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New Insights into the Formation of Aroma-Active Strecker Aldehydes from 3-Oxazolines as Transient Intermediates

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Supporting Information

ABSTRACT: 2-Substituted-5-methyl-3-oxazolines, a novel class of aroma precursors that are able to release the respective Strecker aldehydes by hydrolysis, were identified. Hydrolysis can take place after the addition of water or with human saliva during mastication, respectively. 2-Isobutyl-, 2-*sec*-isobutyl-, 2-isopropyl, and 2-benzyl-5-methyl-3-oxazolines were synthesized and structurally identified by means of gas chromatography−mass spectrometry (GC-MS) in the electron impact mode and in the chemical ionization mode as well as by one- and two-dimensional NMR experiments. With these compounds at hand, a variety of stability experiments were performed using headspace-GC-MS or proton transfer reaction−MS techniques on the basis of stable isotope dilution assays, proving the ability to release the respective Strecker aldehydes was dependent on the pH value as well as on the hydrolysis time. After the addition of water at 37 °C, for example, >70 mol % of 3-methylbutanal or >40 mol % of phenylacetaldehyde was liberated from a solution of 2-isobutyl-5-methyl-3-oxazoline or 2-benzyl-5-methyl-3-oxazoline, respectively, after 5 min. Furthermore, the presence of 2-isobutyl-5-methyl-3-oxazoline in dark chocolate containing 70% cocoa was proven by GC-MS.

KEYWORDS: *Strecker aldehydes, aroma precursors, aroma release, 5-methyl-3-oxazolines, PTR-MS*

INTRODUCTION

Nearly 150 years ago, Strecker[1] reported on the formation of 3-methylbutanal and carbon dioxide when leucine was reacted with the tricarbonyl compound alloxan. However, the important impact of such aldehydes, later designated "Strecker" aldehydes,[2] was not reported until Keeney and Day[3] were able to generate a toasted cheese aroma by reacting a protein hydrolysate with ninhydrine. Shortly after this finding, the thermally induced formation of methylpropanal and 3-methylbutanal was also suggested as an important step in cocoa flavor formation during roasting.[4]

Today, it is well accepted that the formation of Strecker aldehydes from their parent amino acids is initiated by various α-dicarbonyl compounds formed by carbohydrate degradation, such as 2,3-butanedione, 2-oxopropanal, or deoxyosones as well as quinones enzymatically formed by polyphenol oxidation.[5] The reaction pathway is exemplified in Figure 1 for the formation of phenylacetaldehyde (**E**) from phenylalanine, starting from intermediate **A**. Besides phenylalanine, also valine, leucine, isoleucine, methionine, and alanine are known to serve as precursors for odor-active aldehydes following the same pathway (Table 1). However, aldehydes are not the only compounds formed by a Strecker degradation of α-amino acids. As previously shown,[7,8] also phenylacetic acid (**F** from intermediate **D**; Figure 1) and 2-phenylethylamine (**B** from intermediate **A**; Figure 1), showing the partial structure of the parent amino acid phenylalanine, were confirmed to be formed via a Strecker degradation. In addition, besides the free amino acids, also the Amadori reaction products have been confirmed as direct precursors of Strecker aldehydes without the need of an α-dicarbonyl compound as catalyst of the reaction.[9,10]

In a previous study on the isolation of aroma compounds from milk chocolate,[10] it was observed that in comparison to a distillation performed by solvent-assisted flavor evaporation (SAFE) technique[11] phenylacetaldehyde and 3-methylbutanal were increased by factors of ∼120 and ∼13, respectively, when steam distillation was applied in volatile isolation. Although a formation of both Strecker aldehydes from the amino acid/carbohydrate reaction might have occurred during the thermal treatment, these data suggested for the first time the presence of unknown precursors for Strecker aldehydes showing a distinct instability in the presence of hot water. This assumption was later corroborated by the results of Schuh and Schieberle,[13] who reported on the generation of Strecker aldehydes simply by the addition of hot water to black tea leaves. Furthermore, in a recent publication,[14] the significant generation of Strecker aldehydes from dry processed foods, such as malt, bread crust, crackers, and, in particular, cocoa and chocolate, upon heating in water was confirmed by quantitative studies.

More than 40 years ago,[15] it was observed that, as expected, the reaction of valine with 2,3-butanedione led to the formation of methylpropanal. However, probably because the reaction was performed in diglyme in the absence of water, 2-isopropyl-4,5-dimethyl-3-oxazoline was shown to be additionally formed, but because no synthesis of the latter compound was performed, the role of the oxazoline as a possible intermediate in aldehyde formation was not elucidated. The aim of this study was,

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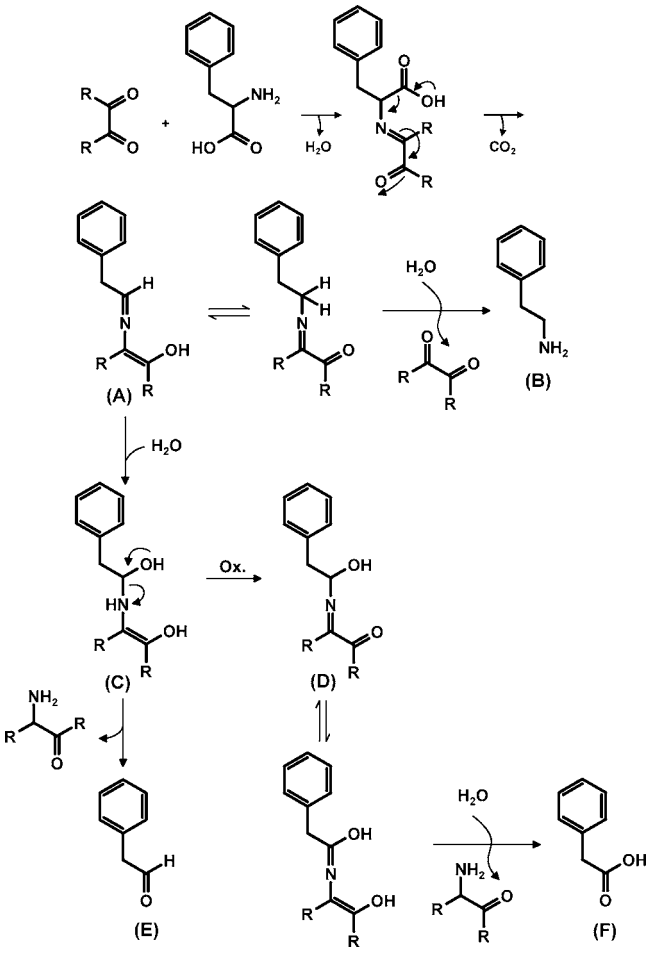

Figure 1. Formation pathway leading to the Strecker aldehyde (**E**), the Strecker acid (**F**), and the Strecker amine (**B**) as exemplified for the reaction of phenylalanine with an α-dicarbonyl compound.

therefore, to get an insight into the possible role of such oxazolines as precursors of Strecker aldehydes, able to liberate the aroma compounds in the presence of water.

MATERIALS AND METHODS

Food Samples. Chocolate was purchased at a local supermarket.

Chemicals. (*R*)-1-Amino-2-propanol, (*S*)-1-amino-2-propanol, methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, 2-methyl-2-propen-1-ol, 2-methyl-3-buten-1-ol, 3-methyl-3-buten-1-ol, [$^{13}C_2$]-phenylethanol, Dess–Martin periodinane, and tris(triphenylphosphine) rhodium(I) chloride were from Sigma-Aldrich (Taufkirchen, Germany). Deuterium gas was from Messer Griesheim (Krefeld, Germany). All other chemicals were of analytical grade.

Synthesis of 2-Substituted-5-methyl-3-oxazolines. The general reaction pathway, which was developed as a retrosynthetic approach, involved the formation of imines from Strecker aldehydes and 1-amino-2-propanol, which immediately cyclized into the 5-methyl-3-oxazolidines. These were finally oxidized by Dess–Martin periodinane to yield the respective 5-methyl-3-oxazolines (Figure 2A–D), which are identical with a possible cyclic tautomer of intermediate **A** in the Strecker reaction (Figure 1).

General Reaction Conditions. (*R*)-1-Amino-2-propanol (20 mmol) was dissolved in dichloromethane (80 mL; dried over anhydrous sodium sulfate) and was singly reacted with either 3-methylbutanal, 2-methylbutanal, methylpropanal, or phenylacetaldehyde (each 20 mmoL; phenylacetaldehyde freshly prepared by distillation) (Figure 2A–D). The reaction mixture was stirred at room temperature for 30 min (phenylacetaldehyde) or for 12 h (for all other aldehydes) to obtain the respective 5-methyl-3-oxazolidine in yields of ∼15% (phenylacetaldehyde) and ∼50% (all other aldehydes), respectively. The diastereoisomers of the 3-oxazolidines were separated neither for further synthesis nor for NMR experiments. Aliquots of the oxazolidines (2.5 mmol) were then dissolved in dichloromethane (60 mL), and Dess–Martin periodinane (3.5 mmol) was added. The oxidation into the corresponding 5-methyl-3-oxazolines was finished within 30 min. After the addition of pentane (25 mL), the mixture was filtered and submitted to high-vacuum distillation at ∼50 °C.[11] The distillate was evaporated to obtain ∼1 mL of an oily residue. After the addition of a pentane/diethyl ether mixture (3 mL; 70:30 by vol), the respective target compound was isolated by column chromatography (22 × 2 cm) as detailed below using a diol phase (40 μm particle size; Bakerbond, Mallinckrodt Baker, Griesheim, Germany) suspended in pentane.

