On the Hodge Mechanism of the Formation of the Bread Flavor Component 6-Acetyl-1,2,3,4-tetrahydropyridine from Proline and Sugars

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The so-called Hodge mechanism for the generation of 6-acetyl-1,2,3,4-tetrahydropyridine, a major Maillard flavor compound in processed foods, from proline and 1,2-propanedione most probably does not involve the intermediacy of *N*-acetonyl-4-aminobutanal. This finding is based on model experiments in which suitably and doubly protected *N*-acetonyl-4-aminobutanal was hydrolyzed into the parent compound and in which no trace of the flavor compound could be detected.

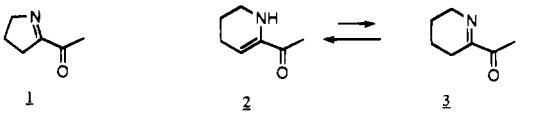
Keywords: *Bread flavor; Maillard reaction; Hodge mechanism; 6-acetyl-1,2,3,4-tetrahydropyridine; flavor formation*

INTRODUCTION

The Maillard reaction or the nonenzymatic browning reaction is a condensation between the free amino groups of amino acids or proteins and reducing sugars, which occurs during baking, cooking, and preservation of foods (Ledl and Schleicher, 1990). The Maillard reaction is very important for the development of flavors in foods and, therefore, it is of major interest in food and flavor science. A recent aspect of the Maillard reaction is that it may be accompanied by a reduction in the nutritive value of foods and the formation of toxic compounds (Ledl and Schleicher, 1990). Accordingly, various model studies on the generation of flavor compounds from the condensation of reducing sugars and amino acids, e.g., proline and 4-hydroxyproline, have been reported (Ledl and Schleicher, 1990; Tressl et al., 1981; Hodge, 1953; Mills and Hodge, 1976). These investigations revealed various proline-specific (Helak et al., 1989; Tressl et al., 1985; Schieberle, 1990a,b) and 4-hydroxyproline-specific (Tressl et al., 1989) Maillard products.

Sugars undergo retro-aldol-type reactions producing a variety of reactive intermediates and compounds derived therefrom, including α-hydroxyaldehydes, α-hydroxyketones, α-diones, α-ketoaldehydes, etc. (Ledl and Schleicher, 1990a,b; Tressl et al., 1981). Upon condensation of these carbonyl compounds with the primary amino group of amino acids, various labile imines are formed which suffer further transformations. Quite often, heterocyclic flavor compounds are produced. Classical examples of such flavor compounds are pyrazines, 2-acetyl-1-pyrroline (**1**), pyrroles, 6-acetyl-1,2,3,4-tetrahydropyridine (**2**), etc. 2-Acetyl-1-pyrroline (**1**) and 6-acetyl-1,2,3,4-tetrahydropyridine (**2**) [which occurs in tautomeric equilibrium with 6-acetyl-2,3,4,5-tetrahydropyridine (**3**)] are considered the major flavor components of rice (Schieberle, 1990a; Buttery et al., 1982, 1983, 1986, 1988), and bread (Schieberle, 1990a; Schieberle and Grosch, 1983, 1984), respectively (Scheme 1). Both compounds contribute significantly to the flavor of bakery products. The formation of the bread flavor component 6-acetyl-1,2,3,4-tetrahydropyridine (**2**) is suggested to originate from proline (**4**) and 1,2-propanedione (**5**), the latter being a degradation product of reducing sugars (Tressl et al., 1981). The mechanism is referred to as the Hodge mechanism (Scheme 2). It concerns the nucleophilic addition of the proline nitrogen atom across the formyl group of 1,2-propanedione (**5**) to form an adduct **6**, which suffers dehydration to form iminium species **7**. A sequence of reactions involving decarboxylation, hydrolysis, ring opening, and ring closure explains the formation of the bread flavor component **2**. Although never explicitly formulated as such, modern mechanistic organic chemistry may explain these transformations as visualized in Scheme 2. It concerns a decarboxylation of iminium intermediate **7** into the azomethine ylide **8**, which is in mesomeric equilibrium with the resonance form **9**. Addition of water to **9** affords the unstable adduct **10**, which undergoes ring opening to the key intermediate **12**. An alternative representation involves a concerted process for the conversion of **7** into **10** via **11**. An intramolecular aldol-type condensation of this ketoaldehyde **12** produces the ring transformation into the aldol intermediate **15**, which dehydrates into 6-acetyl-1,2,3,4-tetrahydropyridine (**2**). Faulty variants of this sequence with incorrect formulation of intermediates have been proposed in the past. It is clear from the above picture that 4-(2-oxopropyl)aminobutanal (**12**) is the cornerstone of the hypothesis of Hodge. We here report on an investigation on the intermediacy of 4-(2-oxopropyl)aminobutanal (**12**) in the Hodge mechanism.

