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Savory flavors

Reaction Systems

Identification and formation of thiazolidines and thiazolines in fried chicken liver

Claus O. Schmidt, Gerhard E. Krammer, Berthold Weber, Detlef Stöckigt, Klaus Herbrand, Frank Ott, Günter Kindel, Stefan Brennecke, Ian L. Gatfield and Heinz-Jürgen Bertram, Symrise GmbH & Co. KG

Processed flavors contain a huge variety of volatile organic compounds which yield characteristic flavor types, like roasted, fried, caramel or popcorn.

During frying of meat, many different chemical reactions take place.[1] Meat is a source of fat, proteins and carbohydrates. During thermal treatment, fatty acids, amino acids, sugars and other degradation products are formed which are able to undergo further reactions. Well-known reactions include the Maillard reaction between reducing sugars and amino acids and the Strecker degradation of amino acids.[2]

In the past, to elucidate the complex Maillard reaction different model systems were analyzed with selected amino acids and carbohydrates in the presence or absence of fat.[3] Those model systems give an insight in the formation of so-called processed flavors.[4] Processed flavors contain a huge variety of volatile organic compounds which yield characteristic flavor types, like roasted, fried, caramel or popcorn. However, the transferability of model systems for real food systems has to be verified. Therefore, systems like fried meat products represent an interesting counterpart in order to study the formation of certain potent aroma compounds, e.g. thiazolines and thiazolidines. The aim of the following study was to identify thiazoline and thiazolidines in a natural product and postulate their mechanism of formation. Moreover, this study was conducted in order to verify our data from previous model studies for culinary food systems.[5]

Results and Discussion

The results of the GC/MS analysis are summarized in T-1. Sensorial evaluation of the compounds was achieved by GC/O analysis. Most of the identified compounds contribute to a typical savory flavor.

The flavor extract is dominated by degradation products of proteins, carbohydrates and fats. Maillard reaction products especially yield a variety of nitrogen- and sulfur-containing compounds.[6]

Earlier research on Maillard reaction model systems indicated that thiazolidines can be formed by reaction between different Strecker degradation products and/or fatty acid degradation products.[5] After isolation by preparative gas chromatography, we identified several thiazolines and thiazolidines that have

At a Glance

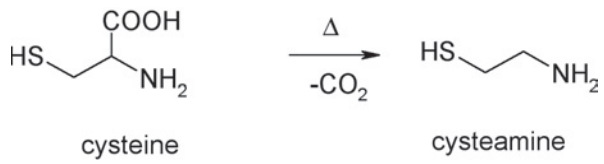
Chicken liver was fried under typical frying conditions in the kitchen. After thermal treatment, the flavor was extracted by solvent assisted flavor extraction (SAFE) and the extract was analyzed by GC/MS and GC-olfactometry (GC/O). Several compounds were isolated by preparative gas-chromatography. Structure elucidation by NMR and IR-techniques identified among others thiazolidine, 2-methyl-thiazolidine, 2-ethyl-thiazolidine, 2-pentyl-thiazolidine, 2-isobutyl-thiazolidine, 2-(1-methyl-propyl)-thiazolidine, 2-isopropyl-thiazolidine, and 2-propionyl-2-thiazoline. These compounds are known to be formed in model Maillard reaction systems. Some of those compounds were now detected for the first time in a natural food product. In order to verify the identity of the compounds, the above-mentioned compounds were synthesized and their spectroscopic and chromatographic data were compared with the isolated substances. The formation of above-mentioned compounds can be explained by the degradation of cysteine to cysteamine and subsequent other Maillard reactions under thermal treatment in the presence of a fat matrix and reducing sugars.