Analytical Data for 2-Substituted-5-methyl-3-oxazolidines and 2-Substituted-5-methyl-3-oxazolines. *2-Isobutyl-5-methyl-3-oxazolidine (1; Figure 2A).* MS-EI, *m/z* (%) 86 (100), 42 (80), 56 (69), 41 (31), 58 (31), 84 (19), 44 (18), 43 (15), 98 (15), 101 (14), 128 (10), 45 (9), 57 (9), 39 (7), 59 (7), 40 (6), 55 (6), 87 (6), 54 (5), 85 (5), 100 (5), 99 (4), 142 (4), 82 (3), 142 ([M − H]$^+$; 3), 140 (M$^+$; tr); MS-CI, *m/z* (%) 144 ([M + H]$^+$; 100), 145 (9); ^{1}H NMR (400 MHz; CD_2Cl_2) δ 0.91–0.95 [m, 12 H, H–C(9, 9′, 10, 10′)], 1.14 [d, *J* = 6.1 Hz, 3H, H–C(6 or 6′)], 1.15 [d, *J* = 6.1 Hz, 3H, H–C(6′ or 6)], 1.37–1.53 [m, 4H, H–C(7, 7′)], 1.73–1.82 [m, 2H, H–C(8, 8′)], 2.47 [dd, *J* = 11.9, 7.5 Hz, 1H, H–C(4a′)], 2.66 [dd, *J* = 11.5, 4.9 Hz, 1H, H–C(4a)], 3.05 [dd, *J* = 11.6, 7.2 Hz, 1H, H–C(4b)], 3.31 [dd, *J* = 11.9, 6.2 Hz, 1H, H–C(4b′)], 3.87–3.95 [m, 2H, H–C(5, 5′)], 4.34 [t, *J* = 5.9 Hz, 1H, H–C(2)], 4.46 [t, *J* = 5.9 Hz, 1H, H–C(2′)]; ^{13}C NMR (100 MHz, CD_2Cl_2) δ 20.21 [C(6 or 6′)], 21.99 [C(6′ or 6)], 22.91 [C(9, 9′ or 10, 10′)], 23.19 [C(10, 10′ or 9, 9′)], 25.84 [C(8, 8′)], 44.08 [C(7 or 7′], 53.21 [C(4)], 54.53 [C(4′)], 72.30 [C(5 or 5′)], 72.43 [C(5′ or 5)], 90.56 [C(2′)], 91.69 [C(2)].

Purification of 2-Isobutyl-5-methyl-3-oxazoline (2; Figure 2A). Stepwise elution was performed as follows: pentane (100 mL; fraction A), pentane/diethyl ether (50 mL; 95:5 by vol; fraction B), and pentane/diethyl ether (200 mL; 95:5 by vol; fraction C). Because two peaks were detected in fraction C (Figure 3), these were separated by rechromatography using the same diol phase: pentane (100 mL; fraction A), pentane/diethyl ether (50 mL; 95:5 by vol; fraction B), pentane/diethyl ether (50 mL; 95:5 by vol; fraction C); pentane/

Table 1. Six of the 20 Proteinogenic Amino Acids Acting as Precursors in the Formation of Odor-Active Strecker Aldehydes

amino acid	Strecker aldehyde	odor quality	odor threshold (μg/kg of water)
methionine	→ 3-(methylthio)propanal	cooked potato-like	0.4[a]
leucine	→ 3-methylbutanal	malty	0.5[a]
valine	→ methylpropanal	malty	0.5[a]
isoleucine	→ 2-methylbutanal	malty	1.5[a]
phenylalanine	→ phenylacetaldehyde	honey-like	5.2[b]
alanine	→ acetaldehyde	fresh, green	25[a]

[a]According to ref 6. [b]Threshold was determined following the procedure described in ref 6.

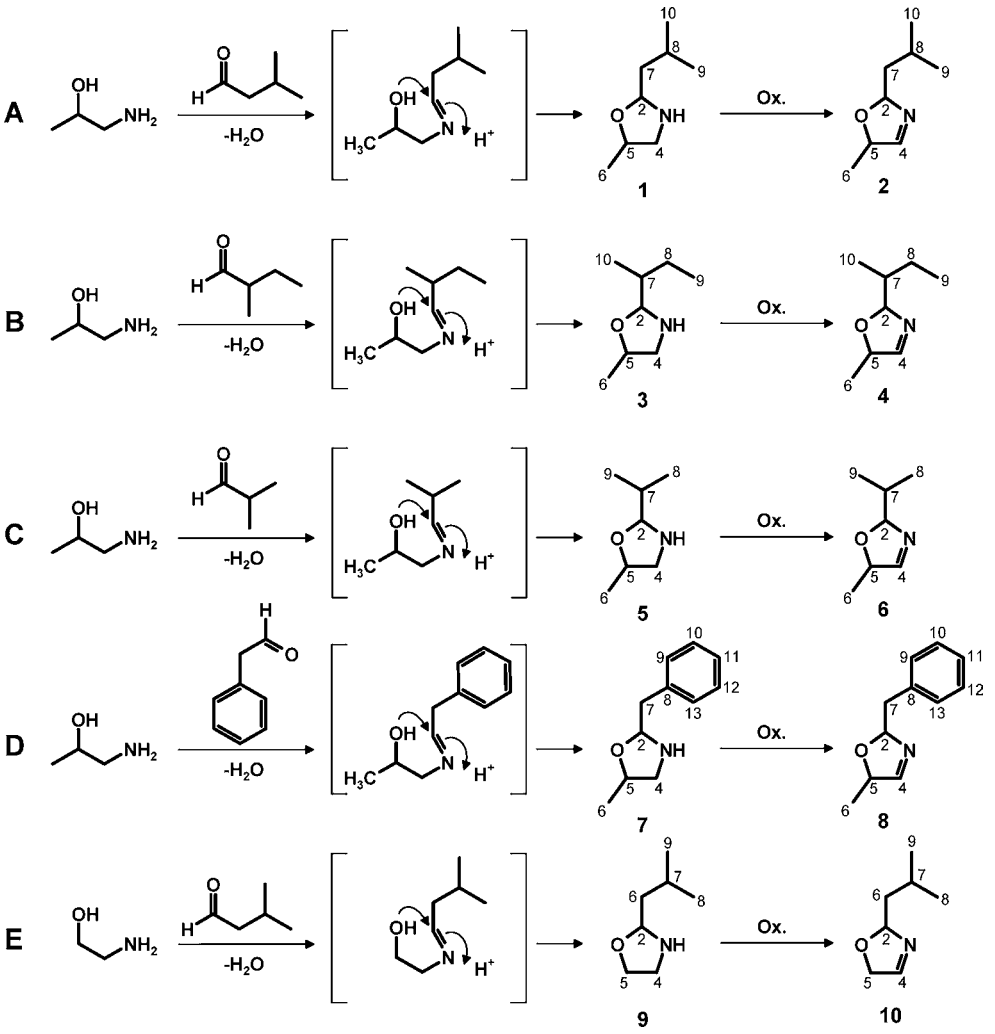

Figure 2. Synthetic routes used in the preparation of 2-substituted-5-methyl-3-oxazolines: (A) 2-isobutyl- (**2**); (B) 2-*sec*-isobutyl- (**4**); (C) 2-isopropyl- (**6**); (D) 2-benzyl-5-methyl-3-oxazoline (**8**); (E) 2-isobutyl-3-oxazoline (**10**).

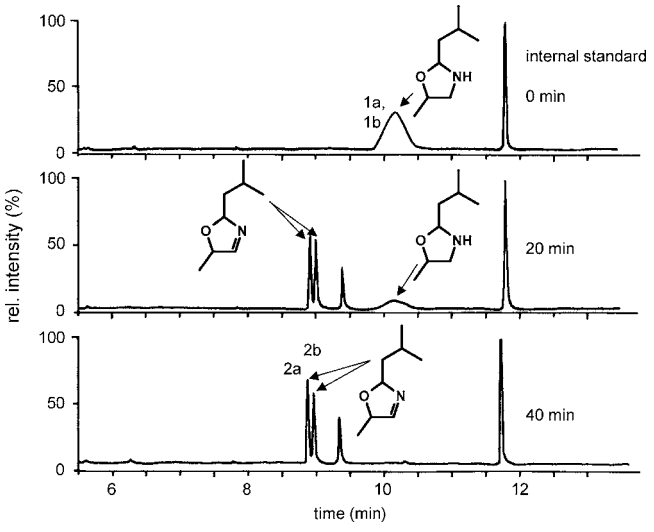

Figure 3. GC-FID chromatograms showing the formation of two isomers of 2-isobutyl-5-methyl-3-oxazoline (**2a** and **2b**) from 2-isobutyl-5-methyl-3-oxazolidine (**1a** and **1b**) over time.

diethyl ether (50 mL; 95:5 by vol; fraction D), and pentane/diethyl ether (50 mL; 95:5 by vol; fraction E). Isomer **2a** [(*R*,*R*) or (*R*,*S*); the absolute configuration was not determined] was present in fraction C, a mixture of isomers **2a** and **2b** was found in fraction D, and isomer **2b** [(*R*,*S*) or (*R*,*R*)] was isolated in fraction E. MS-EI, *m*/*z* (%) 84 (100), 57 (26), 54 (15), 41 (14), 56 (11), 85 (11), 70 (10), 82 (9), 71 (8), 99 (8), 39 (7), 83 (7), 97 (6), 42 (5), 55 (5), 126 (4), 44 (3), 53 (3), 140 ([M − H]$^+$; 1), 141 (M$^+$; tr); MS-CI, *m*/*z* (%) 142 ([M + H]$^+$; 100), 143 (8); ^{1}H NMR (400 MHz; CD_2Cl_2) δ 0.97 [d, J = 6.7 Hz, 6H, H−C(9, 10)], 1.30 [d, J = 6.7 Hz, 3H, H−C(6)], 1.42−1.48 [m, 1H, H−C(7a)], 1.57−1.63 [m, 1H, H−C(7b)], 1.83−1.92 [m, 1H, H−C(8)], 4.69−4.74 [m, 1H, H−C(5)], 5.55−5.59 [m, 1H, H−C(2)], 7.36 [d, J = 2.6 Hz, 1H, H−C(4)]; ^{13}C NMR (100 MHz, CD_2Cl_2) δ 19.75 [C(6)], 22.78 [C(9)], 23.30 [C(10)], 25.27 [C(8)], 46.80 [C(7)], 82.07 [C(5)], 105.61 [C(2)], 163.94 [C(4)].

