

Components Contributing to Beef Flavor

Analysis of the Headspace Volatiles of Beef Broth

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A method is described for the isolation of flavor compounds from the headspace of simmering beef broth. Several interesting flavor components could be condensed in one of three cooled traps when the headspace was flushed with pure nitrogen gas. After separation and purification by gas-liquid chromatography, these components were identified by their mass, infrared, and nmr spectra. Of the identified compounds 3-methylbutanal, benzaldehyde, and 3,5-dimethyl-1,2,4-trithiolane (cis and

trans) are known to be beef flavor components, whereas 2,3-pentanedione, methional, 1-methylthioethanethiol, and 2,4,6-trimethyl-perhydro-1,3,5-dithiazine (thialdine) are not. 1-Methylthioethanethiol, which smells like fresh onions, had not been described in the literature before. Evidence is obtained that the trithiolanes and the thialdine are formed for the main part during the isolation procedure.

Work on the identification of the volatile flavor compounds of meat has been reviewed by Herz (1968).

In the same publication Herz describes the isolation of several novel cyclic compounds containing nitrogen, sulfur, or oxygen from the volatile fraction of boiled beef. The identification of two of these, namely 2,4,5-trimethyl-3-oxazoline and 3,5-dimethyl-1,2,4-trithiolane, was reported separately by Chang *et al.* (1968).

Isolation of flavor components from beef broth at our laboratory led to the identification by Tonsbeek *et al.* (1968) of 4-hydroxy-5-methyl-3(2*H*)-furanone and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. Tonsbeek *et al.* (1969) went on to identify the natural precursors of the 5-methyl homolog in broth.

In this article we describe the isolation of volatile components from the headspace of beef broth and the identification of those volatiles with interesting odors.

EXPERIMENTAL

Materials and Methods. The apparatus for the isolation of the headspace volatiles is shown in Figure 1. The apparatus, which is connected together with conical joints and ball joints, is made entirely of glass, except for the Teflon tube (E) for the nitrogen gas inlet. The large 8.5-l. saucer-shaped flask (G) was made from a 20-l. round-bottomed flask. Pure nitrogen gas (A) (ex Loosco, Amsterdam), used to carry off the headspace, was further purified by leading it through a trap (B) filled with lead shot (diameter 2 mm) and cooled with liquid nitrogen (C). All glassware was regularly cleaned with chromic acid or Decontamin (ex Apiel, Brussels) and rinsed carefully before use with distilled water.

Lean shin of beef was supplied by a local butcher and ground with a Braun mincing machine. Pentane and diethyl ether, the extraction solvents, were distilled before use at a reflux ratio of 3:1 through a 100-cm column (diameter 2.5 cm) packed with Fenske glass helices (diameter 4 mm). Extracts were concentrated by distillation on a 100-cm micro spinning-band distillation column made of Teflon (ex Normag, Hofheim am Taunus) at a reflux ratio of 5:1.

Preparation of Beef Broth. Beef broth was prepared by simmering 8 kg of ground lean shin of beef with 8 l. of distilled water for 1.5 hr at 96°C. The broth was cooled to

room temperature and suction-filtered to remove the meat and the solidified fat. The resulting clear broth was stored at 4°C and worked up within 3 days.

Isolation of the Headspace Volatiles of Beef Broth. Eight liters of beef broth was brought into the saucer-shaped flask (G), which was placed in an oil bath (K) heated to 103–105°C. The broth was agitated with the magnetic stirrer (L and H) at 95–97°C and a stream of purified nitrogen (200 ml/min) was led over the broth for 14 hr (a 7-hr period on each of two successive days). The nitrogen inlet (F) was directed to the outside of the flask to ensure an adequate flushing of the relatively small headspace volume. The nitrogen stream, together with the headspace vapors, left the flask through the outlet (M) in the neck of the flask and entered the cooler (N) which was cooled with tap water. The condensed components (mainly water) were collected in a 500-ml round-bottomed flask (P). The latter was cooled in a bath (O) at –10°C, which caused the condensate to freeze. The nitrogen stream was led into the next trap (R), cooled with liquid nitrogen (S). A third trap (U), containing liquid ethyl chloride (ex Mathieson Comp. Inc., East Lutherford) through which the nitrogen stream was bubbled, could be connected to trap R to remove any volatiles not yet condensed. Trap U was kept at –80°C in a Dewar flask filled with ethanol and solid carbon dioxide (T). The flow rate of the nitrogen stream could be measured with a calibrated flowmeter (Q), placed on trap U or trap R. A water lock (D) is placed in the nitrogen line before the inlet (F) to prevent pressure build-up in the apparatus in the case of blockage of one of the traps by condensate.