Volatile organic compounds of the chicken liver extract

T-1

No.	Compound	RI on DB-1	RI on DB-WAX	Flavor description
1	acetic acid ethyl ester	-	877	rum, estery, sweet
2	2-butanone	-	914	chocolate, rum, chemical
3	butanal	-	870	chocolate, green
4	2-methylbutanal	634	922	cocoa, chocolate, fruity
5	3-methylbutanal	-	924	chocolate, tomato
6	2-pentanone	645	998	sweet, lemon, fruity
7	2,3-pentadienone	655	1051	buttery, cream, fatty
8	hexanal	782	1094	leaf-like, green, aldehyde
9	2-methylpropanol	615	1081	whisky, rum, sweet
10	2-pentanol	685	1108	banana, fermented, rum
11	butanol	-	1146	fermented, candy sugar
12	1-penten-3-ol	674	1161	garlic, fatty (oily), onion
13	2-heptanone	873	1192	roquefort, cheesy, mushroom-like
14	pyridine	734	1201	fish, chemical
15	heptanal	883	1194	aldehyde, greasy
16	3-methyl-2-butenal	753	1208	cocoa, chemical, chocolate
17	2-methylbutanol	720	1205	fusel, fermented
18	3-methylbutanol	726	1201	fruity, fermented, overripe
19	2-oxo-propanol	-	1299	sweet, juicy, cream, fruity
20	2-methyl-2-thiazoline	852	1314	green, herbal, tropical fruit
21	2,5-dimethylpyrazine	892	1336	nut, peanut
22	2,6-dimethylpyrazine	891	1339	nut (hazelnut), peanut, cocoa
23	hexanol	852	1358	green, apple, cherry
24	nonanal	1085	1396	aldehydic, peely
25	trimethylpyrazine	981	1415	roasted note, cocoa, candy sugar
26	2-methyl-thiazolidine	900	1435	fatty (oily), sweet
27	2E-octenal	1038	1434	aldehyde, cucumber, chicken
28	acetic acid	-	1437	sour, fruity, fresh
29	1-octen-3-ol	966	1444	mushroom, earthy, green
30	heptanol	949	1450	fatty, coconut, waxy
31	thiazolidine	878	1490	bloody, fish, cocoa
32	2-acetylfuran	887	1502	sweet, astringent, vanilla
33	2,3-diethyl-5-methylpyrazine	1135	1497	cocoa, chocolate, pyrazine
34	benzaldehyde	934	1524	kernel, almond, marzipan
35	2-ethyl thiazolidine	997	1524	chemical
36	2-methyl thioethanol	814	1530	potato, vegetable
37	propionic acid	-	1538	fruity, blueberry
38	2-isopropyl-thiazolidine	1064	1563	cocoa, chocolate, onion
39	isobutyric acid	743	1545	fruity, butter, sweet
40	butyric acid	748	1627	cheesy, sour, butter
41	2-acetyl-thiazol	988	1651	musty, roasted note
42	2-isobutyl-thiazolidine	1147	1648	sweet, cocoa, chocolate
43	2-hydroxymethylfuran	858	1648	mushroom, phenolic, tomato
44	2-(1-methyl-propyl)-thiazoline	1162	1659	peanut, cocoa, chocolate
45	3-methylbutyric acid	806	1686	isovaleric acid, blueberry
46	2-methylbutyric acid	826	1666	fruity, strawberry, apple
47	3-methylthiopropanol	950	1715	potato, vegetable, green
48	2-acetyl-2-thiazoline	1068	1759	pandan, crust
49	2E,3Z-decadienal	1271	1770	aldehyde, chicken, fatty
50	2-propionyl-2-thiazoline	1170	1838	roasty, sweet
51	2-pentyl-thiazolidine	1304	1838	nut (hazelnut), fatty (oily)
52	hexanoic acid	956	1844	lamb/mutton, overripe, animalic
53	2-ethyl-hexanoic acid	1112	1948	watery, juicy, cheesy
54	2-acetyl-pyrrole	1029	1959	chocolate, butter
55	2-formyl-pyrrole	983	2013	metallic, juicy, fruity

Formation of cysteamine in fried chicken liver **F-1**



so far never been found in a food product, i.e. in a natural product. Most likely, the thiazolidines were formed from the precursor cysteamine, which undergoes a reaction with degradation products from fatty acids or amino acids.[7] F-1 explains the formation of cysteamine from the amino acid cysteine via decarboxylation.

