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Structure determination of 4-methyl-3-thiazoline in roasted sesame flavour

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An aroma extract dilution analysis has recently revealed a garlic, carbide-like smelling compound with a comparatively high flavour dilution factor among the 41 odorants detected in the roasted sesame sample. By application of high-resolution mass spectrometry and NMR measurements followed by synthesis, this flavour compound has been identified as 4-methyl-3-thiazoline (**1**). The analytical data and details on the enrichment of **1** from roasted sesame are reported. The odorant exhibiting the very low odour threshold of 1 μg/L in water has been identified for the first time in a food flavour. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The odour-active compounds in a moderately roasted sesame sample have recently been evaluated by using aroma extract dilution analysis (Schieberle, 1995; this journal). Among the 41 flavour compounds, the garlic, carbide-like smelling 4-methyl-3-thiazoline (**1**) contributed with a comparatively high FD factor to the overall flavour of the roasted sesame. This heterocyclic flavour component has to the best of our knowledge not yet been reported in food flavours though it is known in general in chemistry literature. Therefore, we were not able to identify (**1**) directly in our sesame extracts by means of its mass spectrum. The details of its enrichment from the sesame sample and the identification experiments along with the respective analytical data of 4-methyl-3-thiazoline (**1**) are presented in this paper.

MATERIAL AND METHODS

Isolation of 1 from roasted sesame

White sesame seeds (Mexico) were heated for 10 min at 180°C in small glass vessels (4.5 cm × 1.0 cm i.d.). After freezing with liquid nitrogen, the material (1.5 kg) was crushed by means of a commercial blender, extracted with diethyl ether (total volume: 4 litre) at room temperature and, finally, the extract was concentrated to 2 L by using a Vigreux column (100 cm × 2 cm). The volatiles and the solvent were isolated from aliquots (400 ml) of the oily mixture by high vacuum stripping according to Guth & Grosch (1989). The combined sublimates were concentrated to 3 mL and applied onto five glass columns (20 cm × 1.6 cm) at 10–12°C, filled with a slurry of silica gel in *n*-pentane. The silica had been purified by treatment with hydrochloric acid (36% by weight) for 24 h and adjusted to a water content of 7% (Esterbauer, 1968). After flushing each column with mixtures of *n*-pentane/diethyl ether (95 + 5 by vol.: 70 mL; 85 + 15 by vol.: 160 mL; 7 + 3 by vol.: 160 mL), (**1**) was isolated with *n*-pentane/diethyl ether (4 + 6; 200 mL). The combined etheral solutions were then concentrated to 250 μL, and, after addition of *n*-pentane (250 μL), (**1**) was enriched by HPLC.

HRGC analysis was performed by using the columns described in Schieberle (1995).

High performance liquid chromatography (HPLC)

Aliquots (25 μL) of the above solution were repeatedly injected via a Rheodyne 7125 injection valve onto a stainless steel column (50 cm × 0.46 cm) filled with Hypersil (Shandon Products, Eastmore, GB; 5 μm) which was connected to an HPLC pump 110 B (Beckman, Munich, Germany). Elution was performed with *n*-pentane/diethyl ether (1 + 1 by vol.; 50% saturation with water) at a flow rate of 2.0 mL/min. The effluent was monitored at 220 nm (UV Detector SPD-2A; Gynkotek, Munich, Germany) and compounds eluting between 9.5 and 11 mL were collected (20 runs). After drying over Na_2SO_4, the eluate was concentrated to 500 μL and rechromatographed by

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using *n*-pentane/diethyl ether (75+25 by vol.; 50% saturation with water).

Preparative high resolution gas chromatography (pHRGC)

For spectral measurements, in particular NMR, **1** had to be separated from the solvent and purified. This was performed by repetitive pHRGC on a wide-bore DB-1 fused silica column (30 m×0.52 mm i.d.; film thickness: 3.0 μm) in an all-glass system. The condensed and collected compound (about 5 μg) was analysed by HRGC prior to NMR analysis in order to check its purity (>99%).

Nuclear magnetic resonance spectroscopy (NMR)

^{1}H-NMR measurements were performed on a Varian VXR-400S instrument at 399.952 MHz in C_6D_6 with TMS as the internal standard. The ^{13}C-NMR spectrum was recorded at 100.577 MHz in the same solvent.

Synthesis

The synthesis of 4-methyl-3-thiazoline was performed following closely the method published by Asinger *et al.* (1957).

