

Determination of the Chemical Structure of the Intense Roasty, Popcorn-like Odorant 5-Acetyl-2,3-dihydro-1,4-thiazine

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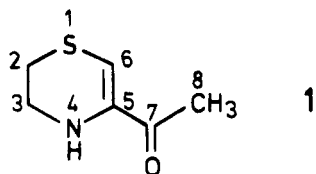
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The chemical structure of a novel intense popcorn-like odorant isolated from the reaction of cysteine and ribose was studied by one- and two-dimensional NMR spectroscopy and high-resolution mass spectrometry, and the structure elucidation was confirmed by synthetic experiments. Thus, the odorant was identified as 5-acetyl-2,3-dihydro-1,4-thiazine (ADT). A sensory study including 5-propionyl- (PDT) and 5-butanoyl-2,3-dihydro-1,4-thiazine (BDT) revealed that also PDT and BDT elicited roasty, popcorn-like odors. However, the odor threshold of the BDT was by a factor of 50 000 higher than those of ADT and PDT (0.06 and 0.1 ng/L of air, respectively).

Keywords: 5-Acetyl-2,3-dihydro-1,4-thiazine; 5-propionyl-2,3-dihydro-1,4-thiazine; 5-butanoyl-2,3-dihydro-1,4-thiazine; odor threshold; synthesis; cysteamine; 2,3-butanedione

INTRODUCTION

The most intense odorants in a thermally treated aqueous ribose/cysteine solution have recently been evaluated (Hofmann and Schieberle, 1995), and it has been shown that 5-acetyl-2,3-dihydro-1,4-thiazine (**1**)



significantly contributed to the overall flavor of the model mixture. The compound elicits an intense roasty, popcorn-like odor at the low odor thresholds of 0.06 ng/L of air and 0.6 µg/L of water (retronasally), respectively.

Since the odorant to the best of our knowledge has not yet been reported in the literature, the details on the identification of **1** are given in the present paper.

EXPERIMENTAL PROCEDURES

Isolation of **1 from the Ribose/Cysteine Mixture.** A mixture of D-ribose (250 mmol; Sigma, Munich, Germany) and L-cysteine (82.5 mmol; Sigma) was reacted in phosphate buffer (2.5 L, 0.5 mol/L, pH 5.0) as recently described (Hofmann and Schieberle, 1995). The mixture was extracted with diethyl ether (total volume 3 L), and the extract was concentrated to 100 mL by distilling off the solvent on a Vigreux column (60 cm × 1 cm). The extract was then treated with an aqueous sodium bicarbonate solution (0.5 mol/L, total volume 100 mL) to remove acidic compounds. To isolate **1** and further basic reaction products, the ethereal layer was extracted with hydrochloric acid (1 mol/L, total volume 100 mL), and the organic layer containing the neutral volatiles was discarded. After the

aqueous phase pH was adjusted to 10.0 (sodium hydroxide, 2 mol/L), the basic compounds were re-extracted into diethyl ether (total volume 150 mL). After drying over Na₂SO₄, the ethereal extract was concentrated to 4 mL on a Vigreux column and the target compound isolated by flash chromatography using a glass column (15 × 1.9 cm; J. T. Baker BV, Deventer, The Netherlands, no. 7022-01) filled with a slurry of Bakerbond-Diol (J. T. Baker) in *n*-pentane. After flushing with pentane (150 mL) followed by pentane/diethyl ether (95 + 5 by vol; 150 mL), the title compound was eluted from the stationary phase with *n*-pentane/diethyl ether (9 + 1 by vol; 150 mL). The flow rate was 6 mL/min.