Alternatively, the formation of cysteamine can be explained by degradation of coenzyme A.[8] However, the concentration of coenzyme A in chicken liver compared to the concentration of cysteine is relatively low. Therefore, it can be assumed, that most cysteamine is formed from cysteine as a precursor.

Amino acids can form aldehydes via Strecker degradation under thermal treatment in the presence of reducing sugars. These aldehydes can react with cysteamine in order to form thiazolidines.[9] F-2 explains the formation of 2-isopropyl-thiazolidine (I), 2-isobutyl-thiazolidine (II) and 2-(1-methyl-propyl)-thiazolidine (III) in fried chicken liver from cyteamine and Strecker degration products as precursors.

During the frying process of chicken liver, oxidation of unsaturated fatty acids also occurs. The products of this oxidation process are aldehydes, which can react with cysteamine to form thiazolidines like 2-pentyl-thiazolidine (IV) (see F-3).

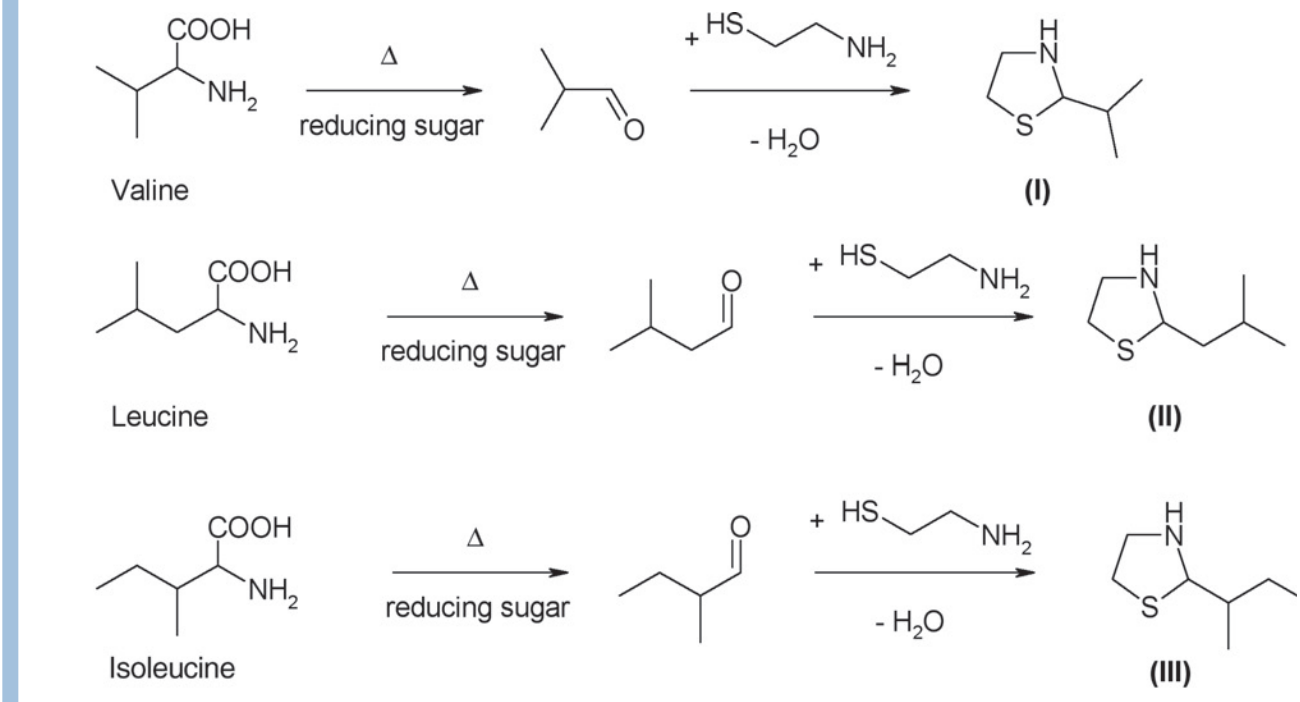
Propanal is a common degradation product either from fatty acids or from carbohydrates, and it can react with cysteamine to form 2-ethyl-thiazolidine (V) (see F-4).

