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Quantitative Model Studies on the Efficiency of Precursors in the Formation of Cooling-Active 1-Pyrrolidinyl-2-cyclopenten-1-ones and Bitter-Tasting Cyclopenta-[*b*]azepin-8(1*H*)-ones

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The yields of the cooling-active compounds 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**1**) and 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**2**) as well as the bitter tastants 7-methyl-2,3,6,7-tetrahydrocyclopenta-[*b*]azepin-8(1*H*)-one (**3**) and 7-methyl-2,3,4,5,6,7-hexahydrocyclopenta-[*b*]azepin-8(1*H*)-one (**4**) obtained by heating mixtures of possible Maillard-type precursors in model systems varying in temperature, pH value, or water content were determined quantitatively. The results showed that hexose-derived cyclotene is the common precursor for all four tastants and that the formation of each individual tastant is strongly determined by the structure of the nitrogen-containing precursor, e.g., reaction of cyclotene with pyrrolidine formed by thermal decarboxylation of L-proline produced the cooling compounds **1** and **2** only, whereas in the presence of 1-pyrroline formed upon Strecker reactions of L-proline, the bitter tasting azepinone **3** was produced exclusively. In contrast, the structure of the secondary amino acid L-proline enabled the formation of compound **4**, whereas the pyrrolidine and 1-pyrroline, respectively, do not generate this tastant. In addition, a nonvolatile, tasteless intermediate, (*S*)-3-methyl-2-[(2′-carboxy)-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**), was isolated from the cyclotene/L-proline reaction mixture and could be confirmed as an efficient precursor for the cooling compound **1**. The data, obtained by these studies, are the scientific basis to tailor the desired overall flavor of foods by means of a more controlled Maillard-type technology.

KEYWORDS: Taste compounds; flavor precursors; 1-pyrroline, cyclotene

INTRODUCTION

The Maillard reaction between reducing carbohydrates and amino acids is chiefly responsible for the development of the unique aromas and typical tastes during thermal processing of foods, such as roasting of meat or coffee, baking of bread, or kiln-drying of malt. In comparison to the aroma-active volatiles, the information available on the structure and the formation of taste compounds generated during thermal food processing is as yet very limited.

To study the taste compounds formed from Maillard reactions during the roasting of malt, we recently mimicked the tastant formation by roasting the predominant Maillard-type precursors present in green malt, namely, the secondary amino acid L-proline and glucose (*1*). Application of the recently developed taste dilution analysis (*2*) on this strongly taste-active mixture led to the identification of 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one, **1** (**Figure 1**), and 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one, **2** (**Figure 1**), exhibiting strong cooling properties in the oral cavity (*1*, *3*). Both compounds could be shown by ^{13}C-labeling experiments to be formed from the reaction of L-proline with the hexose degradation product 2-hydroxy-3-methyl-2-cyclopenten-1-one, the so-called cyclotene (*1*). This intermediate was also reported as the common precursor in the formation of the bitter tasting compounds

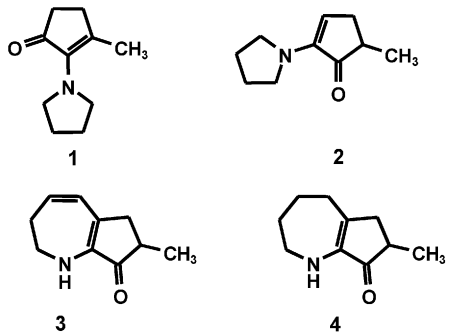

Figure 1. Structures of 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**1**), 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**2**), 7-methyl-2,3,6,7-tetrahydro-cyclopenta-[*b*]azepin-8(1*H*)-one (**3**), and 7-methyl-2,3,4,5,6,7-hexahydro-cyclopenta-[*b*]azepin-8(1*H*)-one (**4**).

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7-methyl-2,3,6,7-tetrahydrocyclopenta-[*b*]azepin-8(1*H*)-one, **3** (**Figure 1**) and 7-methyl-2,3,4,5,6,7-hexahydrocyclopenta-[*b*]-azepin-8(1*H*)-one, **4** (**Figure 1**) from hexoses and L-proline (*4*, *5*). Despite their different oxidation states, the authors suggested that compound **1** and **2** might be formed from cyclotene and L-proline via a common enaminol intermediate (*4*). This intermediate was suggested to give compound **4** directly upon water elimination and compound **3** upon water elimination and oxidation (*4*). Model experiments to prove these suggestions have as yet, however, not been performed.

With respect to tailoring desirable food flavors by controlling product formation more efficiently, detailed information is required on how the yields of these taste compounds can be influenced, i.e., how the yields of the bitter-tasting compounds can be decreased to the benefit of the more desirable cooling compounds **1** and **2**.