*2-sec-Butyl-5-methyl-3-oxazolidine (**3**; Figure 2B; Mixture of Four Diastereoisomers Due to Three Chiral Carbon Atoms).* MS-EI, *m*/*z* (%) 86 (100), 41 (55), 70 (49), 69 (44), 43 (42), 98 (41), 58 (30), 42 (28), 44 (25), 84 (23), 56 (21), 39 (20), 59 (19), 115 (18), 55 (11), 57 (10), 68 (10), 53 (8), 128 (8), 67 (7), 82 (7), 99 (7), 54 (6), 78 (6), 97 (6), 100 (4), 142 ([M − H]$^+$; 3), 143 (M$^+$; tr); MS-CI, *m*/*z* (%) 144 ([M + H]$^+$; 100), 145 (9); ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.90−0.98 [m, 24H, H−C(9, 10)], 1.11−1.27 [m, 16H, H−C(6, 8a)], 1.50−1.68 [m, 8H, H−C(8b, 7)], 2.51−2.56 [m, 2H, H−C(4a, 4a′)], 2.67−2.71 [m, 2H, H−C(4a″, 4a‴)], 3.09−3.14 [m, 2H, H−C(4b″, 4b‴)], 3.33−3.38 [m, 2H, H−C(4b, 4b″)], 3.87−4.05 [m, 4H, H−C(5)], 4.21−4.23 [m, 2H, H−C(2″, 2‴)], 4.32−4.35 [m, 2H, H−C(2, 2′)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.36, 11.38, 11.50, 11.51, 13.87, 13.96, 14.29, 14.38 [C(9, 10)], 19.77, 19.78, 20.66, 20.67 [C(6)],

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25.09, 25.19, 25.49, 25.60 [C(8)], 38.55, 38.57, 38.62, 38.64 [C(7)], 52.69 [C(4″, 4‴)], 54.00, 54.01 [C(4, 4′)], 71.99, 72.04, 72.30, 72.32 [C(5)], 94.91, 94.99 [C(2, 2′)], 95.95, 95.98 [C(2″, 2‴)].

*Purification of 2-sec-Butyl-5-methyl-3-oxazoline (**4**; Figure 2B).* Elution was performed as follows: pentane (100 mL; fraction A), pentane/diethyl ether (150 mL; 95:5 by vol; fraction B), pentane/diethyl ether (20 mL; 95:5 by vol; fraction C), pentane/diethyl ether (50 mL; 95:5 by vol; fraction D), and pentane/diethyl ether (100 mL; 90:10 by vol; fraction E). Fraction B contained isomer **4a**, and fractions D and E contained isomer **4b**. No further rechromatography was needed. MS-EI, *m/z* (%) 84 (100), 56 (56), 57 (42), 112 (34), 85 (28), 41 (27), 70 (21), 68 (15), 86 (15), 55 (13), 83 (13), 126 (11), 42 (9), 43 (8), 97 (7), 39 (6), 58 (5), 140 (4), 140 ($[M - H]^+$; 3), 141 (M^+; tr); MS-CI, *m/z* (%) 142 ($[M + H]^+$; 100), 143 (10); ^{1}H NMR (400 MHz; CD_2Cl_2) δ 0.89 [d, J = 6.8 Hz, 3H, H−C(10)], 0.95 [t, J = 7.4 Hz, 3H, H−C(9)], 1.16−1.29 [m, 1H, H−C(8a)], 1.29 [d, J = 6.7 Hz, 3H, H−C(6)], 1.51−1.63 [m, 1H, H−C(8b)], 1.66−1.78 [m, 1H, H−C(7)], 4.79−4.87 [m, 1H, H−C(5)], 5.63−5.69 [m, 1H, H−C(2)], 7.48 [d, J = 2.6 Hz, 1H, H−C(4)]; ^{13}C NMR (100 MHz, CD_2Cl_2) δ 11.88 [C(9)], 13.83 [C(10)], 18.60 [C(6)], 24.97 [C(8)], 40.74 [C(7)], 82.35 [C(5)], 109.80 [C(2)], 164.41 [C(4)].

*2-Isopropyl-5-methyl-3-oxazolidine (**5**; Figure 2C).* MS-EI, *m/z* (%) 84 (100), 86 (94), 41 (48), 70 (41), 58 (32), 55 (28), 56 (27), 42 (20), 59 (20), 43 (19), 85 (11), 39 (9), 45 (9), 57 (8), 68 (5), 83 (5), 40 (4), 54 (4), 69 (4), 82 (4), 87 (4), 114 (4), 128 ($[M - H]^+$; 3), 129 (M^+; tr); MS-CI, *m/z* (%) 130 ($[M + H]^+$; 100), 131 (8); ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.96−1.01 [m, 12H, H−C(8, 8′, 9, 9′)], 1.19 [d, J = 6.2 Hz, 3H, H−C(6 or 6′)], 1.21 [d, J = 6.2 Hz, 3H, H−C(6′ or 6)], 1.72−1.81 [m, 2H, H−C(7, 7′)], 2.54 [dd, J = 11.9, 7.7 Hz, 1H, H−C(4a′)], 2.68 [dd, J = 11.5, 5.0 Hz, 1H, H−C(4a)], 3.12 [dd, J = 11.5, 7.0 Hz, 1H, H−C(4b)], 3.35 [dd, J = 11.8, 6.1 Hz, 1H, H−C(4b′)], 3.89−4.04 [m, 2H, H−C(5, 5′)], 4.15 [d, J = 5.8 Hz, 1H, H−C(2)], 4.26 [d, J = 5.8 Hz, 1H, H−C(2′)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.88 [C(8 or 8′ or 9 or 9′)], 17.94 [C(8 or 8′ or 9 or 9′)], 18.12 [C(8 or 8′ or 9 or 9′)], 18.17 [C(8 or 8′ or 9 or 9′)], 19.76 [C(6 or 6′)], 20.64 [C(6′ or 6)], 32.12 [C(7 or 7′)], 32.24 [C(7′ or 7)], 52.74 [C(4)], 54.03 [C(4′)], 72.22 [C(5 or 5′)], 72.46 [C(5′ or 5)], 96.05 [C(2′)], 97.04 [C(2)].

*Purification of 2-Isopropyl-5-methyl-3-oxazoline (**6**; Figure 2C).* Elution was performed as follows: pentane (100 mL; fraction A), pentane/diethyl ether (150 mL; 95:5 by vol; fraction B), pentane/diethyl ether (100 mL; 90:10 by vol; fraction C), and pentane/diethyl ether (50 mL; 90:10 by vol; fraction D). Fractions C and D contained both isomers, but these could not be separated further. MS-EI, *m/z* (%) 84 (100), 56 (36), 57 (35), 112 (26), 83 (20), 41 (15), 70 (12), 68 (11), 43 (9), 85 (9), 55 (5), 39 (4), 126 ($[M - H]^+$; 3), 127 (M^+; tr); MS-CI, *m/z* (%) 128 ($[M + H]^+$; 100), 129 (8); ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.93 [d, J = 6.8 Hz, 3H, H−C(8)], 0.96 [d, J = 6.8 Hz, 3H, H−C(9)], 1.29 [d, J = 6.7 Hz, 3H, H−C(6)], 1.91−1.99 [m, 1H, H−C(7)], 4.80−4.86 [m, 1H, H−C(5)], 5.56−5.59 [m, 1H, H−C(2)], 7.48 [d, J = 2.5 Hz, 1H, H−C(4)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.61 [C(8 or 9)], 17.26 [C(9 or 8)], 18.26 [C(6)], 33.52 [C(7)], 82.10 [C(5)], 110.25 [C(2)], 163.87 [C(4)].