Scheme 1



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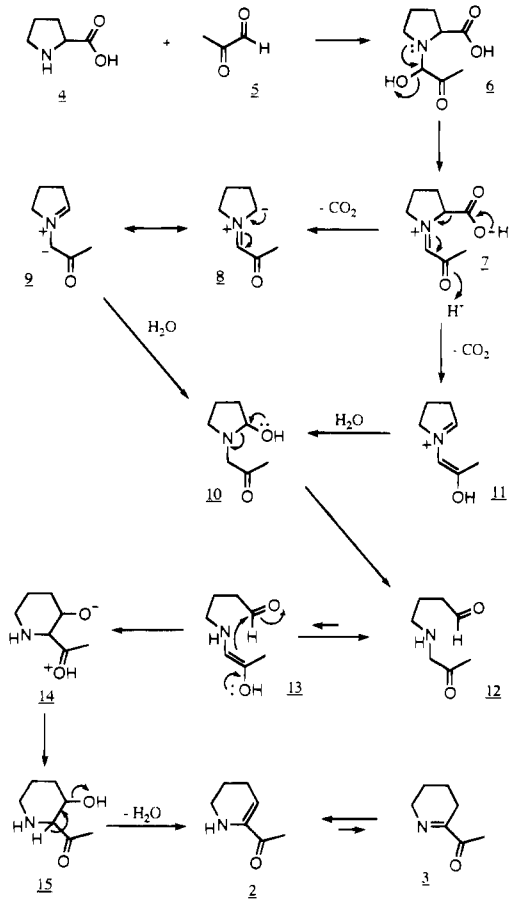
Scheme 2



EXPERIMENTAL PROCEDURES

3-Chloro-2-(methoxymethoxy)-1-propene (**18**) was synthesized from epichlorohydrine (**16**) according to the literature (Gu et al., 1987a,b).

Synthesis of *N*-[2-(Methoxymethoxy)-2-propenyl]-4,4-diethoxybutylamine (20). In a 100-mL round-bottom flask, equipped with a magnetic stirrer, a reflux condenser, and a $CaCl_2$ tube, were placed 40 mL of dry dimethylformamide, 4.1 g (0.03 mol) of 3-chloro-2-(methoxymethoxy)-1-propene (**18**), 4.8 g (0.03 mol) of 4-aminobutanal diethylacetal (**19**), and 4.1 g (0.03 mol) of dry K_2CO_3. The stirred mixture was heated at 70 °C in an oil bath for 15 h and then poured into 100 mL of water. Three ether extractions each with 200 mL of ether were performed; the combined extracts were washed with 400 mL of brine and dried ($MgSO_4$). Evaporation in vacuo gave 5.92 g of an oil which was distilled to afford 2.82 g (36%) of compound **20**, bp 90–100 °C/0.05 mmHg. A higher boiling fraction (1.97 g) contained mainly (80%) *N,N*-bis[2-(methoxymethoxy)-2-propenyl)]-4-aminobutanal diethylacetal (**21**), bp 100–115 °C/0.05 mmHg. Purification of the latter was performed by means of flash chromatography (Si gel; ether–pentane 1:1), giving 1.3 g (24%) of **21**.