The following quantitative model studies were, therefore, undertaken to gain more detailed insights into the efficiency of possible precursors and also of the reaction conditions governing the formation of the cooling-active compounds **1** and **2** and the bitter-tasting compounds **3** and **4** during food processing.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), glucose, L-proline, sodium metaperiodate, 2-methyl-1-pyrroline, 2-acetyl-4-methylpyridine, and copper(II) acetate (Aldrich, Steinheim, Germany). Solvents were HPLC-grade (Aldrich, Steinheim, Germany). Deuterated solvents were obtained from Isocom (Landshut, Germany). 3-Methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**1**), 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**2**), and (*S*)-3-methyl-2-[(2′-carboxy)-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**) were synthesized as reported previously (*3*).

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC/MS). HRGC was performed with a Type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) by using a 30 m × 0.32 mm i.d., 0.25 μm, DB-5 fused silica capillary (J&W Scientific, Fisons, Mainz, Germany). The samples were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C and held for 2 min isothermally and then raised at 6 °C/min to 230 °C and held for 5 min. The flow of the carrier gas helium was 2.5 mL/min. MS analysis was performed with an MS 95 S (Finnigan, Bremen, Germany) in tandem with the HRGC. Mass chromatography in the electron impact mode (MS/EI) was performed at 70 eV.

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (Kontron, Eching, Germany) consisted of two pumps (type 422), a gradient mixer (M 800), a Rheodyne injector (100 μL loop), and a diode array detector (DAD type 540) monitoring the effluent in a wavelength range 220−500 nm. Separations were performed on a stainless steel column packed with 250 × 10 mm^2 RP-18, ODS−Hypersil (5 μm, 10 nm, Shandon, Frankfurt, Germany) in a semipreparative scale at a flow rate 1.6 mL/min. After injection of the sample (20−100 μL), chromatography was first performed isocratically with a mixture (10/90, v/v) of methanol and aqueous ammonium formate buffer (pH 7.0; 0.1 mol/L); after 10 min the methanol content was increased to 100% within 10 min.

Liquid Chromatography/Mass Spectrometry (LC/MS). An Nucleosil 100-5C18 analytical HPLC column (Macherey and Nagel, Dürren, Germany) was coupled to an LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), respectively. After injection of the sample (2−20 μL), analysis was performed using the gradient detailed above.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H, ^{13}C, DQF−COSY, HMQC, and HMBC spectroscopy were performed on a Bruker-AC-200 and a Bruker-AM-360 spectrometer (Bruker, Rheinstetten, Germany).

Syntheses. *7-Methyl-2,3,6,7-tetrahydrocyclopenta-[b]azepinon-8(1H)-one (3).* Cyclotene (125 mmol), L-proline (500 mmol), copper-(II) acetate (5 mmol), and silica gel (25 g) were intimately mixed in a mortar and then dry-heated for 10 min at 180 °C in a cabinet drier. After cooling to room temperature, the reaction mixture was taken up in hot water (1500 mL) and filtered, the pH was adjusted to 9.0 with aqueous NaOH (1 mol/L), and the aqueous solution was then extracted with dichloromethane (5 × 400 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed in vacuo (45 mbar), and the residue was dissolved in ethyl ether (10 mL) and then applied onto a column (500 × 30 mm) filled with a slurry of Al_2O_3 (basic activity III−IV, Merck, Darmstadt, Germany) in *n*-pentane. Chromatography was performed using pentane (150 mL; fraction A), pentane/diethyl ether (9/1, v/v; 150 mL; fraction B), pentane/diethyl ether (8/2, v/v; 300 mL; fraction C), pentane/diethyl ether (7/3, v/v; 300 mL; fraction D), pentane/diethyl ether (6/4, v/v; 150 mL; fraction E1), and pentane/diethyl ether (6/4, v/v; 150 mL; fraction E2), followed by pentane/diethyl ether (5/5, v/v; 300 mL, fraction F). Removing the solvent from fractions E2 and F afforded the bitter tasting compound **3** (200 mg) as a white solid with a purity of more than 99%. HRGC/MS (EI): *m/z* 163 (70; $[M]^+$), 162 (100), 148 (12), 120 (12), 106 (11), 93 (10), 79 (10), 77 (10). LC/MS (ESI): *m/z* 164 (100; $[M + 1]^+$). ^{1}H NMR (360 MHz; $CDCl_3$, COSY): δ 1.19 (d, 3H, $J = 7.5$ Hz, H−C(8)), 2.13 (d, 1H, $J = 16.8$, H_a−C(6)), 2.46 (m, 1H, $J = 7.5$ Hz, H−C(7)), 2.64 (m, 2H, H−C(2)), 2.80 (dd, 1H, $J = 16.8$, 6.6 Hz, H_b-C(6)), 3.31 (m, 2H, H−C(1)), 4.51 (br s, 1H, NH), 6.03 (d, 1H, $J = 10.5$ Hz, H−C(4)), 6.03 (m, 2H, H−C(3), H−C(4)). ^{13}C NMR (360 MHz, $CDCl_3$, DEPT, HMQC, HMBC): δ 16.6 (CH_3, C(8)), 34.8 (CH_2, C(2)), 36.0 (CH_2, C(6)), 38.7 (CH, C(7)), 42.8 (CH_2, C(1)), 126.4 (CH, C(3)), 131.6 (CH, C(4)), 135.2 (C, C(5)), 141.8 (C, C(10)), 205.7 (CO, C(9)).