The degradation of carbohydrates during frying will result in oxidation, dehydration and decarboxylation processes.[10] The fragments of the carbohydrates can undergo further reactions leading to α-carbonyl-aldehydes. 2-Propionyl-2-thiazolidine can be formed by a reaction of cysteamine with different carbohydrate degradation products. Afterwards, oxidation yields 2-propionyl-2-thiazoline (VI) as shown in F-5.[9]

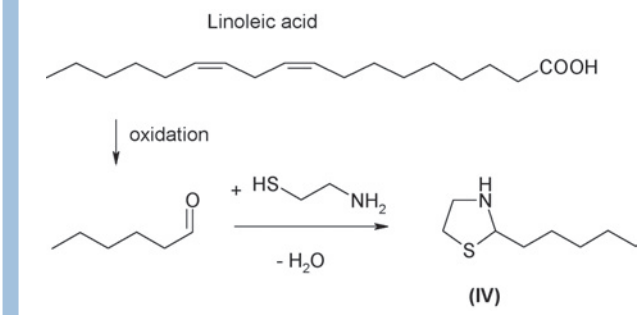
Obviously, the formation of 2-acetyl-2-thiazoline might be easier, because cysteamine can react with 2-oxo-propanal, which is directly derived from fructose (see F-6).[9]

This formation pathway is in accordance with our findings, because 2-acetyl-2-thiazoline is obtained in higher concentration than 2-propionyl-2-thiazoline. Aroma relevant amounts of 2-acetyl-2-thiazoline in wine and from cysteine/methylglyoxal solution were reported by de Revel et al.[11]

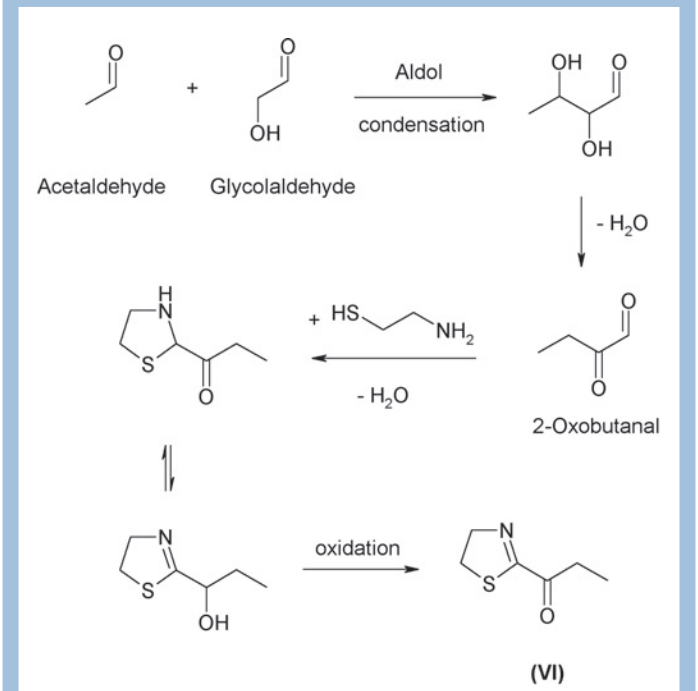
Formation of 2-isopropyl-thiazolidine (I), 2-isobutyl-thiazolidine (II) and 2-(1-methyl-propyl)-thiazolidine (III) after thermal treatment **F-2**