*2-Benzyl-5-methyl-3-oxazolidine (**7**; Figure 2D).* MS-EI, *m/z* (%) 86 (100), 132 (54), 91 (37), 59 (22), 41 (17), 58 (17), 105 (16), 130 (12), 133 (12), 77 (10), 92 (10), 65 (9), 103 (9), 117 (9), 104 (8), 39 (6), 87 (6), 177 (M^+; 6), 53 (4), 176 ($[M - H]^+$; 2); MS-CI, *m/z* (%) 178 ($[M + H]^+$; 100), 179 (12); ^{1}H NMR (400 MHz; CD_2Cl_2) δ 0.89 [d, J = 6.1 Hz, 3H, H−C(6)], 0.96 [d, J = 6.1 Hz, 3H, H−C(6′)], 2.17−2.24 [m, 2H, H−C(4a, 4a′)], 2.69−2.76 [m, 2H, H−C(4b, 4b′)], 2.79−2.90 [m, 4H, H−C(7, 7′)], 3.59−3.69 [m, 2H, H−C(5, 5′)], 4.55 [t, J = 4.9 Hz, 1H, H−C(2)], 4.69 [t, J = 4.8 Hz, 1H, H−C(2′)], 7.05−7.09 [m, 2H, H−C(11, 11′)], 7.16−7.17 [m, 4H, H−C(10, 10′, 12, 12′)], 7.25−7.28 [m, 4H, H−C(9, 9′, 13, 13′)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.76 [C(6′)], 20.56 [C(6)], 40.45 [C(7′)], 40.91 [C(7)], 52.61 [C(4)], 53.94 [C(4′)], 72.27 [C(5′)], 72.33 [C(5)], 91.36 [C(2′)], 92.41 [C(2)], 126.40 [C(11)], 126.44 [C(11′)], 128.10 [C(10, 12)], 128.23 [C(10′, 12′)], 130.10 [C(9′, 13′)], 130.16 [C(9, 13)].

*Purification of 2-Benzyl-5-methyl-3-oxazoline (**8**; Figure 2D).* Elution was performed as follows: pentane (100 mL; fraction A), pentane/diethyl ether (200 mL; 90:10 by vol; fraction B), pentane/diethyl ether (150 mL; 90:10 by vol; fraction C), and pentane/diethyl ether (50 mL; 90:10 by vol; fraction D). Fraction C containing **8** was used for rechromatography as follows: pentane (100 mL; fraction A), pentane/diethyl ether (250 mL; 90:10 by vol; fraction B), pentane/diethyl ether (125 mL; 90:10 by vol; fraction C), pentane/diethyl ether (100 mL; 80:20 by vol; fraction D), and pentane/diethyl ether (100 mL; 80:20 by vol; fraction E). Isomer **8a** was present in fraction C, a mixture of compounds **8a** and **8b** was found in fraction D, and **8b** was isolated in fraction E. MS-EI, *m/z* (%) 84 (100), 91 (71), 57 (39), 92 (38), 65 (17), 77 (10), 41 (9), 104 (9), 131 (9), 103 (7), 78 (6), 130 (6), 39 (5), 44 (5), 105 (5), 40 (4), 42 (4), 43 (4), 54 (4), 55 (4), 56 (4), 85 (4), 173 (3), 175 (M^+; 3), 174 ($[M - H]^+$; 2); MS-CI, *m/z* (%) 176 ($[M + H]^+$; 100), 177 (8); ^{1}H NMR (400 MHz; CD_2Cl_2) δ 1.25 [d, J = 6.7 Hz, 3H, H−C(6)], 2.92 [dd, J = 13.8 Hz, 5.7 Hz, 1H, H−C(7a)], 3.08 [dd, J = 13.8 Hz, 5.0 Hz, H−C(7b)], 4.67−4.70 [m, 1H, H−C(5)], 5.95−5.98 [m, 1H, H−C(2)], 7.22−7.33 [m, 5H, H−C(9, 10, 11, 12, 13)], 7.40 [d, J = 2.4 Hz, 1H, H−C(4)]; ^{13}C NMR (100 MHz, CD_2Cl_2) δ 18.43 [C(6)], 42.11 [C(7)], 82.39 [C(5)], 106.58 [C(2)], 126.75 [C(11)], 128.46 [C(9, 13)], 130.40 [C(10, 12)], 106.58 [C(8)], 164.70 [C(4)].

Synthesis of 2-Isobutyl-3-oxazoline. 2-Isobutyl-3-oxazoline (**10**; Figure 2E) was synthesized following the same approach described above for 2-isobutyl-5-methyl-3-oxazoline (**2**) but using 2-aminoethanol instead of (*R*)-1-amino-2-propanol in the reaction with 3-methylbutanal.

Analytical Data for 2-Isobutyl-3-oxazolidine and 2-Isobutyl-3-oxazoline. *2-Isobutyl-3-oxazolidine (**9**; Figure 2E).* MS-EI, *m/z* (%) 72 (100), 56 (60), 42 (48), 44 (39), 87 (34), 41 (28), 45 (24), 114 (18), 39 (16), 43 (16), 55 (8), 84 (8), 98 (8), 40 (7), 54 (7), 57 (7), 88 (6), 69 (6), 89 (6), 53 (5), 68 (5), 96 (5), 128 ($[M - H]^+$; 3), 129 (M^+; tr); MS-CI, *m/z* (%) 130 ($[M + H]^+$; 100), 131 (7); ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.91 [d, J = 6.7 Hz, 6H, H−C(8, 9)], 1.38−1.51 [m, 2H, H−C(6)], 1.69−1.78 [m, 1H, H−C(7)], 2.89−2.96 [m, 1H, H−C(4a)], 3.13−3.29 [m, 1H, H−C(4b)], 3.59−3.63 [m, 2H, H−C(5)], 4.25−4.28 [m, 1H, H−C(2)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.61 [C(8 or 9)], 22.99 [C(9 or 8)], 25.45 [C(7)], 43.17 [C(6)], 46.22 [C(4)], 64.58 [C(5)], 91.11 [C(2)].

*Purification of 2-Isobutyl-3-oxazoline (**10**; Figure 2E).* Stepwise elution was performed as follows: pentane (100 mL; fraction A), pentane/diethyl ether (50 mL; 95:5 by vol; fraction B), and pentane/diethyl ether (100 mL; 95:5 by vol; fraction C). Due to the fact that only one chiral center is present in the molecule, only one peak was obtained in fraction C and, thus, no rechromatography was necessary. MS-EI, *m/z* (%) 70 (100), 42 (21), 69 (19), 71 (19), 41 (18), 85 (14), 43 (12), 54 (12), 56 (9), 57 (8), 39 (8), 55 (4), 72 (4), 82 (4), 126 ($[M - H]^+$; 1), 127 (M^+; tr); MS-CI, *m/z* (%) 128 ($[M + H]^+$; 100), 129 (7); ^{1}H NMR (400 MHz; CD_2Cl_2) δ 0.97 [d, J = 6.7 Hz, 6H, H−C(8, 9)], 1.42−1.47 [m, 1H, H−C(6a)], 1.55−1.60 [m, 1H, H−C(6b)], 1.82−1.90 [m, 1H, H−C(8)], 4.46−4.50 [m, 1H, H−C(5a)], 4.57−4.61 [m, 1H, H−C(5b)], 5.64−5.69 [m, 1H, H−C(2)], 7.55 [d, J = 2.5 Hz, H−C(4)]; ^{13}C NMR (100 MHz, CD_2Cl_2]) δ 22.73 [C(8)], 23.32 [C(9)], 25.29 [C(7)], 45.13 [C(6)], 75.16 [C(5)], 106.05 [C(2)], 160.31 [C(4)].

Synthesis of Isotopically Labeled Standards. *[$^2H_2$]-3-Methylbutanal.* 3-Methyl-3-buten-1-ol was deuterated using the Wilkinson catalyst to obtain [3,4-$^2H_2$]-3-methylbutanol, which was subsequently oxidized into [3,4-$^2H_2$]-3-methylbutanal by Dess−Martin periodinane,[16,17] following the approach reported previously for the synthesis of [5,5,6,6-$^2H_4$]-hexanal.[18]

[$^2H_2$]-2-Methylbutanal. Starting from 2-methyl-3-buten-1-ol, the target compound was synthesized following the two-step procedure described for [$^2H_2$]-3-methylbutanal.

[$^2H_2$]-Methylpropanal. Starting from 2-methyl-2-propen-1-ol, the target compound was synthesized following the two-step procedure described for [$^2H_2$]-3-methylbutanal.

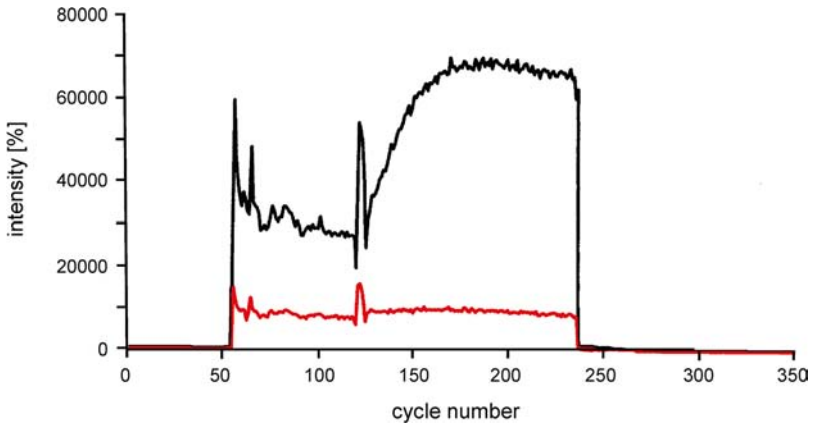

Figure 4. Release of 3-methylbutanal after the addition of water (black line, 3-methylbutanal; red line, [$^2H_2$]-3-methylbutanal as internal standard).

[$^{13}C_2$]-Phenylacetaldehyde. [$^{13}C_2$]-Phenylacetaldehyde was synthesized by oxidation of [1,2-$^{13}C_2$]-2-phenylethanol by Dess−Martin periodinane.