7-Methyl-2,3,4,5,6,7-hexahydrocyclopenta-[b]azepinon-8(1H)-one (4). Following a procedure reported earlier (*4*), a solution of cyclotene (90 mmol) and L-proline (90 mmol) in water (90 mL) was heated for 90 min at 150 °C in a laboratory autoclave (Roth, Germany). After cooling to room temperature, the pH was adjusted to 9 with sodium hydroxide solution (30% in water), the solution was extracted with diethyl ether (5 × 150 mL), and the combined organic layers were washed with aqueous Na_2CO_3 (200 mL; 0.5 mol/L), dried over Na_2SO_4, and then freed from solvent in vacuo (45 mbar). The residual oil was dissolved in pentane/ethyl ether (6/4, v/v; 10 mL) and then applied onto a column (500 × 30 mm) filled with a slurry of Al_2O_3 (basic activity III−IV, Merck, Darmstadt, Germany) in *n*-pentane. Chromatography was performed using pentane (300 mL; fraction A), pentane/diethyl ether (9/1, v/v; 150 mL; fraction B), pentane/diethyl ether (8/2, v/v; 300 mL; fraction C), pentane/diethyl ether (7/3, v/v; 150 mL; fraction D1) and pentane/diethyl ether (7/3, v/v; 150 mL; fraction D2), followed by pentane/diethyl ether (6/4, v/v; 150 mL; fraction E1) and pentane/diethyl ether (6/4, v/v; 150 mL; fraction E2). Removing the solvent from fraction D2 and E1 yielded compound **4** (1450 mg) as a

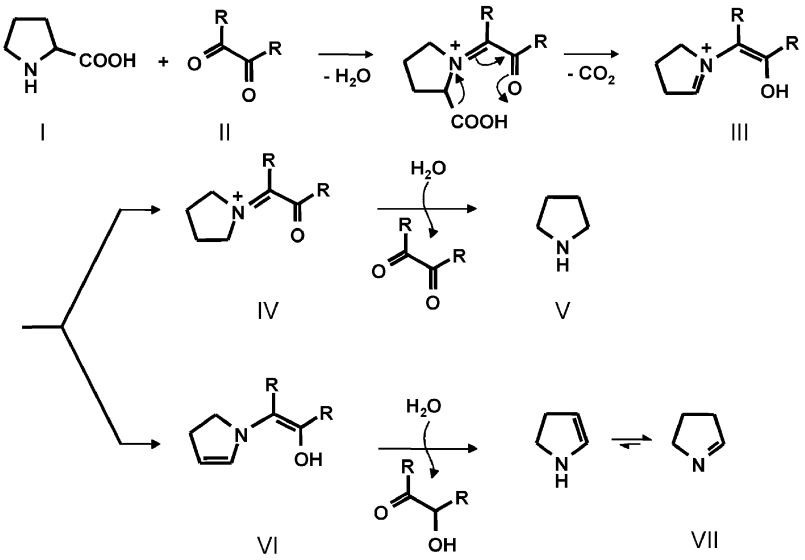

Figure 2. Reaction sequence proposed for the formation of pyrrolidine and 1-pyrroline upon Strecker-type reaction of L-proline and dicarbonyls.