Compound 20: ^{1}H NMR (CCl_4) δ 1.13 [6H, t, J = 7 Hz, $(OCMe)_2$], 1.3–1.8 (4H, m, $NCH_2CH_2CH_2$), 2.53 (2H, t, J = 6 Hz, NCH_2CH_2), 3.12 (2H, s, $NCH_2C{=}C$); 3.36 (3H, s, OMe), 3.3–3.7 [4H, m, $(OCH_2Me)_2$], 4.09 and 4.17 (each 1H, each d, J = 1.5 Hz, $C{=}CH_2$), 4.40 (1H, t, J = 5.5 Hz, $OCHO$), 4.90 (2H, s, OCH_2OMe); ^{13}C NMR ($CDCl_3$) δ 15.34 (q, 2Me), 25.36 and 31.46 (each t, CH_2CH_2), 52.72 (t, NCH_2), 56.14 (t, $NCH_2C{=}C$), 56.14 (q, OMe), 60.97 (t, 2 × OCH_2Me), 85.62 (t, $C{=}CH_2$), 93.66 (t, $MeOCH_2O$), 102.63 [d, $CH(OEt)_2$], 158.45 (s, $C{=}CH_2$); IR (NaCl) 1630 cm^{-1} (C=C); mass spectrum, *m/z* (%) 261 (M^+, 3), 260 (3), 246 (4), 232 (31), 216 (19), 200 (14), 186 (8), 184 (8), 170 (71), 154 (21), 137 (15), 130 (25), 128 (19), 126 (33), 124 (31), 115 (27), 114 (16), 110 (21), 103 (39), 99 (21), 98 (31), 97 (100), 87 (21), 86 (21), 84 (27), 82 (15), 81 (30), 75 (26), 72 (10), 71 (53), 70 (31), 69 (15), 59 (13), 58 (17), 57 (16), 56 (17), 55 (17).

Compound 21: ^{1}H NMR ($CDCl_3$) δ 1.20 [6H, t, J = 7 Hz, $(O\text{-}CH_2Me)_2$], 1.4–1.7 (4H, m, $NCH_2CH_2CH_2$), 2.57 (2H, t, J = 6 Hz, NCH_2CH_2), 3.17 (4H, s, 2 × $NCH_2C{=}C$), 3.41 (6H, s, 2 × OMe), 3.4–3.7 (4H, m, 2 × OCH_2CH_3), 4.23 and 4.28 (each 2H, each d, J = 1.6 Hz, 2 × $C{=}CH_2$), 4.49 [1H, t, J = 5.5 Hz, $CH(OEt)_2$], 4.98 (4H, s, 2 × OCH_2O); ^{13}C NMR ($CDCl_3$) δ 15.37 (q, 2 × OCH_2Me), 22.41 and 31.37 (each t, $NCH_2CH_2CH_2$), 53.15 (t, NCH_2CH_2), 56.10 (q, 2 × OMe), 56.79 (t, $NCH_2C{=}C$), 60.87 (t, 2 × OCH_2), 87.16 (t, 2 × $C{=}CH_2$), 93.55 (t, 2 × OCH_2O), 102.87 [d, $CH(OEt)_2$], 158.10 (s, $C{=}CH_2$); mass spectrum, *m/z* (%) 361 (M^+, 0.5), 360 (0.5), 330 (2), 316 (6), 300 (2), 274 (3), 270 (2), 240 (2), 230 (12), 228 (3), 214 (2), 210 (2), 198 (2), 194 (2), 184 (7), 182 (3), 180 (2), 170 (5), 154 (10), 152 (2), 143 (2), 140 (3), 138 (3), 136 (2), 128 (3), 115 (6), 110 (3), 103 (6), 101 (5), 100 (3), 99 (24), 98 (10), 96 (5), 87 (6), 86 (3), 85 (3), 84 (4), 82 (3), 81 (3), 75 (5), 73 (3), 71 (20), 70 (3), 69 (3), 59 (4), 57 (3), 55 (3), 47 (5), 46 (4), 45 (100), 43 (14), 42 (5), 41 (5).