Preparative Gas Chromatography. For spectral measurements, **1** was purified by preparative GC (GC type 5700 A; Hewlett-Packard) on a stainless steel column (3 m × 2.4 mm) packed with SE-30 (10% w/w) on Chromosorb W-AWD-MCS (80–100 mesh) followed by rechromatography on an FFAP column of the same size. Helium was used as the carrier gas (30 mL/min). Aliquots of the extract (100 µL) were repeatedly injected, and the effluent in the retention index region of 1320–1380 (FFAP) was condensed into a self-constructed helical glass trap (30 cm × 2 mm i.d.) via a 13:1 effluent splitter. During the condensation, the traps were maintained at –50 °C. After every four runs, the material was flushed with 200 µL of either CDCl₃ or benzene-*d*₆ into an NMR tube. To avoid condensation of water, the traps were connected to CaCl₂ tubes between the GC runs.

NMR Spectroscopy. NMR spectra were recorded with an AM 360 (¹H; ¹³C; ¹³C-DEPT) and an AMX 500 (C,H correlation experiments: HMQC; HMBC) (Bruker, Karlsruhe, Germany) at 298 K in a Wilmad 535-PP tube. ¹H NMR: transmitter frequency, 360.13 MHz; 30° pulse, 3 µs; repetition time, 3.2 s; spectral width, 7.2 kHz; 32K data set; for resolution enhancement, the FID was multiplied with a Lorentz Gauss function prior to Fourier transformation; δ relative to CDCl₃ (7.24 ppm) or benzene-*d*₆ (7.15 ppm). ¹³C NMR: transmitter frequency, 90.56 MHz; 30° pulse, 2 µs; repetition time, 2.5 s; spectral width, 21.7 kHz; 64K data set; ¹H composite pulse decoupling (WALTZ-16); 1-Hz line broadening; δ relative to CDCl₃ (77.0 ppm); for spectral editing of the ¹³C signals, the 135° DEPT experiments were used. ¹H-detected heteronuclear multiple bond correlation: the pulse sequence described by Bax et al. (1986), with an additional bilinear (BIRD) pulse to suppress protons which are not coupled to ¹³C, was used; the experiment with magnetization transfer was optimized for coupling constant 7.14 Hz, giving a delay of 70 ms; the heteronuclear multiple bond correlation was started 400 ms after the BIRD pulse; 256 scans preceded by 4 dummy scans were recorded

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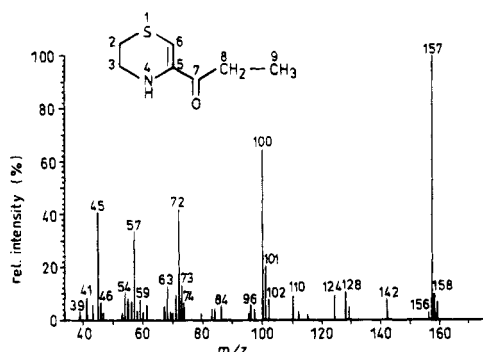
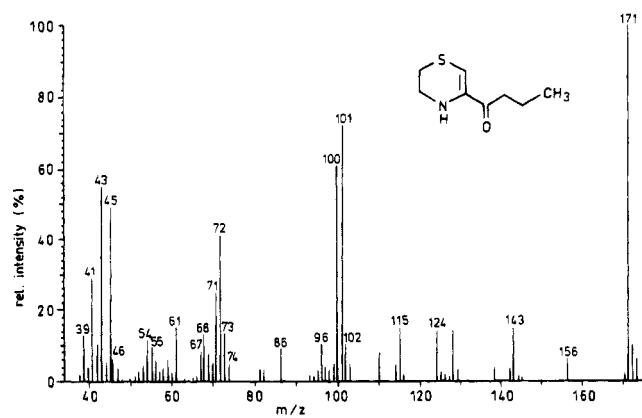
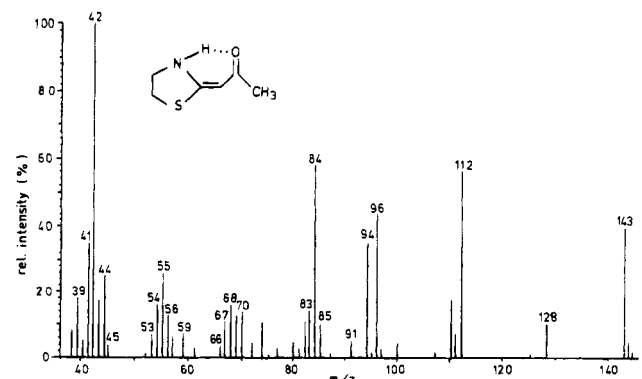
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Table 1. Key Ions and Sum Formulas Obtained by High-Resolution Mass Spectrometry of 1^a