pure white solid. HRGC/MS (EI): *m/z* 165 (100; $[M]^+$), 164 (38), 150 (17), 137 (31), 136 (36), 122 (55), 109 (34), 108 (41), 95 (37), 94 (26), 81 (16), 80 (16), 68 (10), 67 (28), 55 (12), 53 (15). LC/MS (ESI): *m/z* 166 (100; $[M + 1]^+$). ^{1}H NMR (360 MHz; $CDCl_3$, COSY): δ 1.16 (d, 3H, J = 7.1 Hz, H−C(8)), 1.65 (m, 2H, H−C(3)), 1.75 (m, 2H, H−C(2)), 2.05 (d, 1H, J = 17.3, H_a-C(6)), 2.41 (m, 3H, J = 5.3 Hz, H−C(4), H−C(7)), 2.70 (dd, 1H, J = 17.3, 5.3 Hz, H_b-C(6)), 3.03 (m, 2H, H−C(1)), 4.12 (br s, 1H, NH). ^{13}C NMR (360 MHz, $CDCl_3$, DEPT, HMQC, HMBC): δ 16.3 (CH_3, C(8)), 27.0 (CH_2, C(3)), 30.7 (CH_2, C(2)), 32.8 (CH_2, C(4)), 38.0 (CH_2, C(6)), 38.3 (CH, C(7)), 47.4 (CH_2, C(1)), 142.3 (C, C(5)), 142.4 (C, C(10)), 206.6 (CO, C(9)).

1-Pyrroline. Following a procedure reported in the literature (*6*), L-proline (5.0 g) dissolved in water (70 mL) was added to an aqueous solution of sodium metaperiodate (100 mL; 0.3 mmol/L). After the mixture was stirred for 2h in the dark at room temperature, the pH was adjusted to 9 with an aqueous sodium hydroxide solution (1 mol/L), and the solution was extracted with diethyl ether (5 × 10 mL). The combined organic layers were washed with a saturated NaCl solution (20 mL) and, after drying over Na_2SO_4, concentrated to 5 mL on a Vigreux column (60 cm × 1 cm) at 37 °C. After the solvent was evaporated in a gentle stream of nitrogen, the residue was taken up in water (10 mL). The 1-pyrroline was characterized by its MS/EI spectrum, which was in good agreement with the data reported by Schieberle (*6*). MS/EI: *m/z* 41 (100), 69 (66), 42 (41), 68 (31). The amount of 1-pyrroline was calculated gas chromatographically using 2-methyl-1-pyrroline as the internal standard.

Quantitation of Tastants 1−4 in Maillard Reaction Mixtures. After cooling to room temperature, defined amounts of 2-acetyl-4-methylpyridine were added to the model reaction mixtures (**Tables 1**−**3**) as the internal standard. The pH value of the aqueous model mixtures and dry-heated model mixtures, which were taken up in hot water (500 mL), respectively, was adjusted to 9.0 using an aqueous NaOH solution (1mol/L), and the solutions were then extracted with methylene chloride (5 × 150 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and then quantitation of the tastants **1**−**4** was performed by HRGC/MS. MS response factors for **1** (0.92), **2** (0.90), **3** (0.75), and **4** (0.70) were calculated from mixtures containing known amounts of the synthetic analytes and the internal standard.

Table 1. Influence of Carbohydrate Precursors and Processing Parameters on the Formation of Cooling Compounds **1** and **2** and Bitter Compounds **3** and **4** in the Presence of L-Proline

	carbohydrate	amount [μg] of compound			
expt.	precursor	**1**	**2**	**3**	**4**
1	glucose[a]	5	1	441	393
2	cyclotene[a]	1500	6	5000	227600
3	cyclotene[b]	1610	9	5095	234200
4	glucose[c]	66	32	614	26
5	cyclotene[c,d]	1700	6000	800	6700
6	cyclotene[c,e]	1100	100	1500	4400
7	cyclotene[c,f]	200	3	1200	2600

[a] A solution of the precursor (10 mmol) and L-proline (10 mmol) in water (30 mL; pH 7.0) was heated for 60 min at 150 °C in a laboratory autoclave. [b] Reaction detailed under *a* was performed under an atmosphere of argon. [c] A mixture of the precursor (10 mmol) and L-proline (10 mmol) was dry-heated for 10 min at 180 °C in an cabinet drier. [d] Al_2O_3 (2 g) was added as a neutral/basic matrix prior to heat treatment. [e] SiO_2 (2 g) was added as an acidic matrix prior to heat treatment. [f] SiO_2 (2 g) and copper(II) acetate (0.5 mmol) were added prior to heat treatment.