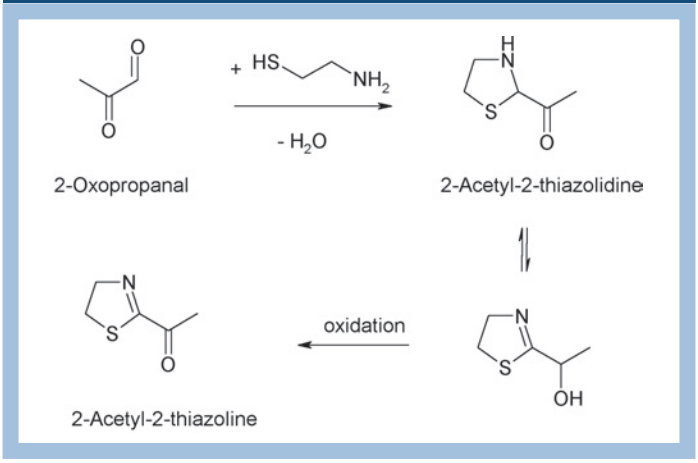
Formation of 2-pentyl-thiazolidine (IV) **F-3**



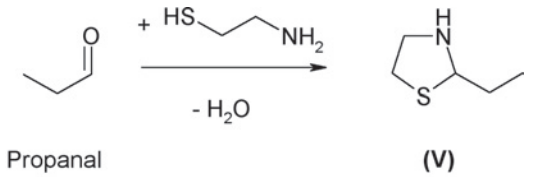
Formation of 2-propionyl-thiazoline (VI) **F-5**



Formation of 2-acetyl-thiazoline **F-6**



Formation of 2-ethyl-thiazolidine (V) **F-4**



Conclusions

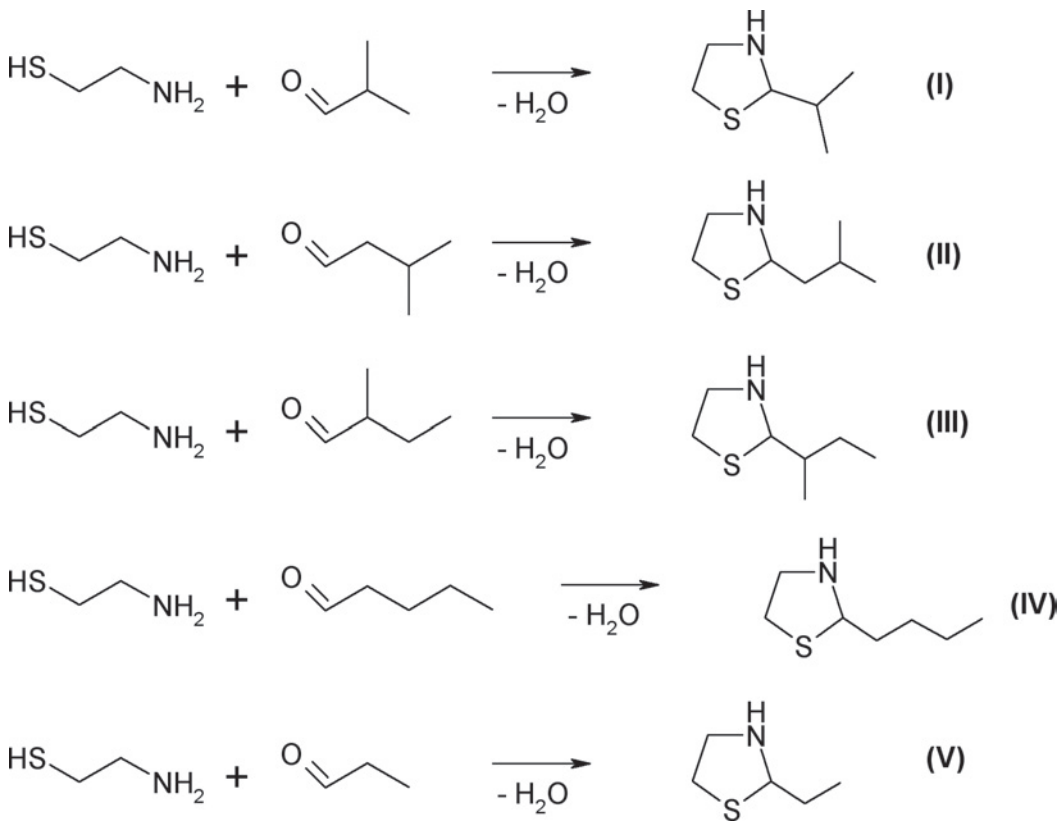
The results indicate that during frying of chicken liver a variety of compounds are formed that contribute to the overall roasty flavor of fried liver. Different classes of molecules are formed during the heating process and several reaction types can be observed, such as dehydration, decarboxylation, aldol condensation, oxidation, Strecker degradation and Maillard reaction. Mostly acids, ketones, aldehydes, alcohols, pyridines, thiazolidines, thiazolines, furans and pyrrols were identified.