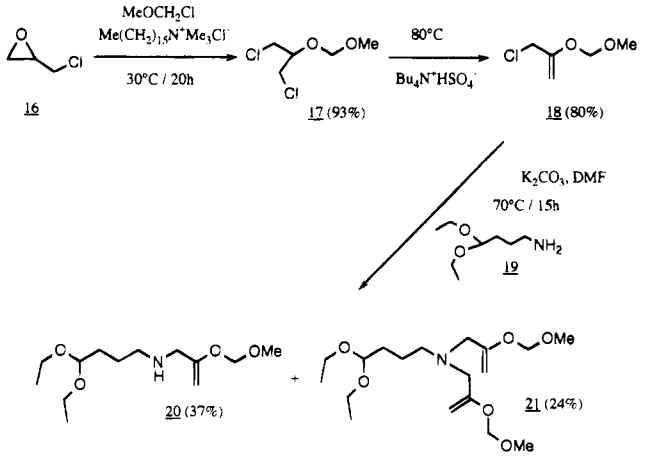
Hydrolyses of Compound 20. All hydrolyses of compound **20** were run as described in the discussion of the results. The reaction of compound **20** with aqueous acids or bases was performed under magnetic stirring. After the given reaction time, extraction was performed with ether or dichloromethane. For some acidic hydrolysis experiments, the reaction mixture was made alkaline or neutral with aqueous sodium hydroxide (2 N) and the reaction mixture was left under these conditions for several hours (vide supra). Afterward, a similar extraction was performed. After drying of all extracts ($MgSO_4$), evaporation of the solvent gave an oily residue that was analyzed by ^{1}H NMR and GC.

Selective Hydrolysis of Compound 21. A solution of 1.08 g (0.003 mol) of compound **21** in 10 mL of dichloromethane was treated with 0.76 g (0.006 mol) of oxalic acid 2 aq and 6 mL of water. The stirred mixture was refluxed for 1 h, poured into 1 N sodium hydroxide, and extracted twice with dichloromethane. After drying ($MgSO_4$) of the organic extracts, evaporation of the solvent afforded an oil (0.79 g, 92%) consisting of almost pure (>96%) *N,N*-bis[2-methoxymethoxy-2-propenyl]-4-aminobutanal (**22**): ^{1}H NMR ($CDCl_3$) δ 1.76 (2H, quintet, J = 6.5 Hz, NCH_2CH_2), 2.47 (2H, txt, J = 7.5 Hz, J = 2 Hz, CH_2CHO), 2.58 (2H, t, J = 7 Hz, NCH_2CH_2), 3.10 (4H, s, 2 ×Yen $NCH_2C{=}C$), 3.40 (6H, s, 2 × OMe), 4.21 and 4.30 (each 1H, AB, J = 1.5 Hz, 2 × $C{=}CH_2$), 4.95 (4H, s, 2 × OCH_2O), 9.81 (1H, t, J = 2 Hz, CH=O); ^{13}C NMR ($CDCl_3$) δ 20.59 (t, CH_2CH_2CHO), 41.56 and 52.58 (each t, $NCH_2CH_2CH_2$-CHO), 55.90 (q, OMe), 56.84 (t, $NCH_2C{=}C$), 87.24 (t, $C{=}CH_2$), 93.65 (t, OCH_2O), 158.16 (s, $C{=}CH_2$), 201.79 (d, CH=O); mass spectrum, *m/z* (%) 287 (M^+, 0.4), 272 (1), 244 (1), 242 (1), 230 (3), 214 (3), 200 (3), 198 (3), 184 (3), 182 (2), 170 (1), 168 (1), 167 (1), 158 (7), 156 (2), 154 (7), 152 (1), 144 (2), 140 (2), 138 (2), 130 (1), 126 (3), 124 (2), 114 (2), 112 (2), 110 (1), 100 (2), 99 (2), 98 (8), 97 (2), 96 (3), 86 (1), 85 (1), 84 (4), 83 (2), 82 (2), 72 (2), 71 (23), 70 (3), 69 (1), 61 (1), 58 (1), 57 (2), 56 (1), 55 (1), 46 (3), 45 (100), 44 (5), 43 (8), 42 (4), 41 (4).