Isolation of (*S*)-3-Methyl-2-[(2′-carboxy)-1-pyrrolidinyl]-2-cyclopenten-1-one (5) from the Dry-Heated Cyclotene/Proline Mixture. A mixture of the cyclotene (10 mmol) and L-proline (10 mmol) was dry-heated for 10 min at 180 °C in a cabinet drier. After cooling, the mixture was suspended in water (30 mL) and filtered, and the aqueous layer was freeze-dried. The residue was taken up in a mixture (15 mL; 5/95, v/v) of methanol and aqueous ammonium formate solution (pH 7.0; 0.1 mol/L) and, then, fractionated by flash chromatography on RP-18 material (15.0 g; Lichroprep 25−40 μm, Merk, Darmstadt, Germany) using the same solvent mixture as the mobile phase. After application of the crude material and chromatography with an eluent flow of 1.5 mL/min, the effluent of a peak detected at 300 nm after 5h was collected. After evaporation of the solvent and freeze-drying, the material was finally purified by RP-HPLC. Monitoring the effluent at 300 nm gave a peak at 9 min, which was collected in several runs. The combined eluates were freeze-dried, yielding 3-methyl-2-[(2′-carboxy)-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**, 1.0 mmol; 2% yield), which showed the identical spectroscopic data as recently reported for the synthetic reference compound (*3*). LC/MS ($APCI^+$): *m/z* 210 (100, $[M+1]^+$), 166 (45), 164 (39), 192 (10). ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.80 (m, 2H,

Table 2. Influence of the Nitrogen-Containing Precursor on the Formation of Cooling Compounds **1** and **2**, and Bitter Compounds **3** and **4** from Cyclotene[a]

	nitrogen		amount [μg] of compound			
expt.	precursor	pH	**1**	**2**	**3**	**4**
1	L-proline	7	1500	6	5000	227600
2	1-pyrroline[b]	7	n.d.	n.d.	2112	3
3	pyrrolidine	5	9250	5	n.d.	n.d.
4	pyrrolidine	7	12320	1233	n.d.	n.d.
5	pyrrolidine	9	15600	21300	n.d.	n.d.

[a] A solution of cyclotene (10 mmol) and a nitrogen precursor (10 mmol each) in water (30 mL; pH 5.0, 7.0 or 9.0) was heated for 60 min at 150 °C in a laboratory autoclave. [b] 1-Pyrroline (2 mmol) was used in the experiment. n.d.: not detectable.

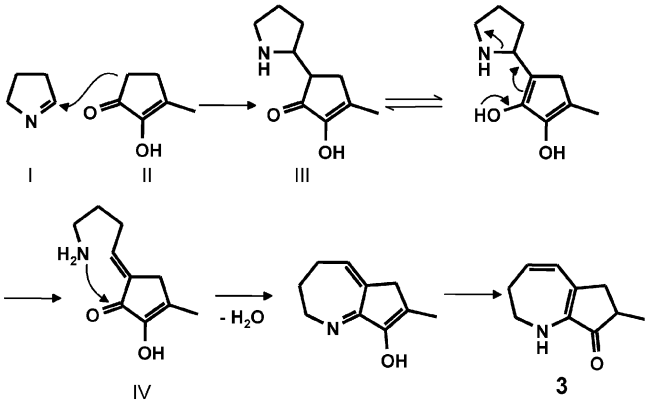

Figure 3. Proposed reaction pathway leading to the formation of bitter tastant **3** via the key intermediates cyclotene and 1-pyrroline.

CH_2), 1.95 (s, 3H, CH_3), 2.01 (m, 2H, CH_2), 2.27 (m, 2H, CH_2), 2.34 (m, 2H, CH_2), 3.23 (m, 2H, CH_2,), 4.60 (m, 1H, CH).

RESULTS AND DISCUSSIONS

Influence of Carbohydrate Precursors and Reaction Parameters. To gain first insights into the role of 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) as a common intermediate in the formation of the tastants **1**−**4** (**Figure 1**) from hexoses, we reacted L-proline in aqueous solution either with glucose, or in the presence of cyclotene, and the amounts of the tastants formed were quantified (**Table 1**). When reacted with L-proline in aqueous solution, glucose produced compound **3** as the major taste compound, closely followed by compound **4** (expt. 1 in **Table 1**). In contrast, both the cooling-active 1-pyrrolidinyl-2-cyclopenten-1-ones were formed in comparatively low amounts (expt. 1 in **Table 1**). Substitution of the hexose with cyclotene generated compound **4** in by far the highest amounts, e.g., the yields of compound **4** were 45 times above the amount quantified for tastant **3** (expt. 2 in **Table 1**). To study the role of atmospheric oxygen, in a additional experiment, we performed the reaction under an atmosphere of argon (expt. 3 in **Table 1**). The yields, however, did not differ significantly from those measured in the oxygen-containing model mixture (expt. 2 in **Table 1**).