ion <i>m/z</i>	intensity (%)	sum formula
143	100	C ₆ H ₉ NOS
128	15	C ₅ H ₈ NOS (M ⁺ - CH ₃)
100	30	C ₄ H ₆ SN (M ⁺ - CH ₃ CO)
72	22	C ₂ H ₂ SN (M ⁺ - CH ₃ CO and C ₂ H ₂)
43	60	C ₂ H ₃ O

^a Analysis was performed with a 8230 mass spectrometer (Finnigan, Bremen, Germany) running in the electron impact mode by using perfluorokerosine as the reference.

**Figure 1.** Mass spectrum (MS/EI) of 5-propionyl-2,3-dihydro-1,4-thiazine.**Figure 2.** Mass spectrum (MS/EI) of 5-butanoyl-2,3-dihydro-1,4-thiazine.**Figure 3.** Mass spectrum (MS/EI) of 2-acetyliden-1,3-thiazolidine.

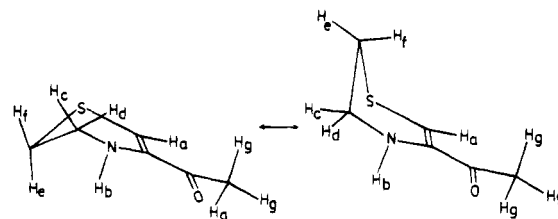
for 100 *t*₁ values and zero-filled to 256; phase sensitive acquisition using TPPI, no zero-filling in *t*₂, squared cosine bell window function in both dimensions, and magnitude calculation only in F2 dimension was used; the sample was not rotated to suppress *t*₁ noise.

Synthesis. 5-Acetyl-2,3-dihydro-1,4-thiazine. Cysteamine (2 mmol) and 2,3-butanedione (2 mmol) were dissolved in phosphate buffer (100 mL; 0.5 mol/L; pH 6.0) and heated for

Table 2. Assignment of ¹H-NMR Signals (CDCl₃) in Connection with the Structure Proposed for 1

H at	δ	multiplicity	<i>J</i> (Hz)
C8	2.28	s; 3H	
C2	3.01	m; 2H	6.6; 2.9; -12 gem ^a ; 0.8 ₆₋₂
C3	3.51	m; 2H	6.6; 2.9; -12 gem ^a
N	4.62	m; 1H	
C6	6.19	t; 1H	0.8 ₆₋₂

^a Geminal coupling constants were calculated from a computer simulated AA'XX' spin system.

**Figure 4.** Energy-minimized conformations calculated for 1 by a computer simulation.

20 min in a laboratory autoclave at 145 °C. After cooling, the solution was extracted with diethyl ether (total volume 125 mL). The basic reaction products were then isolated by treatment of the ethereal extract with hydrochloric acid (0.1 mol/L; total volume 100 mL), subsequently liberated from their salts by adjusting the aqueous phase to pH 10 (sodium hydroxide; 1 mol/L), and re-extracted into diethyl ether (total volume 150 mL). After drying over Na₂SO₄, the solution was concentrated to about 1 mL by distilling off the solvent on a Vigreux column and purified by preparative GLC as described above. Mass spectral data (MS/EI; cf. Table 1) were in agreement with those reported earlier for 1 isolated from the ribose/cysteine mixture (Hofmann and Schieberle, 1995).

5-Propionyl-2,3-dihydro-1,4-thiazine. The compound was prepared from 2,3-pentandione and cysteamine as described above for the homologous acetyl compound. The target compound was purified for NMR measurements by column chromatography and preparative GC as described above for the isolation of the acetyl homologue.