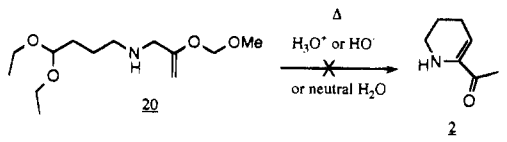
RESULTS AND DISCUSSION

The study of the behavior of the intermediate 4-(2-oxopropyl)aminobutanal (**12**), neat or under protected form, should shed light on the position of this substance in the generation of the Maillard compound **2**. The lability of the γ-aminoaldehyde **12** precludes straightforward experiments. Alternatively, suitable protection of both carbonyl moieties of the γ-aminoaldehyde allows generation of compound **12** under a variety of conditions and verification of its behavior with respect to the formation of 6-acetyl-1,2,3,4-tetrahydropyridine (**2**). To this end, a doubly protected form of compound **12** was synthesized; i.e., the aldehyde function was protected as an acetal and the acetonyl moiety was masked as an enol ether (Scheme 3). The target molecule, i.e., *N*-[2-(methoxymethoxy)-2-propenyl]-4,4-diethoxybutylamine (**20**), was prepared by reaction of epichlorohydrine (**16**) with chloromethyl methyl ether in the

Scheme 3



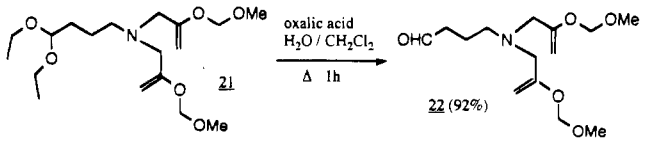
Scheme 4



presence of a quaternary ammonium salt and phase-transfer-catalyzed dehydrochlorination of the resulting acetal **17** into the functionalized allyl chloride (**18**) (Gu et al., 1987a,b). Condensation of 3-chloro-2-(methoxymethoxy)-1-propene (**18**) with 4,4-diethoxybutylamine (**19**) in dimethylformamide in the presence of potassium carbonate produced the protected key intermediate **20** from the Hodge mechanism. In addition, some *N,N*-dialkylated product **21** was formed, which was separated from the monoalkylated compound **20** by vacuum distillation.

The diprotected *N*-acetonyl-4-aminobutanal **20** was then subjected to a variety of hydrolytic conditions. Under these conditions, *N*-acetonyl-4-aminobutanal (**12**) should be formed and then give rise to at least some 6-acetyl-1,2,3,4-tetrahydropyridine (**2**) if the Hodge mechanism would be operative. To cover a broad range of hydrolytic conditions of which some might have similarity with the production of Maillard-type flavor compounds, compound **21** was subjected to acidic, alkaline, and neutral hydrolytic conditions. Heating compound **20** (2 mmol) in distilled water at 80 °C for 24 h did not hydrolyze compound **20**. The reaction of compound **20** with aqueous oxalic acid (2 molar equiv of 2 N solution) under reflux for 1 h hydrolyzed the starting material completely. However, the acidic, neutral, or basic extracts (CH_2Cl_2) did not contain any compound that could be identified. Several analogous hydrolyses of compound **20** (2 mmol) with aqueous sulfuric acid (10 mL; catalytic amount, or 1%, 2%, or 10% solution) at 80 °C for 1–3 h and then at room temperature for 24–96 h did not afford any compound that could be identified by spectroscopic methods (^{1}H NMR, GC–MS). Acidic hydrolysis as described above followed by neutralization or basification with sodium hydroxide in aqueous medium and subsequent heating to 80 °C or standing at room temperature for up to 72 h did not give any isolable product. Instead, very complex reaction mixtures were obtained. Finally, the reaction of 5% aqueous sodium hydroxide with compound **20** at 80 °C for 24 h and subsequent standing at room temperature for 96 h did not result in any change of the starting material. In all experiments mentioned above there was no formation of even the slightest trace

Scheme 5



of 6-acetyl-1,2,3,4-tetrahydropyridine (**2**), as could be verified additionally by the absence of its typical cracker-like flavor. Due to the extremely low odor threshold value of the flavor compound **2** (1.4 ppb in water), a detection by odor would be easy (our research group has great experience in handling and working with 6-acetyl-1,2,3,4-tetrahydropyridine). It should be stressed that both protective groups of compound **20**, namely the methoxymethylenol ether function and the diethyl acetal function, are known to hydrolyze under the given acid hydrolytic reaction conditions. The methoxymethylenol ether function hydrolyzes readily with 1% sulfuric acid (Gu et al., 1987a), while the hydrolysis of a diethyl acetal is also generally known to occur under these conditions. Even aqueous oxalic acid was able to hydrolyze selectively the diethyl acetal functionality in the presence of the bis(enol ether) function in compound **21**, affording aldehyde **22** in 92% yield (Scheme 5). On the basis of all of these experiments, it seems that the so-called Hodge mechanism for the generation of 6-acetyl-1,2,3,4-tetrahydropyridine (**2**) from proline (**4**) and 1,2-propanedione (**5**) does not involve the intermediacy of *N*-acetonyl-4-aminobutanal (**12**).