To gain more detailed information into factors governing the formation of the tastants under investigation, we reacted glucose with L-proline under dry-heating conditions. These conditions favored the formation of the bitter compound **3**. Factors of 9, 19, and 24 higher amounts were generated in comparison to **4** as well as the cooling compounds **1** and **2**, respectively (expt. 4 in **Table 1**). Substitution of the hexose with its degradation product cyclotene led to significantly higher amounts of all four taste compounds, but the yields were found to be significantly influenced by the reaction conditions (expts. 4−7 in **Table 1**). Heating the reaction mixture under neutral/basic conditions produced most effectively the cooling compound **2** and the bitter tastant **4**, which were formed from cyclotene in 320- or 258-fold higher amounts when compared to glucose (expt. 5 in **Table 1**). Thermal treatment of the cyclotene/L-proline mixture under slightly acidic conditions gave a different picture, e.g., compound **2** was formed in comparatively low amounts of about 100 μg, whereas the bitter tasting compound **4** was still formed predominantly (expt. 6 in **Table 1**). To check the influence of transition metal ions on the yields of the tastants, we repeated the reaction in the presence of trace amounts of copper(II) ions (expt. 7 in **Table 1**). The results show that under these oxidative conditions compound **4** is still significantly favored, clearly indicating that a metal-catalyzed oxidation of a reaction intermediate from cyclotene and L-proline as proposed in the literature (*4*) does not play an important role in the formation of compound **3**. The key intermediates involved in the formation of compound **3** are, therefore, still an open question.

Influence of the Nitrogen Precursor on Tastant Formation. To elucidate alternative reaction routes for the formation

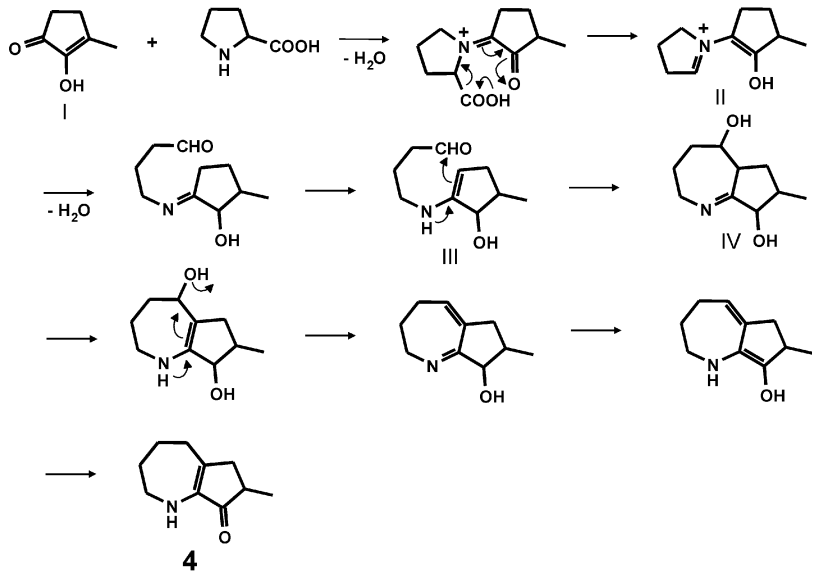

Figure 4. Reaction route proposed for the formation of bitter tastant **4** running via cyclotene and L-proline.

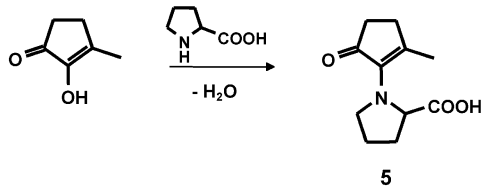

Figure 5. Structure of the tasteless, nonvolatile reaction intermediate **5** formed from cyclotene and L-proline.

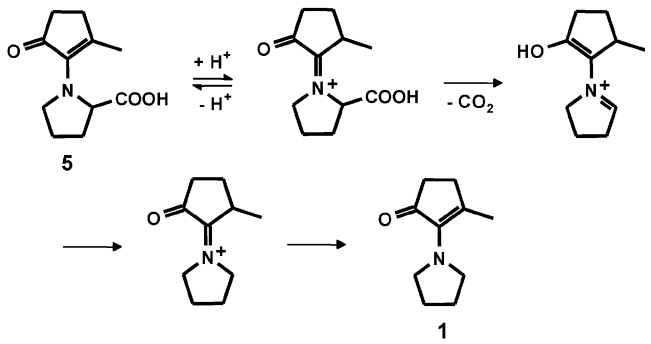

Figure 6. Formation of cooling compound **1** from its nonvolatile precursor **5**.