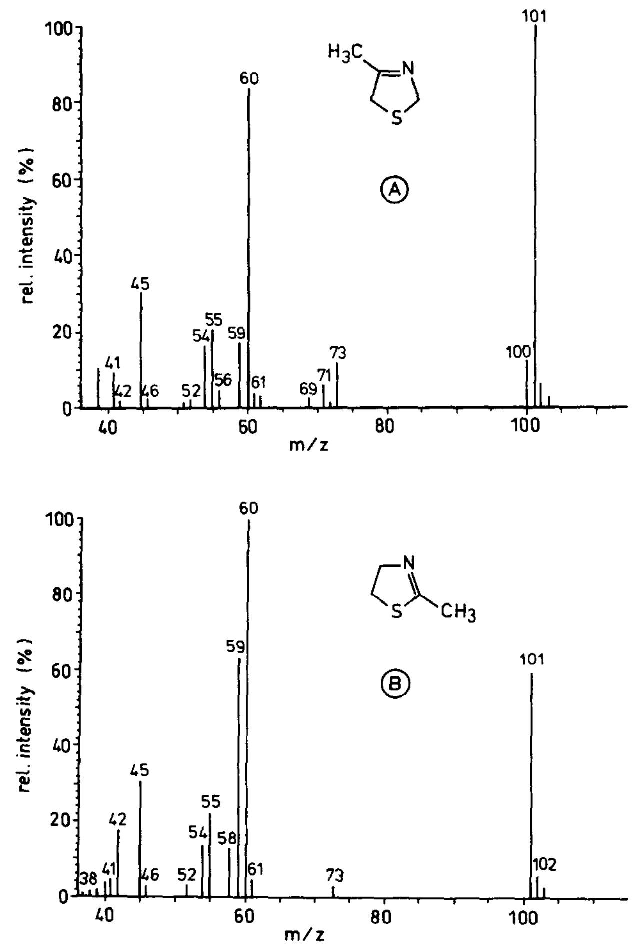

Fig. 1. Mass spectra of **1** (A) and of 2-methyl-2-thiazoline (B).

Determination of odour thresholds

Odour thresholds in air were determined by HRGC/olfactometry as described previously (Ullrich & Grosch, 1987) by using (*E*)-2-decenal (2.7 ng/L air) instead of hexanal as the reference.

RESULTS AND DISCUSSION

With the elution of **1** from the HRGC column, the mass spectrum shown in Fig. 1A was obtained. High resolution mass spectrometry revealed a molecular formula of C_4H_7NS in the molecular ion (m/z 101; Table 1) which was suggested from the mass spectrum obtained by chemical ionization (MS/CI). From the elemental composition follows a value 2 for the total number of

Table 1. Key ions and sum formulas obtained by high resolution mass spectrometry of 1[a]

Ion (m/z)	Intensity (%)	Sum formula
101	100	C_4H_7NS
60	82	C_2H_4S (M^+–CH_3CN)

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

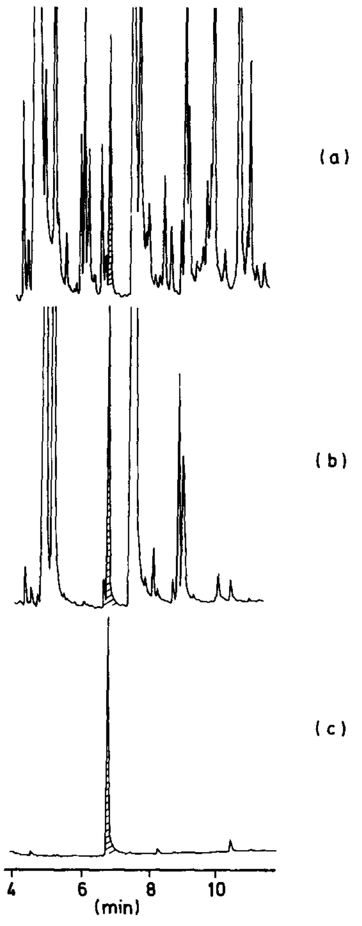

Fig. 2. HRGC chromatograms of fractions obtained by column chromatography (a) and HPLC (b, c) illustrating the purification of **1** for NMR measurements.