In order to evaluate the most important contributors for the typical roast flavor, the extract was analyzed by trained flavorists using GC-olfactometry. The chemical structure of most compounds was identified by comparing their mass spectra with MS libraries. Those compounds that could not be identified were isolated via preparative gas chromatography followed by structure elucidation using NMR and IR. Proposed structures were verified by synthesizing the compounds and comparing their spectroscopic and chromatographic data.

The identified compounds are already known as products from Maillard reaction model systems. The relevance of those model systems can now be established, because the proposed reaction products have been identified in fried chicken liver. [5] The reaction mechanism for the formation of thiazolidines involves a condensation reaction of cysteamine with aldehydes. The reaction mechanism for thiazolines also starts with cysteamine and aldehydes as precursors, but an additional oxidation yields the thiazoline.

In our work, we identified one thiazoline and five thiazolidines

Synthesis of thiazolidines **F-7**



for the first time in a natural food product. While 2-ethyl-thiazolidine only has a chemical flavor, 2-isopropyl-thiazolidine, 2-isobutyl-thiazolidine, 2-(1-methyl-propyl)-thiazolidine, 2-pentyl-thiazolidine and 2-propionyl-2-thiazoline yield a more nut-like and cocoa-like flavor, which cover a broad range of sensorial profiles.

Experimental Procedures

Samples: Raw chicken liver was purchased at a local market in Holzminden, Germany. Some 500 g of the liver were fried for 2 min in a Teflon pan with a thin layer of Palmin fat. Afterwards, the fried liver was ground in a "Grindomix" and extracted with 1 L diethylether/pentane 1:1 for 24 h. The extraction was repeated with 500 mL diethylether/pentane 1:1 for 4 h. The volatile compounds of combined organic extracts were separated from the fat by a solvent assisted flavor extraction (SAFE) for 2 h at approximately 10^{-5}mbar. The organic solvent was removed by distillation at 45°C. The concentrated extract was used for further analysis.

GC/MS: Finnigan MAT8200 MS coupled with a Carlo Erba 5360 Mega Series GC, using a 60 m DB-WAX column (0.25 µm, 0.32 mm).

NMR: Varian Unity Inova (400 Mhz) or Varian Gemini 2000 (200 MHz).