Generation of Strecker Aldehydes from 2-Substituted-5-methyl-3-oxazolines. The respective 5-methyl-3-oxazoline (**2**, **4**, **6**, or **8**; Figure 2A−D) was singly reacted as follows: To the compound dissolved in pentane/diethyl ether (2 mL) was added ethanol (0.5 mL). The pentane/diethyl ether mixture was carefully evaporated, and the residue was finally made up to 5 mL with ethanol. Aliquots (0.5 mL) were then added to water (5 mL) containing the respective isotopically labeled standard, and the solutions were stirred in closed glass vials at 37 °C for 5, 15, or 30 min, respectively. A control experiment was performed without the addition of water.

For quantitation of the respective aldehyde by stable isotope dilution analysis (SIDA), the samples were cooled and either directly subjected to HS-HRGC-MS or extracted with diethyl ether (total volume = 45 mL). Then, the organic phases were combined, dried over anhydrous sodium sulfate, and concentrated to ∼4 mL, and an aliquot (2 μL) was used for HRGC-MS.

Influence of the pH on the Generation of Aldehydes from 2-Substituted-5-methyl-3-oxazolines. Buffers were prepared by mixing solution A (disodium hydrogen phosphate dihydrate; 11.876 g/L of water) and solution B (potassium dihydrogen phosphate; 9.078 g/L of water): pH 5, A + B (0.95 + 99.05 by vol); pH 7, A+B (61.2 + 38.8 by vol); pH 9, an aqueous solution of sodium hydroxide (5 mol/L) was added to A until a pH of 9 was reached. An aliquot of the oxazoline solution in ethanol (0.5 mL) was added to the respective buffer solution (5 mL; either pH 5.0, pH 7.0, or pH 9.0). Then, the respective isotopically labeled standard was added, and the solutions were stirred in closed glass vials at 37 °C for 5, 15, or 30 min, respectively. A control experiment was performed without the addition of the respective buffer solution.

Quantitation of Strecker Aldehydes Generated from Chocolate upon Addition of Water. Chocolate (70% cocoa content) was frozen in liquid nitrogen and ground to a powder by means of a mill. An aliquot (50 g) was melted at 37 °C, and [$^2H_2$]-3-methylbutanal (53 μg, dissolved in 500 μL of dichloromethane) and [$^{13}C_2$]-phenylacetaldehyde (18 μg, dissolved in 100 μL of dichloromethane) were added. After stirring for 5 min, the chocolate ("spiked chocolate") was cooled to room temperature and then used for PTR-MS measurements.

A reference mixture in sunflower oil (10 g) containing methylpropanal (2.5 μg), 3-methylbutanal (6.25 μg), phenylacetaldehyde (25 μg), [$^2H_2$]-3-methylbutanal (4.3 μg), and [$^{13}C_2$]-phenylacetaldehyde (20 μg) served as the control.

Proton Transfer Reaction−Mass Spectrometry (PTR-MS). Spiked chocolate (5.0 g) was weighed into a conical glass flask (200 mL) and sealed with a gastight septum. The chocolate was melted at 37 °C, and then the flask was connected to a PTR-MS (Ionicon Analytik, Innsbruck, Austria) via a peek capillary. After defined times at 37 °C, either acidified water (5 mL, pH 4) or human saliva (5 mL), respectively, was added through the septum via a syringe. Preliminary tests with acetone/air mixtures proved a leak-free connection. Air was sampled from the flask via a continuous flow of 150 mL/min and analyzed as described by Lindinger.[19] The following parameters were used: inlet temperature, 120 °C; chamber temperature, 80 °C; inlet flow, 70 mL/min. With the scan mode applied, samples were scanned for the full mass range from m/z 20 to 180 at specific dwell times. For multiple ion detection, the following mass to charge ratios and individual dwell times were selected: m/z 59 for acetone of breath (100 ms), m/z 73 for methylpropanal (200 ms), m/z 87 for 2- and 3-methylbutanal (200 ms), m/z 89 for [$^2H_2$]-3-methylbutanal (200 ms), m/z 121 for phenylacetaldehyde (500 ms), and m/z 123 for [$^{13}C_2$]-phenylacetaldehyde (500 ms). Signal intensities were normalized to a theoretical intensity of the primary ion H_3O^+ of 1×10^7 counts per second.

Static Headspace High-Resolution Gas Chromatography−Mass Spectrometry (HS-HRGC-MS). Analysis was performed on a Trace GC Ultra (Thermo Scientific, Dreieich, Germany) connected to a mass spectrometer Saturn 2100T ion trap (Varian, Darmstadt, Germany). Aliquots (500 μL) of the headspace volume were injected with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 1 mL gastight syringe (SGE Analytic Science, Darmstadt, Germany) into a hot PPKD injector (Thermo Scientific). After each injection, a possible carry-over into the syringe was eliminated by an automatic syringe flush with helium. Volatiles were trapped in a Cold Trap 915 (Thermo Scientific) at −150 °C and were subsequently transferred by thermal desorption (initial temperature, −150 °C for 0.1 min; heating rate, 12 °C/s; final temperature, 240 °C for 3 min) onto a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific; Agilent, Waldbronn, Germany) starting with a temperature of 0 °C for 2 min and subsequent heating at 6 °C/min to 240 °C (3 min). The oven was heated from −5 °C at 4 °C/min to 50 °C and then to 220 °C at 10 °C/min. The effluent was monitored by a mass spectrometer running in the chemical ionization (CI) mode (ionization energy, 70 eV) with methanol as the reagent gas.

Two Dimensional Gas Chromatography−Mass Spectrometry (TDGC-MS). Analysis was accomplished by means of a GC/GC-MS system with a heart-cut interface. The system consisted of a Trace GC Ultra (Thermo Scientific) equipped with a DB-FFAP column (30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific), a CP-3800 gas chromatograph (Varian) equipped with a DB-1701 column (30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific), and a CombiPal autosampler. The first GC housed a moving column stream switching system (MCSS) dividing the effluent in equal parts to a flame ionization detector and a sniffing outlet or, alternatively, leading the effluent in total to the second GC column. The second column was connected to a mass spectrometer Saturn 2200 (Varian) running in the CI mode with methanol as reactant gas (ionization energy, 115 eV). Mass chromatograms were recorded, and concentrations of the

analytes were calculated from the area counts of specific ions of the analytes and the labeled standards as reported before.[7]

HRGC-MS. HRGC-MS analyses were performed by means of a type 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) coupled to a sector field mass spectrometer MAT 95 S (Finnigan MAT, Bremen, Germany) running either in the electron impact (EI) mode (ionization energy, 70 eV) or in the CI mode using isobutane as the reagent gas (ionization energy, 115 eV). The samples were injected by the cold on-column technique at 40 °C onto a DB-FFAP fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific). The oven temperature was held for 2 min isothermally, then raised at 40 °C/min to 60 °C, held for 1 min, and finally raised at 6 °C/min to 230 °C (3 min).

NMR Spectroscopy. ^{1}H, ^{13}C, and HSQC NMR spectra were recorded using a Bruker 400 MHz DRX spectrometer (Bruker, Rheinstetten, Germany). Samples were dissolved either in CD_2Cl_2 or in $CDCl_3$ with 0.03% of tetramethylsilane (TMS). Chemical shifts were determined using TMS as the internal standard (^{1}H NMR) or the carbon signal of either CD_2Cl_2 (53.9 ppm; ^{13}C NMR) or $CDCl_3$ (77.0 ppm; ^{13}C NMR), respectively.

RESULTS AND DISCUSSION

Measurement of Aroma Release. As reported previously,[14] treatment of chocolate with water at 100 °C led to the formation of Strecker aldehydes in significant amounts. Because it can be assumed that the formation of these potent aroma compounds may also occur in the mouth during mastication, the release of Strecker aldehydes upon addition of either water or saliva to chocolate at 37 °C was measured by PTR-MS.

To simulate this process, chocolate (70% cocoa content) was frozen in liquid nitrogen and ground to a powder. An aliquot (50 g) was melted at 37 °C and [$^{2}H_2$]-3-methylbutanal as well as [$^{13}C_2$]-phenylacetaldehyde were added as internal standards. The "spiked chocolate" was then submitted to PTR-MS measurements. As exemplified for 3-methylbutanal in Figure 4, the signals of the labeled compound (red line) and the analyte (black line) were nearly constant between cycles 52 (start of measurement) and 120 without water added, indicating a constant headspace concentration of standard and analyte. Upon the addition of water (cycles 120−125), 3-methylbutanal increased significantly while the internal standard remained constant (cycles 125−240). This can be seen as proof that 3-methylbutanal is generated from precursors in chocolate, which are susceptible to water. The same results were obtained after the addition of human saliva (data not shown).

To confirm the results, a blank sample consisting of methylpropanal, 3-methylbutanal, phenylacetaldehyde, [$^{2}H_2$]-3-methylbutanal, and [$^{13}C_2$]-phenylacetaldehyde in sunflower oil was measured by PTR-MS. All monitored compounds showed only a small increase (∼10%) after the addition of water due to the change in matrix (data not shown).

To get an initial idea on the amounts of the aldehydes released, headspace-GC-MS was performed. For this purpose, the spiked chocolate was stirred for 5 min at 37 °C. Then, the first injection was done, which was used as blank sample, and the chocolate sample was cooled to approximately 16 °C. After the blank run was finished, human saliva was added through the septum via a syringe and the sample was heated again to 37 °C under stirring for 5 min. Then, the second injection (sample) was done. Addition of saliva caused an increase of methylpropanal by a factor of 43 (from 25.4 to 1103 μg/kg), of 2-methylbutanal by a factor of 8.5 (from 354 to 3009 μg/kg), and of 3-methylbutanal by a factor of 8.9 (502 to 4456 μg/kg).