¹H-NMR signals in benzene-*d*₆ (360 MHz; assignment of the carbon atoms refers to Figure 1): δ 0.78 (t; 3H; *J*₉₋₈ = 7.08 Hz; C-9), 2.21 (q; 2H; *J*₈₋₉ = 7.08; C-8), 2.32 (m; 2H; C-2), 2.75 (m; 2H; C-3), 4.48 (m; 1H; N-4), 5.65 (t; 1H; *J*₆₋₂ = 0.8 Hz; C-6). ¹³C-NMR signals in benzene-*d*₆ (360 MHz, assignment of the carbon atoms refers to Figure 1): δ 24.2 (C-9), 26.9 (C-2), 30.1 (C-8), 42.3 (C-3), 106.5 (C-6). The signals of the two quaternary carbon atoms (C-5; C-7) were too weak for an unequivocal interpretation.

A comparison of the NMR data with those obtained for the acetyl homologue in combination with the mass spectrum confirmed the structure displayed for 5-propionyl-2,3-dihydro-1,4-thiazine in Figure 1.

5-Butanoyl-2,3-dihydro-1,4-thiazine. The compound was prepared from 2,3-hexandione and cysteamine as described above for the homologous acetyl compound. The mass spectrum (MS/EI) is displayed in Figure 2.

2-Acetyliden-1,3-thiazolidine. Cysteamine (2 mmol) and *N*-(acetoacetoxy)-succinimide (2 mmol; Fa. Aldrich, Steinheim, Germany) in methanol/triethylamine (4 + 1; by weight) were reacted for 6 h at 70 °C in an argon atmosphere. After cooling, the pH was adjusted to 7.0 (NaHCO₃) and the triethylamine was carefully distilled off under vacuum. After the addition of water (10 mL), the thiazolidine was isolated by preparative GC, extracted with diethyl ether, and characterized by mass spectrometry (Figure 3).

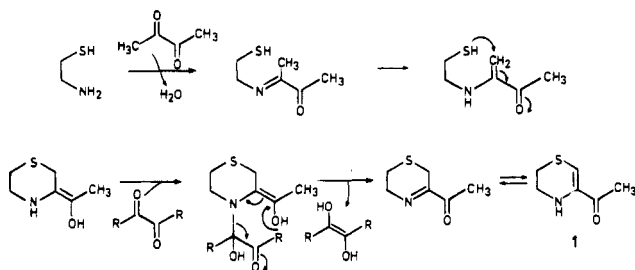
RESULTS AND DISCUSSION

High-resolution mass spectrometry of 1 revealed a sum formula of C₆H₉NOS in the molecular ion (*m/z* 143; Table 1) which had been established by MS-Cl. From

Table 3. Assignment of ^{13}C -NMR Signals (CDCl_3) of 5-Acetyl-2,3-dihydro-1,4-thiazine (1)^a

C atom	δ	DEPT analysis	heteronuclear $^1\text{H}, ^{13}\text{C}$ multiple-quantum coherence	
			via $^1J(\text{C}, \text{H})$ Hz	via $^{2,3,4}J(\text{C}, \text{H})$
2	26.6	CH_2	$2\text{H}-\text{C}2; J = 141.0$	$^2J(2\text{H}-\text{C}3); ^3J(\text{H}-\text{C}6)$
3	40.9	CH_2	$2\text{H}-\text{C}3; J = 145.0$	$^2J(2\text{H}-\text{C}2)$
5	137.6	C		$^2J(\text{H}-\text{C}6); ^3J(2\text{H}-\text{C}3); ^3J(3\text{H}-\text{C}8)$
6	106.5	CH	$\text{H}-\text{C}6; J = 180.0$	$^3J(2\text{H}-\text{C}2); ^4J(3\text{H}-\text{C}8)$
7	190.0	C		$^2J(3\text{H}-\text{C}8); ^3J(\text{H}-\text{C}6)$
8	23.7	CH_3	$3\text{H}-\text{C}8; J = 128.5$	

^a Observed heteronuclear $^1\text{H}, ^{13}\text{C}$ connectivities in 1 by DEPT analysis and inverse $^1\text{H}, ^{13}\text{C}$ multiple-quantum coherence experiments.