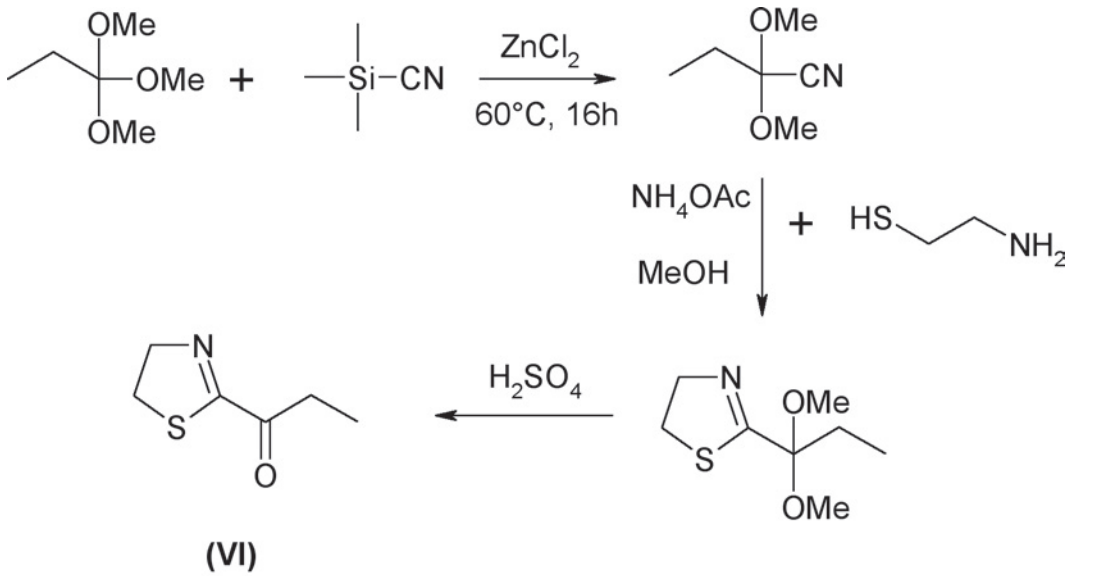
Synthesis: All thiazolidines were prepared by reaction of cysteamine hydrochloride with corresponding aldehydes as described before[12] (see F-7). 2-Propionyl-thiazoline was synthesized via acylcyanide as shown in F-8.[13,14]

Spectroscopic Data

2-isopropyl-thiazolidine (I): ^{1}H-NMR ($CDCl_3$, 200 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity; hydrogen atom at relevant carbon]: 1.05, 1.09 (2 d, J = 6.7, 6.7, 6H, C-2', C-3'), 1.90 (dqq, J = 6.7, 6.7, 7.5, 1H, C-1'), 2.70-3.06 (m, 3H, C-4, C-5), 3.48-3.64 (m, 1H, C-4), 4.29 (d, J = 7.5, 1H, C-2). ^{13}C-NMR, δ (ppm of relevant carbon): 20.5, 20.8 (2 CH_3, C-2', C-3'), 34.0 (CH, C-1'), 34.8 (CH_2, C-5), 52.5 (CH_2, C-4), 79.0 (CH, C-2). MS (70 eV): 131 (7.1 percent, MM); 90 (4.7); 88 (100); 84 (5.8); 70 (9.9); 61 (11.3); 55 (8.5); 44 (8.7); 41 (5.8); 28 (8.5).

2-isobutyl-thiazolidine (II): ^{1}H-NMR ($CDCl_3$, 200 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity; hydrogen atom at relevant carbon]: 0.94, 0.96 (2 d, J = 6.4, 6.3, 6H, C-3', C-4'), 1.52-1.90 (m, 3H, C-1', C-2'), 2.76-3.08 (m, 3H, C-4, C-5),

Synthesis of 2-propionyl-thiazoline (VI) **F-8**



(VI)

3.46-3.64 (m, 1H, C-4), 4.52 (dd, J = 5.8, 7.7, 1H, C-2). ^{13}C-NMR, δ (ppm of relevant carbon): 22.3, 23.0 (2 CH_3, C-3‘, C-4‘), 27.3 (CH, C-2‘), 35.0 (CH_2, C-5), 45.7 (CH_2, C-1‘), 52.5 (CH_2, C-4), 79.0 (CH, C-2). MS (70 eV): 145 (15.5 percent, MM); 130 (8.1); 98 (8.9); 88 (100); 70 (7.7); 56 (20.3); 44 (11.8); 43 (9.9); 42 (10.0); 41 (9.7).