Characterization of Precursors for Strecker Aldehyde Generation. One intermediate proposed in the Strecker reaction following decarboxylation is intermediate **A** (Figure 5), which upon hydrolysis generates the respective Strecker

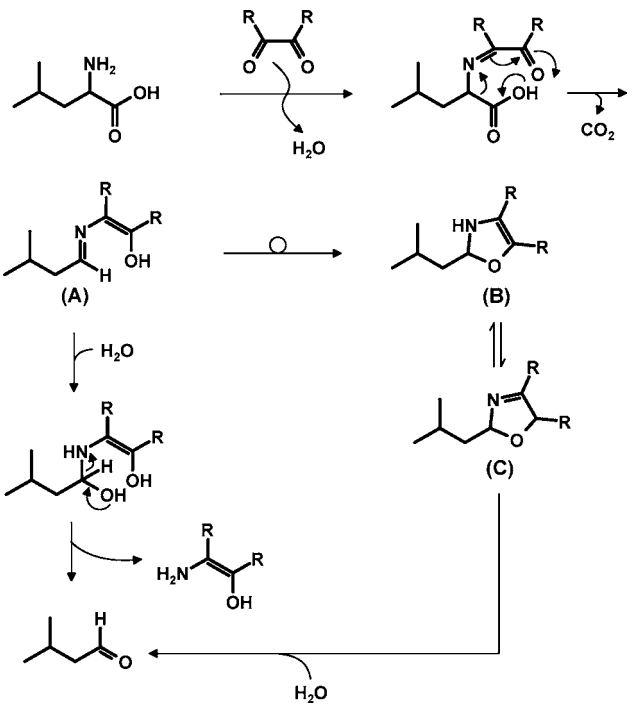

Figure 5. Strecker degradation of leucine catalyzed by an α-dicarbonyl compound; alternative route leading to 3-methylbutanal via a 4-oxazoline (**B**) or a 3-oxazoline (**C**), respectively.

aldehyde (left side). However, it can be assumed that the 4-oxazoline (**B**; Figure 5) and/or the tautomeric 3-oxazoline (**C**; Figure 5) might be formed as additional intermediates. Assuming that these cyclic intermediates are stable in the absence of water, such oxazolines could be the precursors suggested to be present in dry foods[12] and leading to the formation of Strecker aldehydes upon the addition of water. To test this assumption, a common strategy for the synthesis of the 3-oxazolines to be expected from different amino acids was developed. Because numerous α-dicarbonyl compounds may occur in foods, which influence the substituents at positions 4 and 5 in the oxazolines, the synthesis was focused on products to be expected from a reaction of α-amino acids with 2-oxopropanal. The common synthetic route was planned as a retrosynthetic approach. (*R*)-1-Amino-2-propanal was reacted with either 3-methylbutanal, 2-methylbutanal, methylpropanal, or phenylacetaldehyde to first yield the corresponding 5-methyl-3-oxazolidines. These were then oxidized into the corresponding 5-methyl-3-oxazolines (Figure 2A−D). By this approach, four 5-methyl-3-oxazolidines and four 5-methyl-3-oxazolines with different substituents in the 2-position were newly synthesized. In the following, the application of this synthetic strategy is exemplified for the synthesis of 2-isobutyl-5-methyl-3-oxazoline.

The reaction of 3-methylbutanal with (*R*)-1-amino-2-propanol directly led to the formation of 2-isobutyl-5-methyl-3-oxazolidine (**1**, Figure 2A). Due to the two chiral carbon atoms, a pair of diastereoisomers should be expected for **1** [(*R*,*R*) and (*R*,*S*)], but using an FFAP column as stationary GC phase, no separation was achieved (**1a** and **1b**; Figure 3). The

mass spectra (MS-EI; Figure 6A) and MS-CI (data not shown) confirmed the assumed molecular mass of 143. The cyclic

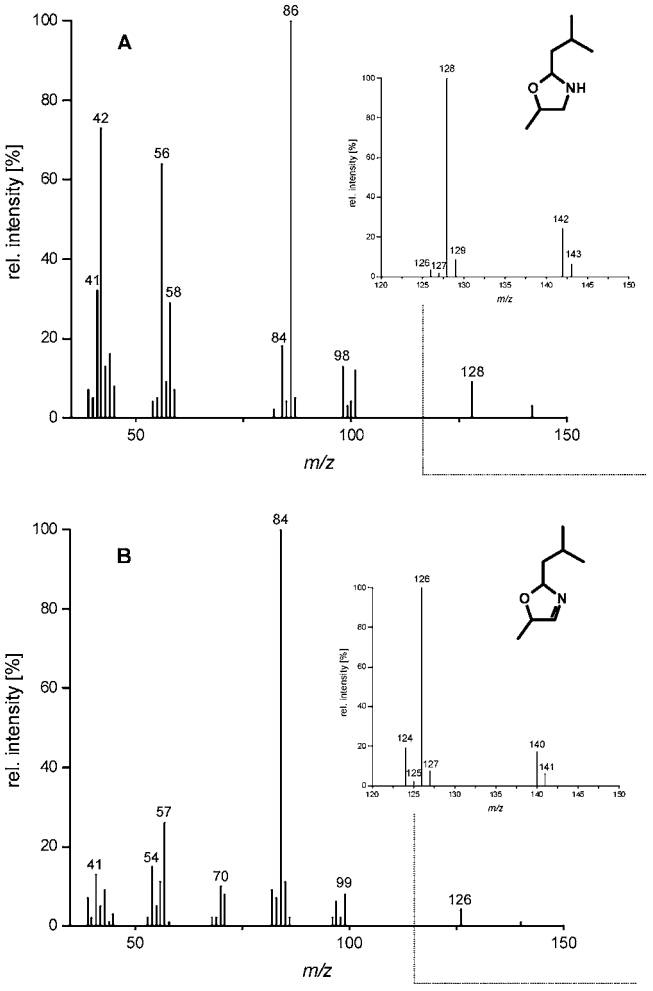

Figure 6. (A) Mass spectrum (MS-EI) of 2-isobutyl-5-methyl-3-oxazolidine (**1**); (B) mass spectrum (MS-EI) of 2-isobutyl-5-methyl-3-oxazoline (**2a**).

structure was derived from NMR measurements. Both the ^{1}H NMR and the two-dimensional HSQC experiment (Figure 7) proved the absence of an olefinic proton, which should be present in an acyclic structure (Figure 2A). For data interpretation, the numbering of the molecules is illustrated in Figure 2.

The oxazolidine was then oxidized, yielding 2-isobutyl-5-methyl-3-oxazoline (**2**; Figure 2A), the respective mass spectrum (MS-EI) is shown in Figure 6B. Due to the two chiral carbon atoms [(*R*,*R*) and (*R*,*S*)], again a pair of diastereoisomers is to be expected for **2**, and two peaks (**2a** and **2b**; Figure 3) showing identical mass spectra in the MS-EI as well as in the MS-CI were obtained. GC-MS proved the expected loss of 2 mass units during the oxidation of 2-isobutyl-5-methyl-3-oxazolidine into 2-isobutyl-5-methyl-3-oxazoline (M^{+}, *m*/*z* 141). The position of the double bond in **2** was determined by NMR. By applying one- and two-dimensional experiments, the double bond between the nitrogen and carbon atom 4 in the ring system could be proven due to the chemical shift of the hydrogen atom at carbon atom 4 into the higher parts per million area. In addition, by means of a HSQC experiment, the change at carbon atom 4 from a methylene group in the oxazolidine (Figure 7) into a methine group in the oxazoline (Figure 8) confirmed the suggested structure. To prove that **2a** and **2b** (Figure 3) are diastereoisomers, a procedure for their separation by column chromatography was developed. By application of ^{1}H NMR and ^{13}C NMR measurements on both isomers, the same spectra showing only a marginal shift of the signals were obtained, thereby confirming that both are diastereoisomers (data not shown). To further corroborate the presence of diastereoisomers, 3-methylbutanal was reacted with (*S*)-1-amino-2-propanol, yielding again two isomers of 2-isobutyl-5-methyl-3-oxazoline [(*S*,*R*) and (*S*,*S*)]. These molecules revealed the same mass spectrometric data as well as the corresponding NMR data, proving the existence of a second pair of diastereoisomers bearing the (*S*) configuration at carbon atom 5 in the ring system (**2c** and **2d**; data not shown). To finally prove the stereochemistry, 1-amino-2-propanol was replaced by 2-aminoethanol, yielding 2-isobutyl-3-oxazoline (**10**; Figure 2E). As **10** contained only one chiral atom, this compound showed only one peak after separation on the achiral GC stationary phase.

The same procedure was applied to synthesize three additional oxazolines, 2-*sec*-butyl-5-methyl-3-oxazoline (**4**; Figure 2B), 2-isopropyl-5-methyl-3-oxazoline (**6**; Figure 2C), and 2-benzyl-5-methyl-3-oxazoline (**8**; Figure 2D).

Generation of Strecker Aldehydes by Treatment of 2-Substituted-5-methyl-3-oxazolines with Water. With the possible precursors of the respective Strecker aldehydes at hand, the generation of the odorants was studied in model systems. The first series of experiments was performed with the leucine-related precursor 2-isobutyl-5-methyl-3-oxazoline (**2**; Figure 2A). In a closed vessel, an aliquot of the oxazoline was dissolved in ethanol, and any contact with water was avoided. Then, an aliquot of the solution was added to water containing the isotopically labeled standard [$^{2}H_{2}$]-3-methylbutanal. The solution was stirred in closed glass vials for 5, 15, or 30 min, respectively, at 37 °C.