Scheme 1. Reaction Pathway Leading from Cysteamine and 2,3-Butanedione to 5-Acetyl-2,3-dihydro-1,4-thiazine**Table 4. Odor Thresholds of Homologous 5-Alkanoyl-2,3-dihydro-1,4-thiazines**

compound	odor threshold ^a (ng/L air)	odor quality
5-acetyl-2,3-dihydro-1,4-thiazine	0.06	roasty, popcorn-like
5-propionyl-2,3-dihydro-1,4-thiazine	0.1	roasty, popcorn-like
5-butanoyl-2,3-dihydro-1,4-thiazine	>2000	roasty, sweet

^a Odor thresholds were approximated by an olfactometric method (Ullrich and Grosch, 1987) using (*E*)-2-decenal (odor threshold = 2.7 ng/L of air) as the reference. Mean values of triplicates are reported.

the ratio of carbon to hydrogen atoms three double-bond equivalents were calculated. Because (i) a ring structure was probable due to the intense molecular ion (100%; Table 1) and (ii) the presence of an acetyl group was indicated by the fragments m/z 43 and 100 (m/z 143 – 43), the molecule was assumed to contain either a thiazine or a thiazoline ring system.

The results of the NMR experiments, which are discussed in detail below, and the mass spectral data are consistent with the structure for 1 proposed above.

In the ^1H -NMR spectrum five signals were detectable, which are assigned in connection with the structure of

5-acetyl-2,3-dihydro-1,4-thiazine (1) in Table 2. The singlet of 3 H atoms at δ 2.28 confirmed the presence of a methyl group neighboring the carbonyl group. Furthermore, the broad resonance at δ 4.62, which disappeared on exchange with D_2O , is in agreement with a secondary amine structure.

Compared with data published for the similar structure 2-acetyl-1,4,5,6-tetrahydropyridine (Büchi and Wüst, 1971), the protons of the corresponding methylene groups in 1 (δ 3.01 and 3.51) appear at a much lower field, corroborating that both groups are located between the nitrogen and the sulfur atom.

A molecular dynamics simulation suggested the existence of two energy minima characterized by the conformations shown in Figure 4. The presence of both conformers was indicated by a complex but almost identical signal pattern obtained for the protons at carbons 2 and 3 appearing with two coupling constants of 6.6 and 2.9 Hz for *ce/df* and *cf/de* (Figure 4). Due to an additional long-range coupling with the olefinic proton at carbon 6, the signal of the methylene protons at C2 (δ 3.01) showed an additional fine splitting with a coupling constant of 0.8 Hz.

Usually, an olefinic proton in the β -position of an enamine appears in the δ region of 3.5–5.0 due to a shielding effect of the nitrogen atom. The comparatively high δ value found for the olefinic proton in 1 (δ 6.19, Table 2) is induced by the deshielding effect of the sulfur in the α -position and the conjugated carbonyl in the β -position and is a further proof for the suggested structure of 1.

In Table 3 the results of $^1\text{H}, ^{13}\text{C}$ correlation experiments are summarized. Connectivities, especially for the two quaternary carbon signals at 137.6 and 190.0, were established by heteronuclear multiquantum bond correlation (HMBC) experiments, optimized for $^2J(\text{C}, \text{H})$ to $^4J(\text{C}, \text{H})$ coupling constants. The data obtained (Table 3) were also well in line with the thiazine structure of 1.

The possible presence of the isomeric 6-acetyl-2,3-dihydro-1,4-thiazine can be ruled out for the following reasons: Due to a higher deshielding effect of the nitrogen compared with the sulfur atom, carbon 3 has to be attributed to the higher δ value of the two methylene carbons (δ 40.9; Table 3). In the possible 6-acetyl derivative, carbon 3 would, therefore, show coupling with the olefinic proton and, additionally, the tertiary carbon would couple with the hydrogen atoms at carbon 2. However, this suggestion is not in line with the experimental data. Furthermore, there was also no indication for the presence of the tautomeric 5-acetyl-2,3,6-trihydro-1,4-thiazine.