2-(1-methyl-propyl)-thiazolidine (III) (mixture of two diastereomers): ^{1}H-NMR ($CDCl_3$, 200 MHz), δ in ppm [multiplicity,

coupling constant (Hz), intensity; hydrogen atom at relevant carbon]: 0.94 (t, J =7.4, 3H, C-3‘), 1.01/1.08 (d, J = 6.8/6.8, 1H, C-4‘), 1.15-1.88 (m, 3H, C-1‘, C-2‘), 2.66-3.04 (m, 3H, C-4, C-5), 3.52-3.65 (m, 1H, C-4), 4.34/4.41 (d, J = 7.4/6.8, 1H, C-2). ^{13}C-NMR, δ (ppm of relevant carbon): 11.3/11.4 (CH_3, C-3‘), 16.4/16.6 (CH_3, C-4‘), 27.7/28.0 (CH_2, C-2‘), 34.5/34.7 (CH_2, C-5), 39.9/40.4 (CH, C-1‘), 52.5/52.5 (CH_2, C-5), 77.8/77.8 (CH, C-2). MS (70 eV): 145 (5.9 percent, MM); 90 (5.0); 88 (100); 84 (4.0); 70 (9.4); 61 (9.2); 44 (6.8); 42 (4.8); 41 (8.5); 30 (4.6).

2-pentyl-thiazolidine (IV): ^{1}H-NMR ($CDCl_3$, 200 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity; hydrogen atom at relevant carbon]: 0.89 (t, J = 7.0, 3H, C-5‘), 1.20-1.58 (m, 6H, C-2‘, C-3‘, C-4‘), 1.60-2.04 (m, 2H, C-1‘), 2.77-3.04 (m, 3H, C-4, C-5), 3.42-3.60 (m, 1H, C-4), 4.46 (dd, J = 7.1, 5.7, 1H, C-2). ^{13}C-NMR, δ (ppm of relevant carbon): 14.0 (CH_3, C-5‘), 22.5 (CH_2, C-4‘), 27.7 (CH_2, C-2‘), 31.7 (CH_2, C-3‘), 35.0 (CH_2, C-5), 36.7 (CH_2, C-1‘), 52.2 (CH_2, C-4), 73.5 (CH, C-2). MS (70 eV): 159 (10.9 percent, MM); 116 (7.3); 112 (17.1); 88 (100); 70 (8.2); 61 (8.1); 56 (21.7); 44 (8.1); 41 (8.3); 30 (7.3).

2-ethyl-thiazolidine (V): ^{1}H-NMR ($CDCl_3$, 200 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity; hydrogen atom at relevant carbon]: 1.06 (t, J = 7.4, 3H, C-2‘), 1.72, 1.95 (2 m, 2H, C-1‘), 2.77-3.06 (m, 3H, C-4, C-5), 3.42-3.60 (m, 1H, C-4), 4.43 (dd, J = 7.3, 5.5, 1H, C-2). ^{13}C-NMR, δ (ppm of relevant carbon): 12.2 (CH_3, C-2‘), 29.6 (CH_2, C-1‘), 35.0 (CH_2, C-5), 52.2 (CH_2, C-4), 73.5 (CH, C-2). MS (70 eV): 117 (26.4 percent, MM); 88 (100); 71 (13.9); 70 (28.6); 61 (13.6); 58 (11.2); 56 (18.3); 44 (9.8); 41 (12.3); 28 (13.7).

2-propionyl-2-thiazoline (VI): ^{1}H-NMR ($CDCl_3$, 200 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity; hydrogen atom at relevant carbon]: 1.14 (t, J = 7.3, 3H, C-3‘), 2.95 (q, J = 7.3, 2H, C-2‘), 3.33 (t, J = 8.8, 2H, C-5), 4.52 (t, J = 8.8, 2H, C-4). ^{13}C-NMR, δ (ppm of relevant carbon): 7.6 (CH_3, C-3‘), 32.1, 32.5 (2 CH_2, C-2‘, C-5), 66.1 (CH_2, C-4), 170.4 (C_q, C-2), 196.2 (C_q, C-1‘). MS (70 eV): 143 (41.7 percent, MM); 115 (43.4); 88 (6.6); 87 (17.4); 60 (29.7); 59 (17.1); 57 (100); 45 (8.5); 29 (52.1); 27 (20.5).

Acknowledgements

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Address correspondence to Claus O. Schmidt, Symrise GmbH & Co. KG, Mühlenfeldstr. 1, D-37603 Holzminden, Germany; claus.oliver.schmidt@symrise.com.

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