO
1		15	1,1	2 	100	4
				3 	80	1
9		-	10	11 	-	40
10		1	2,5	12 	4	8
	C			D		
		PYRR	PRO		PYRR	PRO
4		25	1,2	6 	13	1,5
5		15	0,1	7 	10	0,2
13		-	6,3	15 	-	7,2 1,5[a]
14		0,5	0,6	16 	0,5	1,5 0,4[a]

[a] 2-Hydroxy-2-cyclopenten-1-one (A), 2-hydroxy-5-methyl-2-cyclopenten-1-one (B), 2-hydroxy-5-ethyl-2-cyclopenten-1-one (C), and 2-hydroxy-4,5-dimethyl-2-cyclopentene-1-one (D). The figures represent mg/g.

analogous ring enlargement of the proline system as discussed for tetrahydropyridines. Hexahydrocyclopent(*b*)-

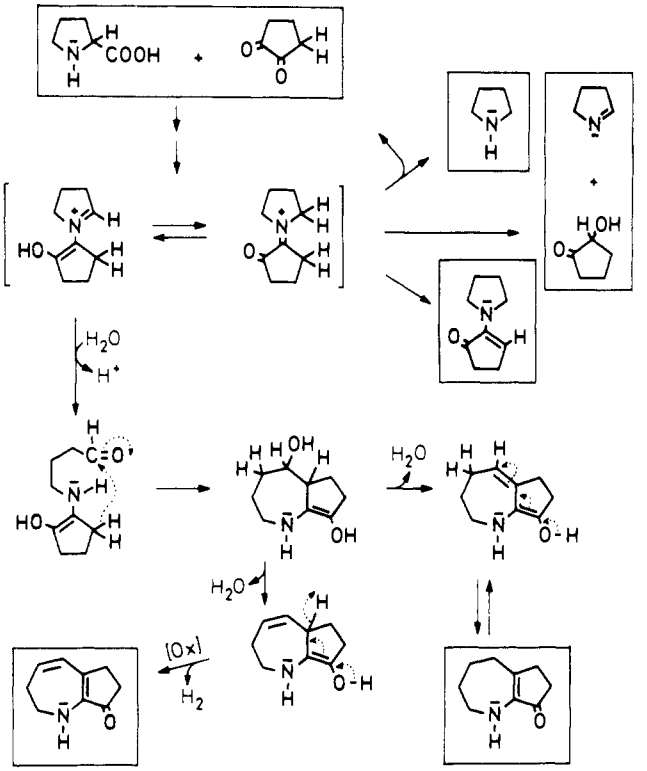


Figure 2. Formation of 2-(1-pyrrolidinyl-2-cyclopenten-1-ones and cyclopent(*b*)azepin-8(1*H*)-ones in proline/cyclic enolone model experiments.

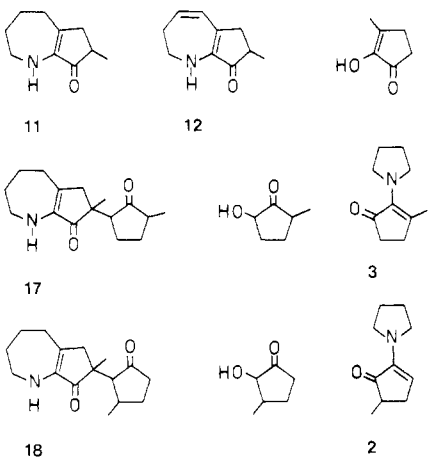


Figure 3. Components characterized in the proline/2-hydroxy-3-methyl-2-cyclopenten-1-one model experiment.

azepin-8(1*H*)-ones and tetrahydrocyclopent(*b*)azepin-8-(1*H*)-ones can be formed from the same intermediate by dehydration or dehydration and oxidation, respectively. Both components are always produced together, and their ratio depends on the reaction conditions. Cyclopent(*b*)-azepin-8(1*H*)-ones characterized in this study possess an active methylene group at C(7) which undergoes aldol condensation with α-hydroxy ketones also formed during the model experiments leading to 7-(2-oxocyclopentyl)-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-ones. Figure 3 presents the structure of components characterized in the proline/cyclotene model experiment.

The isomeric components **17** and **18** were tentatively identified according to mass spectrometric fragmentation as 7-methyl-7-(3(or 5)-methyl-2-oxocyclopentyl)-2,3,4,5,6,7-hexahydrocyclopent(*b*)azepin-8(1*H*)-one. Both compounds possessed identical mass spectra but different retention times. The reaction mixture of the proline/cy-

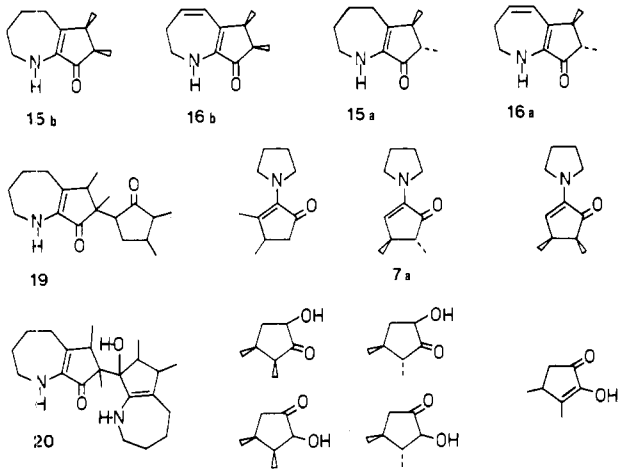


Figure 4. Components characterized in the proline/2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one model experiment.

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

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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-

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