LITERATURE CITED

Buttery, R. G.; Ling, L. C.; Juliano, B. O. 2-Acetyl-1-pyrroline: an Important Aroma Component of Cooked Rice. *Chem. Ind.* **1982**, 958–959.

Buttery, R. G.; Ling, L. C.; Juliano, B. O.; Turnbaugh, J. G. Cooked Rice Aroma and 2-Acetyl-1-pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823–826.

Buttery, R. G.; Ling, L. C.; Mon, T. R. Quantitative Analysis of 2-Acetyl-1-pyrroline in Rice. *J. Agric. Food Chem.* **1986**, *34*, 112–114.

Buttery, R. G.; Turnbaugh, J. G.; Ling, L. C. Contribution of Volatiles to Rice Aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006–1009.

Gu, X. P.; Nishida, N.; Ikeda, I.; Okahara, M. 2-(Chloromethyl)-3,5-dioxahex-1-ene. An Effective Acetonylating Reagent. *J. Org. Chem.* **1987a**, *52*, 3192–3196.

Gu, X. P.; Ikeda, I.; Okahara, M. Regioselective Addition of Organic Chlorides to Epoxides in the Presence of Quaternary Ammonium Chloride. *Bull. Chem. Soc. Jpn.* **1987b**, *60*, 397–398.

Helak, B.; Spengler, K.; Tressl, R.; Rewicki, D. Formation of 7H-Cyclopenta[b]pyridin-7-ones as Proline-Specific Maillard Products. *J. Agric. Food Chem.* **1989**, *37*, 400–404.

Hodge, J. E. Browning Reactions in Model Systems. *J. Agric. Food Chem.* **1953**, *1*, 928–943.

Ledl, F.; Schleicher, E. New Aspects of the Maillard Reaction in Foods and in the Human Body. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 565–706.

Mills, F. D.; Hodge, J. E. Amadori Compounds: Vacuum Thermolysis of 1-Deoxy-1-L-prolino-D-fructose. *Carbohydr. Res.* **1976**, *51*, 9–21.

Schieberle, P. Occurrence and Formation of Roast-smelling Odorants in Wheat Bread Crust and Popcorn. In *Flavour Science and Technology, 6th Weurman Symposium*; Bessiere, Y., Thomas, A. F., Eds.; Wiley: Chichester, U.K., 1990a; pp 105–108; *Chem. Abstr.* **1992**, *116*, 5519.

Schieberle, P. The Role of Free Amino Acids Present in Yeast as Precursors of the Odorants 2-Acetyl-1-pyrroline and 2-Acetyltetrahydropyridine in Wheat Bread Crust. *Z. Lebensm. Unters. Forsch.* **1990b**, *191*, 206–209; *Chem. Abstr.* **1991**, *114*, 100068.

Schieberle, P.; Grosch, W. Identification of Flavor Compounds from the Crust of Rye Bread. *Z. Lebensm. Unters. Forsch.* **1983**, *177*, 173–180.

Schieberle, P.; Grosch, W. Identification of the Flavor Compounds of Rye Bread Crumb and Their Comparison with the Crust Flavor Compounds. *Z. Lebensm. Unters. Forsch.* **1984**, *178*, 479–483.

Tressl, R.; Grünewald, K. G.; Helak, B. Formation of Flavor Components from Proline and Hydroxyproline with Glucose and Maltose and their Importance to Food Flavor. In *Flavor '81, 3rd Weurman Symposium*; Schreier, P., Ed.; de Gruyter: Berlin, 1981; pp 397–416.

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

Tressl, R.; Grünewald, K. G.; Kersten, E.; Rewicki, D. Formation of Pyrroles and Tetrahydroindolizin-6-ones as Hydroxyproline–Specific Maillard Products from Erythrose and Arabinose. *J. Agric. Food Chem.* **1986**, *34*, 347–350.

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