of tastant **3**, we conducted a third set of experiments aimed at studying the role of the nitrogen precursor on the yields of the compounds **1**−**4**. It is well-known in the literature that during Maillard reactions the amino acid L-proline might be degraded via three different pathways: (i) the pyrrolidine ring of L-proline is either irreversibly attached to a carbon backbone, (ii) liberated in course of Strecker-type reactions of L-proline (I in **Figure 2**) and dicarbonyls (II in **Figure 2**) via the imine intermediates III and IV as pyrrolidine (5 in **Figure 2**), or (iii) liberated via the enaminol VI as 1-pyrroline (VII in **Figure 2**) (*7*). To gain insights into the nitrogen-containing precursor of the tastants, we reacted the common hexose-derived intermediate cyclotene in binary mixtures with either synthetic 1-pyrroline or pyrrolidine, and the amounts of the tastants generated were compared with those formed from using L-proline as the precursor (expt. 1 in **Table 2**). Reacting cyclotene in the presence of 1-pyrroline generated exclusively compound **3** in high amounts (expt. 2 in **Table 2**), whereas compounds **1** and **2** were not formed at all, and tastant **4** was present only in trace amounts. These data clearly demonstrate for the first time that the hexose-derived cyclotene and the proline-derived 1-pyrroline are key intermediates in the formation of compound **3**. A reaction pathway explaining how compound **3** might be formed via these intermediates is displayed in **Figure 3**. Nucleophilic attack of the CH-acidic methylene group of cyclotene (II in **Figure 3**) at the 1-pyrroline (I in **Figure 3**) would form intermediate III, which, upon enolization and β-elimination of the amino moiety, would lead to the primary amine IV. Subsequent cyclization would give rise to the bitter tastant **3** (**Figure 3**).

In contrast, the amino acid L-proline generated both cyclopenta-[*b*]azepin-8(*1H*)-ones but drastically favored the formation of **4** (expt. 1 in **Table 2**). These data demonstrate that besides the cyclotene the intact amino acid L-proline is necessary for the formation of compound **4**, fitting well with earlier findings (*4*). A reaction mechanism explaining the formation of tastant **4** is outlined in **Figure 4**. Strecker reaction between the cyclotene (I in **Figure 4**) and L-proline would form the iminium intermediate II upon decarboxylation. Hydrolysis of the iminium ion, followed by imine/enamine tautomerism, would result in intermediate III, which would undergo intramolecular cyclization to give the seven-membered ring in intermediate IV (**Figure 4**). Water elimination of this vinylogous hemi aminal, followed by enolization, gives rise to the bitter compound **4** (**Figure 4**).

Reacting cyclotene in the presence of pyrrolidine generated excusively the cooling-active 1-pyrrolidinyl-2-cyclopenten-1-ones independent of the pH value and did not form any trace amounts of the bitter-tasting cyclopenta-[*b*]azepin-8(*1H*)-ones (expt. 3−5 in **Table 2**). The yields of the cooling compounds **1** and **2** were, however, strongly influenced by the pH value, e.g., at pH 5.0 compound **1** was formed almost exclusively, whereas increasing the pH to 9.0 led to the predominant formation of **2**. These data indicate cyclotene and pyrrolidine as very efficient precursors for the 1-pyrrolidinyl-2-cyclopenten-1-one formation, via a simple hydroxyl/amine-exchange reaction.

Characterization of a Cyclotene/Proline Reaction Intermediate. To check whether the cooling compounds **1** and **2** might be formed exclusively via pyrrolidine liberated from L-proline by decarboxylation or from a cyclotene/L-proline reaction intermediate which undergoes decarboxylation in a