To ensure the reliability of the data, quantitation was performed using two different techniques. In the first approach, the headspace above the solution was withdrawn with a gastight syringe and directly analyzed by GC-MS. The second approach used solvent extraction prior to GC-MS analysis.

The results showed that only 5 min after addition of water, nearly 70 mol % of **2a** or **2b**, respectively, was converted into 3-methylbutanal when reacted singly (Table 2). After 30 min, >90 mol % of the aroma compound was generated. No differences were found between the data obtained with both methods of quantitation, suggesting **2** as a very powerful precursor in the formation of the aroma compound.

The next series of experiments was aimed at getting information on the influence of the pH value on the generation of 3-methylbutanal from 2-isobutyl-5-methyl-3-oxazoline. After 10 min at pH 7, >50 mol % of **2a** was converted into the aroma compound (Table 3), corroborating the key role of the 3-oxazoline in Strecker aldehyde formation. However, the reaction was even faster at pH 5, leading to a nearly complete conversion of **2a** into 3-methylbutanal after only 10 min. On the other hand, alkaline conditions (pH 9; Table 3) partly stabilized the precursor, because only 9 mol % of 3-methylbutanal was formed from **2a** after 10 min.

To confirm that the degradation of **2a** and **2b** was quantitatively correlated with 3-methylbutanal formation, the decrease in their concentrations were measured in parallel with aldehyde formation (Table 4). As expected, for both isomers a

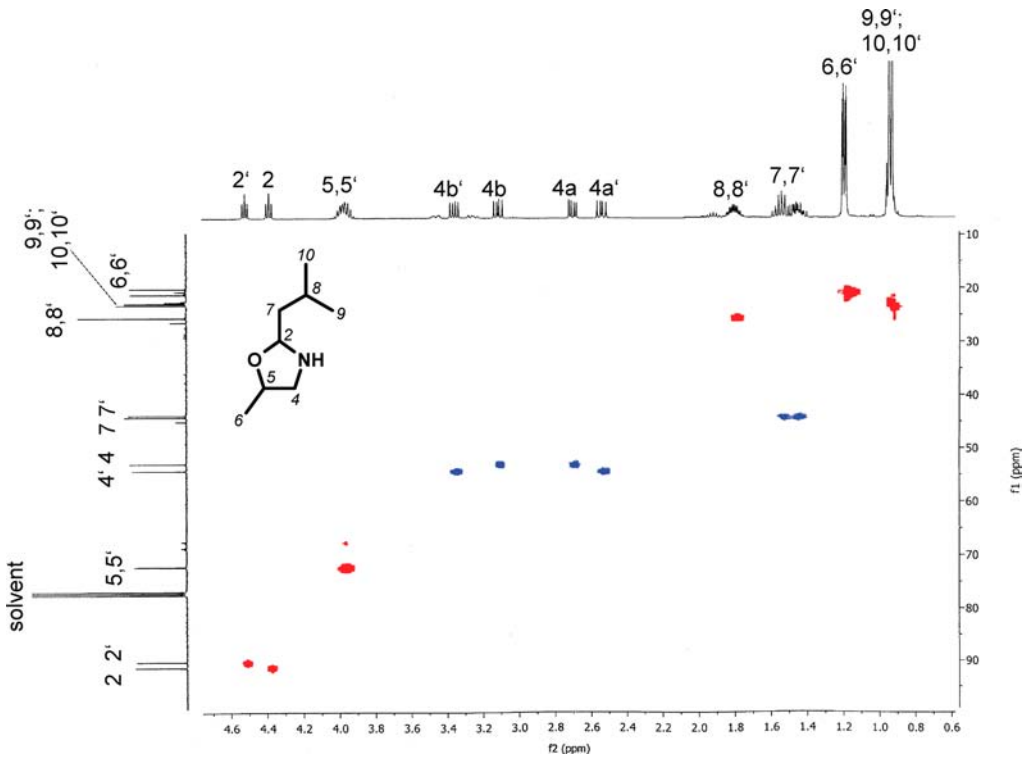

Figure 7. HSQC NMR spectrum of 2-isobutyl-5-methyl-3-oxazolidine (**1**).

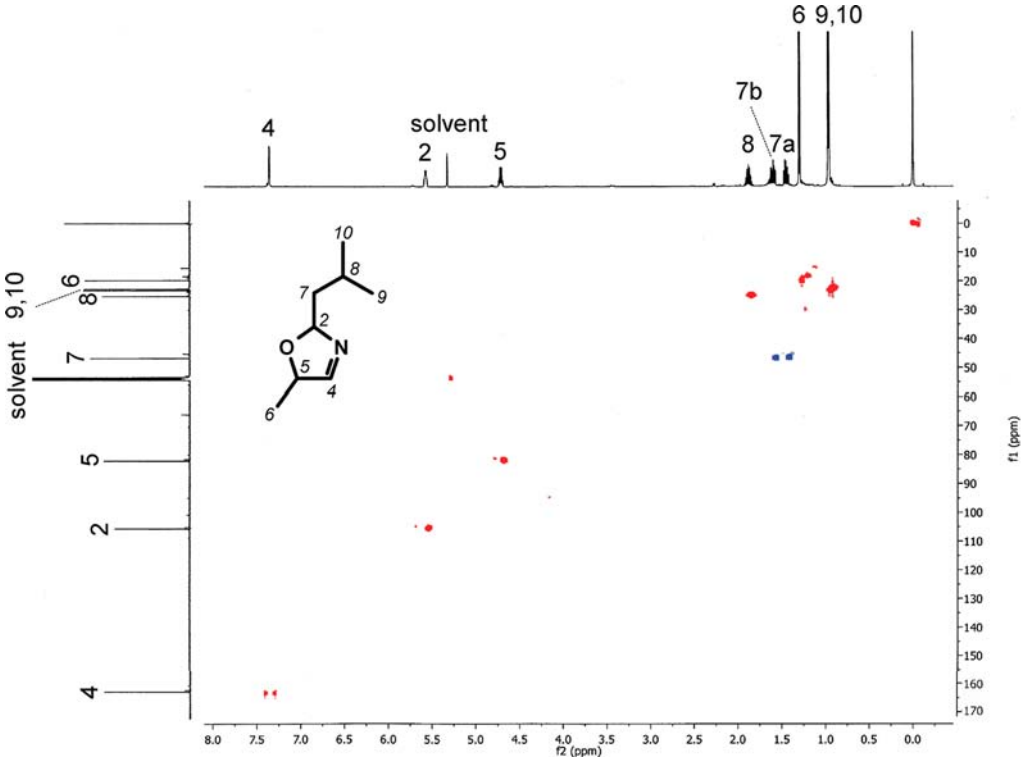

Figure 8. HSQC NMR spectrum of 2-isobutyl-5-methyl-3-oxazoline (**2a**).

quite good correlation of the time course of degradation with aldehyde formation was found (cf. Tables 4 and 2). However, interestingly, both isomers **2a** and **2b** are also converted into each other: **2b** is formed to a certain extent during the degradation of **2a** (expt A; Table 4), and **2a** is formed during the degradation of **2b** (expt B; Table 4). This result suggested the possibility of ring opening/closure during the degradation process.

In the next experiment, the formation of phenylacetaldehyde from both isomers of 2-benzyl-5-methyl-3-oxazoline (**8**; Figure 2D) was studied. After a 5 min treatment with water at 37 °C, ~17 mol % of phenylacetaldehyde was formed from both

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Table 2. Time Course of the Generation of 3-Methylbutanal from 2-Isobutyl-5-methyl-3-oxazoline (2a and 2b; Figure 2A) after the Addition of Water[a]

	3-methylbutanal (mol %)	
time (min)	isomer **2a**	isomer **2b**
control[b]	0.3	0.4
5	72.7	72.5
15	82.4	81.4
30	91.1	92.2

[a]The precursor was stirred in water at 37 °C. [b]Control experiment without addition of water.

Table 3. Influence of the pH Value on the Time Course of the Generation of 3-Methylbutanal from 2-Isobutyl-5-methyl-3-oxazoline (2a; Figure 2A)[a]

	3-methylbutanal (mol %) formed at		
time (min)	pH 5	pH 7	pH 9
control[b]	0.5	0.3	0.3
10	95.1	54.8	9.0
60	98.9	97.9	35.7

[a]The precursor was stirred in the respective buffer solution at 37 °C. [b]Control experiment without addition of the respective buffer solution.

Table 4. Degradation/Isomerization of 2-Isobutyl-5-methyl-3-oxazoline (2; Figure 2A) in Water at 37 °C[a]

	expt A		expt B	
time (min)	compd **2a** unreacted (mol %)	compd **2b** formed (mol %)	compd **2b** unreacted (mol %)	compd **2a** formed (mol %)
control[b]	98.1	1.9	98.3	1.7
5	16.2	5.1	30.2	3.5
15	8.4	10.2	16.7	3.7
30	2.9	4.3	14.4	3.8

[a]The precursor was stirred in water at 37 °C. [b]Control experiment without addition of water.

isomers (Table 5) and the yields passed 40 mol % after 30 min. Thus, also this oxazoline can be regarded as an important precursor of the Strecker aldehyde. As found for **2**, the pH had also a clear influence on the degradation rate of **8**: whereas at pH 5 after 30 min 72 mol % of **8a** and 90 mol % of **8b**, respectively, were converted into phenylacetaldehyde, the yields were much lower at pH 7 (18 mol % of **8a** and 20 mol % of **8b**) as well as at pH 9 (7 mol % of **8a** and 8 mol % of **8b**), respectively (Table 6). However, compared to **2** (cf. Tables 2 and 3), the degradation of **8** was somewhat slower.