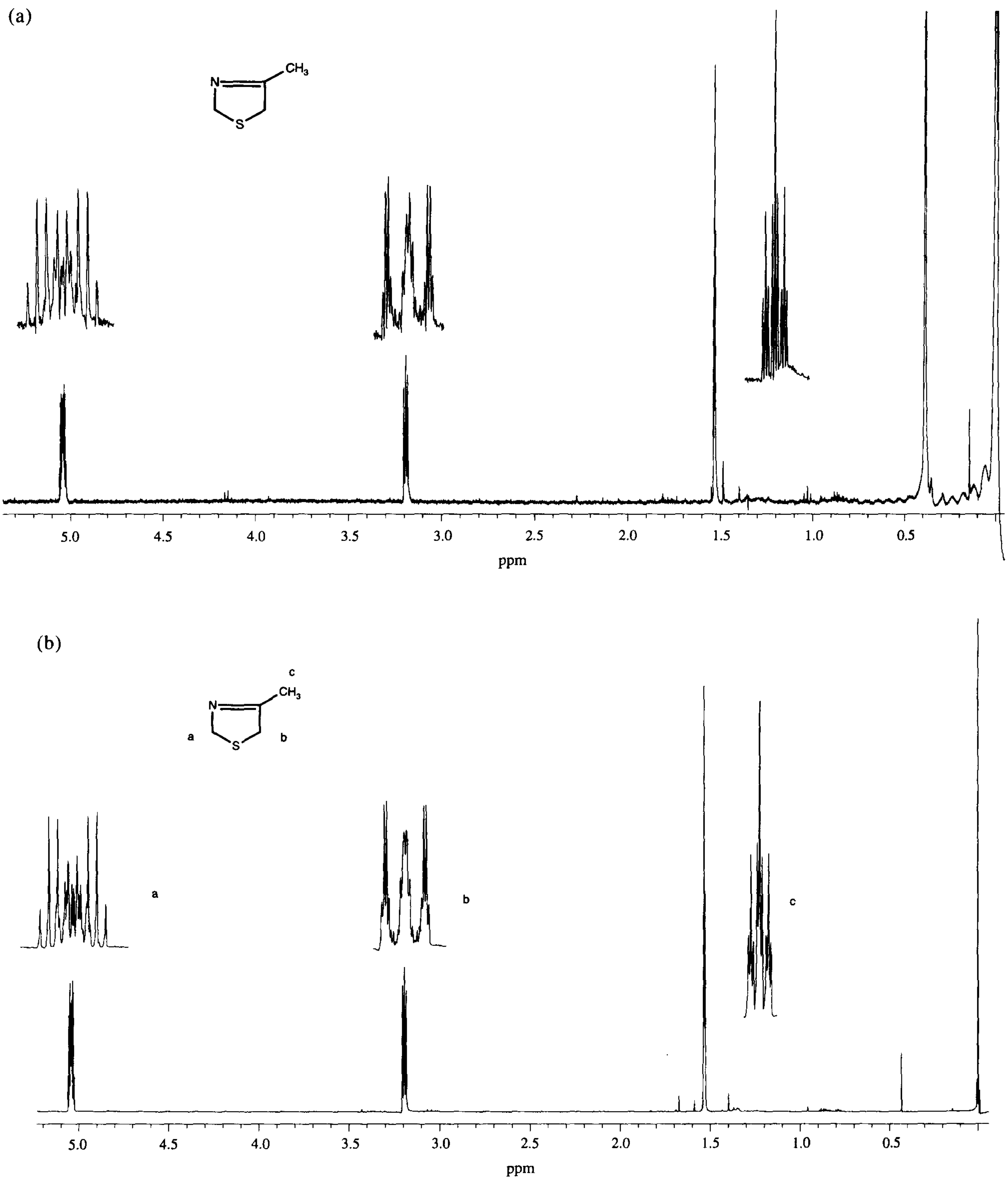

Fig. 3. ^{1}H-NMR spectra of **1** isolated from roasted sesame seeds (a) and of the synthesized 4-methyl-3-thiazoline (b).

rings and/or double bonds. Furthermore, the elimination of CH_3CN (m/z 60: $M^+ - 41$) in the MS/EI (cf. Table 1) suggested a methyl group in **1** attached at a C–N double bond. These data gave strong evidence for a methyl-substituted thiazoline structure.

The mass spectrum of the commercially available 2-methyl-2-thiazoline (Fig. 1B) was similar to the spectrum of **1** (Fig. 1A); however, the retention indices differed significantly. While 2-methyl-2-thiazoline has an index of 1300 (RI_{FFAP}), the much more polar **1** elutes at 1438 (RI_{FFAP}). In addition, the very high odour threshold of 2-methyl-2-thiazoline (> 2000 ng/L) did not correspond to the value determined for **1** which was much lower (0.1 ng/L). For these reasons the structure of 4-methyl-3-thiazoline was proposed for the unknown odorant.

The volatile fraction of roasted sesame was rather complex and, therefore, several chromatographic steps

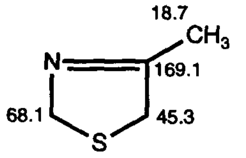

Fig. 4. ^{13}C-NMR data of 4-methyl-3-thiazoline (C_6H_6; chemical shifts in ppm).

were necessary in order to isolate **1** for NMR measurements. As illustrated in Fig. 2, even after pre-separation by column chromatography, numerous compounds were detectable besides **1** in the HRGC chromatogram (dashed peak; Fig. 2a). HPLC purification (Fig. 2b) followed by rechromatography (Fig. 2c) was applied in order to obtain an almost pure compound in solvent. Finally, the last step was purification by preparative HRGC on a thick film column for NMR measurements.

The ^{1}H-NMR spectrum of isolated **1** (Fig. 3A) and the respective spectrum of the synthesized thiazoline (Fig. 3B) are identical and show three signals at 5.045 ppm (2 H), 3.196 ppm (2 H) and 1.535 ppm (3 H) corresponding to a spin system of the $AA'MM'X_3$ type. The coupling pattern of the methyl group is a triplet of triplets with coupling constants of 1.9 and 0.5 Hz. The first value is typical for coupling over four bonds, establishing the methyl group and the double bond at the same carbon atom. In combination with the mass spectral data, this result corresponds well with the proposed structure for **1** as 4-methyl-3-thiazoline.

The ^{13}C-NMR data of the synthesized 4-methyl-thiazoline (Fig. 4) were also consistent with the proposed structure and, thus, the structure could be unambigously identified.

The pure compound smells intensively of garlic and carbide. Its taste threshold was determined as 1 μg/L in water.

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