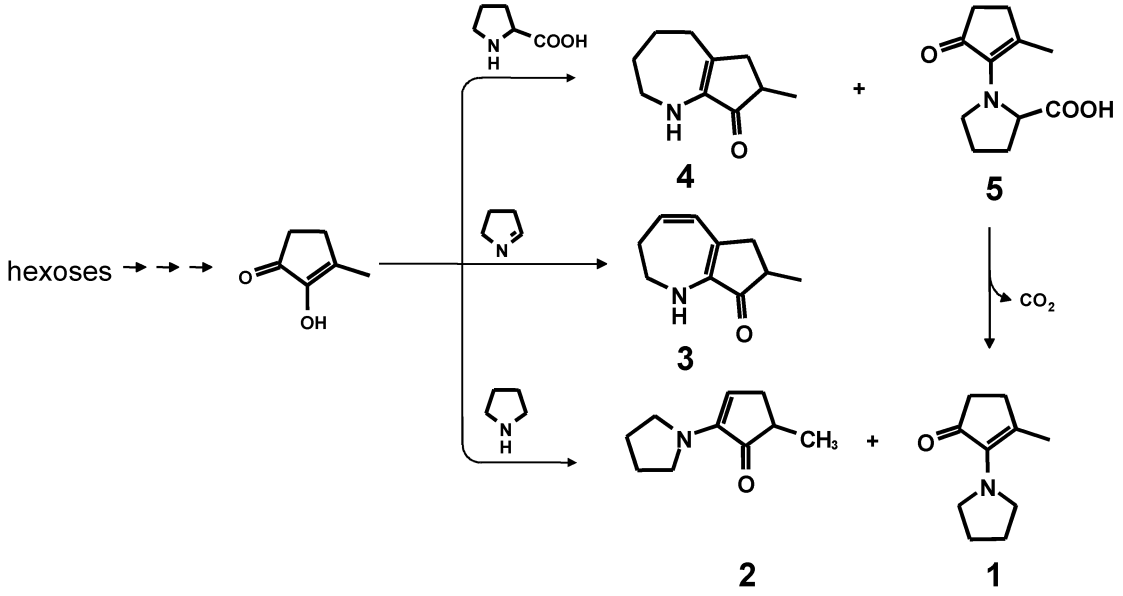

Figure 7. Influence of the structure of the nitrogen precursor on the formation of taste compounds from the hexose-derived cyclotene.

Table 3. Formation of Cooling Compounds (**1**, **2**) and Bitter Compounds (**3**, **4**) upon Thermal Treatment of (*S*)-3-Methyl-2-[(2′-carboxy)-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**)

	amount [μg] of compound			
expt.	**1**	**2**	**3**	**4**
1[a]	5250	4	9	675
2[b]	9410	2	4	14

[a] A solution of compound **5** (10 mmol) in water (30 mL, pH 7.0) was heated for 60 min at 150 °C in a laboratory autoclave. [b] A mixture of compound **5** (10 mmol) and SiO_2 (2 g) was dry-heated for 10 min at 180 °C in a cabinet drier.

second reaction step, we fractionated the water-soluble, nonvolatile fraction from the cyclotene/L-proline mixture by flash-chromatography using RP-18 material as the stationary phase. Monitoring the effluent at 300 nm, a peak was collected and freeze-dried. After the raw material was purified by semi-preparative RP-HPLC, comparison of the retention times (RP-18), LC/MS and NMR data with those obtained for a synthetic reference compound led to the unequivocal identification of the water-soluble major reaction product as (*S*)-3-methyl-2-[(2′-carboxy)-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**), the structure of which is shown in **Figure 5**.

To check the efficiency of the nonvolatile reaction intermediate **5** as a precursor in generating the target tastants **1**−**4**, we heated the freshly purified material under dry-heating as well as under aqueous conditions, and the amounts of the tastants under investigation were determined. As given in expt. 1 (**Table 3**), under aqueous conditions compound **1** was formed predominantly from intermediate **5**, most likely by a simple decarboxylation reaction. In addition, some amounts of the bitter tastant **4** were generated. Considering a possible hydrolysis of intermediate **5**, the formation of **4** might be explained by the reaction between the cyclotene and L-proline liberated from **5**. To prevent a possible hydrolysis of intermediate **5**, we repeated the reaction under dry-heating conditions. As given in expt. 2 (**Table 3**), compound **1** was formed nearly exclusively from its nonvolatile precursor, but only low amounts of the isomeric compound **2**. On the basis of these data, a Strecker-type decarboxylation was proposed for the formation of tastant **1** as displayed in **Figure 6**.

Taking all these quantitative data into consideration, it can be concluded that hexose-derived cyclotene is the common precursor for all four tastants and that the formation of each individual tastant is strongly determined by the structure of the nitrogen-containing precursor. As summarized in **Figure 7**, reaction of cyclotene with pyrrolidine, formed by thermal decarboxylation of L-proline (*7*), produces the cooling compounds **1** and **2** only, whereas the 1-pyrroline favors the formation of the bitter tasting azepinone **3**. The structure of the secondary amino acid L-proline enables the formation of compound **4** exclusively, whereas the pyrrolidine and 1-pyrroline, respectively, do not generate this tastant. In addition, the nonvolatile, tasteless intermediate **5** formed from cyclotene and L-proline acts as an efficient precursor giving rise to the cooling compound **1** upon Strecker-type decarboxylation (**Figure 7**).

Such systematic studies open the possibility to develop more sophisticated processes in flavor manufacturing, which are based on extended knowledge of the yields of desirable tastants, the reaction pathways, and factors governing their formation. Data, obtained therefrom, are very helpful to tailor the desired overall flavor of foods by means of a more controlled Maillard-type technology.

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