Table 5. Generation of Phenyacetaldehyde from 2-Benzyl-5-methyl-3-oxazoline (8a and 8b; Figure 2D) after the Addition of Water[a]

	phenylacetaldehyde (mol %) generated from	
time (min)	isomer **8a**	isomer **8b**
control[b]	0.2	0.3
5	16.6	17.2
15	23.1	32.2
30	42.5	46.5

[a]The precursor was stirred in water at 37 °C. [b]Control experiment without addition of water.

Table 6. Influence of the pH Value on the Generation of Phenylacetaldehyde from 2-Benzyl-5-methyl-3-oxazoline (8a/8b; Figure 2D)[a]

	phenylacetaldehyde (mol %) generated at		
time (min)	pH 5	pH 7	pH 9
control[b]	0.4/0.5	0.3/0.4	0.3/0.4
15	41.2/63.3	7.7/9.4	4.9/5.4
30	72.1/90.3	18.0/19.5	7.1/7.6
60	87.7/93.1	30.2/23.4	14.3/10.8

[a]The precursor was stirred in the respective buffer solution at 37 °C. [b]Control experiment without addition of the respective buffer solution.

In a last series of experiments, also the degradation of **4** (Figure 2B) and **6** (Figure 2C) at 37 °C in the presence of water was studied. Due to the very similar behavior of **2a** and **2b** as well as of **8a** and **8b**, compounds **4** and **6** were not separated into the isomers for these experiments. As found for **2** and **8**, both oxazolines underwent degradation into the respective Strecker aldehydes 2-methylbutanal and methylpropanal. After the addition of water to **4**, 15 mol % of 2-methylbutanal was quantitated after 5 min, 33 mol % after 15 min, and 56 mol % after 30 min, respectively (Table 7). For compound **6**, after the addition of water, 46 mol % of methylpropanal was released after 5 min, 54 mol % after 15 min, and 56 mol % after 30 min, respectively (Table 7).

Table 7. Generation of 2-Methylbutanal from 2-*sec*-Butyl-5-methyl-3-oxazoline (4; Figure 2B) and Methylpropanal from 2-Isopropyl-5-methyl-3-oxazoline (6; Figure 2C)[a]

time (min)	2-methylbutanal (mol %) from isomers **4**	methylpropanal (mol %) from isomers **6**
control[b]	1.1	0.1
5	14.6	46.0
15	33.3	53.5
30	55.6	56.1

[a]The precursor was stirred in water at 37 °C. [b]Control experiment without addition of water.

The next aim was to get a first insight into the presence of such 3-oxazolines in real foods. On the basis of the recent results,[14] a dark chocolate was extracted with dichloromethane, and the extract was subjected to SAFE distillation.[11] The distillate was concentrated and analyzed by two-dimensional GC-MS. Using **2** as the reference, 2-isobutyl-5-methyl-3-oxazoline was identified on the basis of the same retention time as well as the same mass spectrum in the chocolate extract (Figure 9). The concentration of 2-isobutyl-5-methyl-3-oxazoline was quite low, but it has to be taken into account that various α-dicarbonyl compounds, such as glyoxal, 1-deoxyosone, 3-deoxyosone, glucosone, or 2,3-pentandione, are formed by carbohydrate degradation.[20] All of these intermediates would be able to generate the respective 3-oxazolines but having different substituents at positions 4 and 5. Because glucosone and the 3-deoxyosone are formed in the earlier stages of the carbohydrate degradation,[20] more polar 3-oxazolines with polyhydroxy functions at positions 4 and 5 may be the major precursors in chocolate or cocoa, respectively.

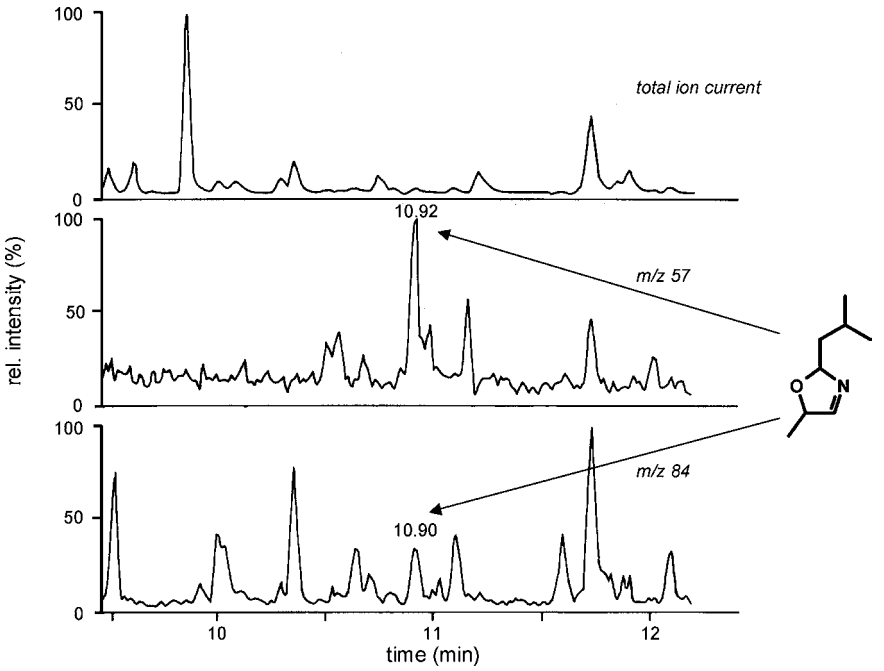

Figure 9. GC-MS chromatogram of an extract obtained from dark chocolate. Arrows indicate the presence of 2-isobutyl-5-methyl-3-oxazoline.

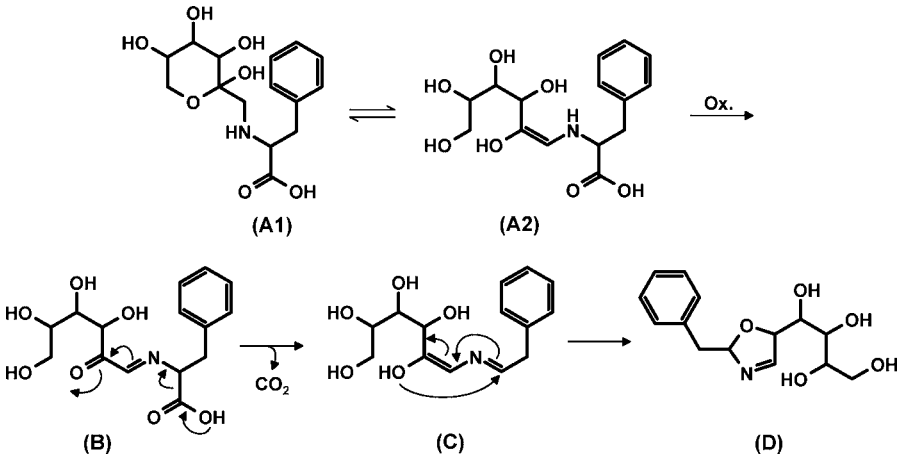

Figure 10. Generation of a 3-oxazoline by an oxidative decarboxylation of the Amadori compound of phenylalanine.

Alternatively, the oxazolines could directly be formed from the Amadori compounds as exemplified for phenylalanine degradation in Figure 10. The key step is the oxidation of the enaminol function in tautomer **A2** to initiate the decarboxylation from **B** into **C**. Cyclization of this tautomer then leads to the formation of the oxazoline (**D**).

A possible pathway for the hydrolytic cleavage of the 3-oxazolines into the respective Strecker aldehydes is suggested in Figure 11. Because the degradation was favored under slightly acidic conditions (cf. Tables 3 and 6), it can be assumed that protonation at the ring nitrogen allows a nucleophilic attack of water at ring carbon atom 4. Ring opening generates an amino acetal, followed by a substitution of the amino group by a hydroxyl group. The semiacetal formed is finally cleaved into phenylacetaldehyde and 2-hydroxypropanal.

In conclusion, 3-oxazolines are a novel class of Strecker aldehyde precursors, which are stable in dry form but easily hydrolyze and liberate the aldehydes in the presence of water. The hydrolysis may take place either by the addition of water to

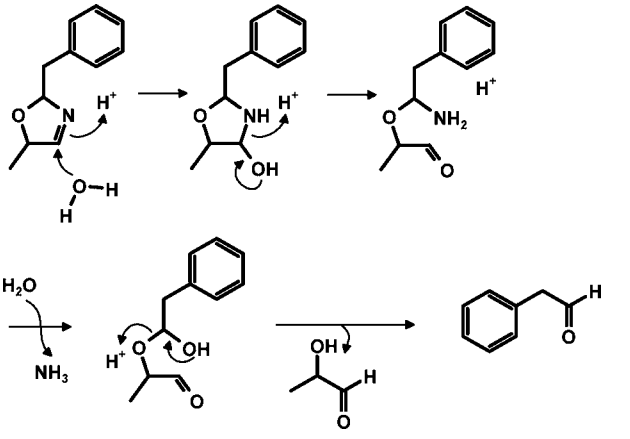

Figure 11. Hypothetical pathway leading to the formation of phenylacetaldehyde from 2-benzyl-5-methyl-3-oxazoline.

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a food product or during mastication. The hydrolysis efficacy depends on the pH value of the solution, but lower hydrolysis speed can be compensated by longer hydrolysis time, for example, during chewing.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1−S18. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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