A further possible structure, 2-acetonylidene-1,3-thiazolidine, was ruled out by synthesis (cf. Figure 3).

Table 5. Nitrogen Compounds Exhibiting Roasty, Popcorn-like Odors

odorant	odor threshold ^a (ng/L of air)	established as key contributor to the odor of
2-acetyl-1-pyrroline	0.02	rice, ^b popcorn, ^c wheat bread crust, ^d proline/carbohydrates ^e
2-propionyl-1-pyrroline	0.02	popcorn ^c
2-acetyl-2-thiazoline	0.05	roast beef ^f
2-acetyltetrahydropyridine	0.06	bread crust, ^d popcorn, ^c proline/carbohydrates ^e
5-acetyl-2,3-dihydro-1,4-thiazine	0.06	
5-propionyl-2,3-dihydro-1,4-thiazine	0.1	
acetylpyrazine	0.4	popcorn, ^c rye bread crust ^d
2-acetylthiazole	4	beef broth ^g

^a Cf. footnote a, Table 4. ^b Buttery and Ling (1982). ^c Schieberle (1991). ^d Schieberle and Grosch (1987). ^e Schieberle (1990); Roberts and Acree (1994). ^f Cerny and Grosch (1992). ^g Gasser and Grosch (1990).

On the basis of these results, **1** was identified as 5-acetyl-2,3-dihydro-1,4-thiazine.

Synthetic Experiments. **1** was prepared synthetically in relatively low yields (1 mol %) by a reaction of cysteamine with 2,3-butanedione. It may be assumed that 5-acetyl-2,3-dihydro-1,4-thiazine is formed from cysteamine and the α -diketone as detailed in Scheme 1. Because the 5-acetyl-2,3,5,6-tetrahydro-1,4-thiazine results as an intermediate after formation of the enamine and subsequent cyclization, an intermolecular disproportionation between this compound and the dione has to be assumed.

To establish the validity of this hypothesis, two further α -dicarbonyl compounds were reacted with cysteamine. The results indicated (cf. Figures 1 and 2) that 5-propionyl-2,3-dihydro-1,4-thiazine is formed from a reaction of cysteine and 2,3-pentanedione and the 5-butanoyl-2,3-dihydro-1,4-thiazine from the corresponding reaction with 2,3-hexanedione, thereby corroborating the synthetic pathway shown in Scheme 1.

Odor Thresholds. The odor thresholds of the three thiazines are compared in Table 4. Low values were found for the acetyl and propionyl derivatives, which differed only slightly. However, the longer alkyl side chain in the butanoyl derivative led to a significant increase in the threshold value by a factor of about 50 000. A similar result was recently obtained by us (Schieberle, 1991) in a sensory study on homologous 2-alkanoyl-1-pyrrolines, indicating that the odor threshold of compounds bearing the following structural element



is significantly influenced by the length of the alkyl side chain **R**.

5-Acetyl- and 5-propionyl-2,3-dihydro-1,4-thiazine are further examples for heterocyclics showing roasty, popcorn-like odors and bearing a structural feature in which the nitrogen atom and the adjacent carbon atom form part of a ring structure (see above). Compounds bearing this structural element and exhibiting roasty, cracker- or popcorn-like odors are summarized in Table 5. The data indicate that 5-acetyl-2,3-dihydro-1,4-thiazine and 5-propionyl-2,3-dihydro-1,4-thiazine, be-

sides 2-acetyl-1-pyrroline, belong to the most odor-active roasty smelling odorants known among the volatiles of food flavors.

Conclusion. NMR and MS data as well as synthetic experiments indicated that compound **1**, isolated from a reaction of cysteine and ribose and exhibiting an intense roasty, popcorn-like flavor, is 5-acetyl-2,3-dihydro-1,4-thiazine.

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