Preparation of Flavor Concentrates. The contents of trap P (2 × 500 ml) were extracted continuously with 100 ml of distilled pentane for 8 hr and the pentane extract was concentrated to 1 ml by distillation. The remaining aqueous phase was then extracted with 100 ml of distilled diethyl ether for 8 hr to extract the more polar volatiles. The condensate in trap R was transferred to a flask and stored at –5°C under nitrogen. The trap was rinsed twice with distilled water and the rinse water was added to the condensate (total volume 25 ml). The isolation procedure was carried out 20 times (160 l. of broth) until 0.5 l. of condensate had been collected from R. This condensate was extracted continuously with 100 ml of pentane for 8 hr and the extract was concentrated to 1 ml by distillation. The content of trap U was collected and concentrated by distillation under cooling with ice water.

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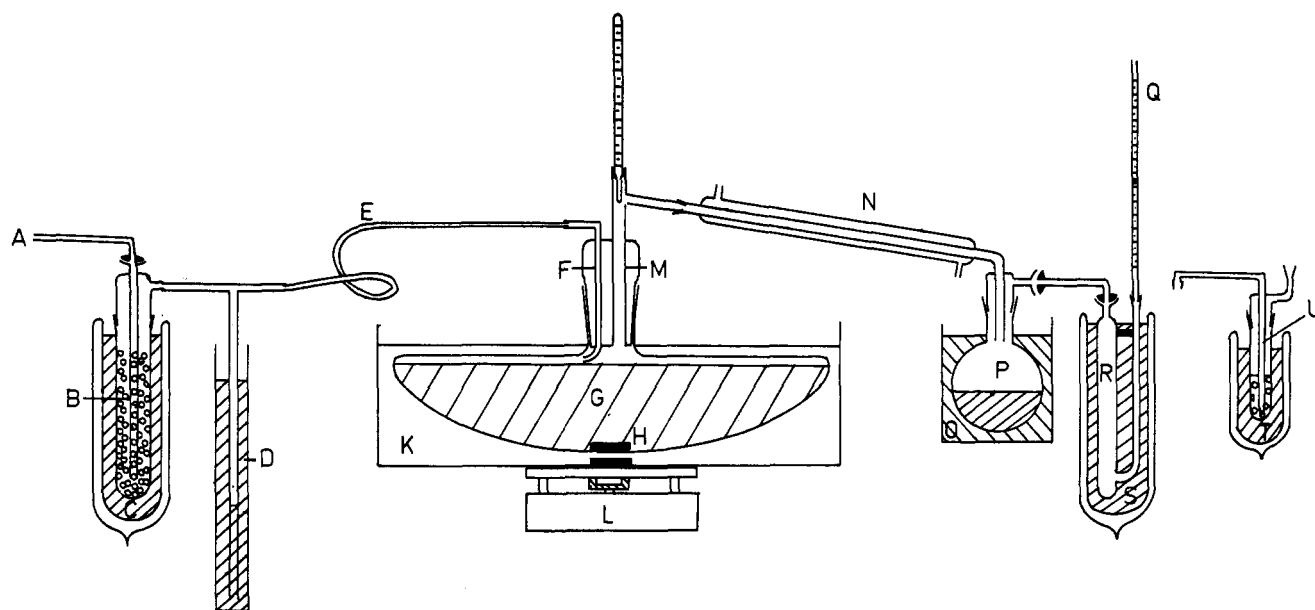


Figure 1. Apparatus for the isolation of headspace volatiles

In this article the investigation of the condensate from trap R only is described. The condensates from traps P and U are still under investigation.

Blank Experiment. The procedure for the isolation of the headspace volatiles was also carried out three times with 8 l. of distilled water in the saucer-shaped flask. The contents of the traps were worked up as described above.

Gas-Liquid Chromatography (glc). The glc analyses were performed on an F&M model 5750 gas chromatograph, equipped with a pre-column, splitting, and trapping system described in detail elsewhere (van de Weerdhof *et al.*, 1970; Copier and Schutte, 1970) and a 200 × 0.3 cm glass column. The column was filled with 20% Carbowax 20M on Diatoport S 80/100 mesh. The oven temperature was programmed linearly at 4°C/min from 50 to 220°C. Carrier gas was nitrogen at a flow rate of 40 ml/min. The injection temperature was 220°C and the detector temperature was 250°C. The trapped components were analyzed spectrometrically.

Infrared Spectra. Spectra were recorded on Perkin-Elmer model 257 and 225 spectrometers. The procedures and

techniques for the transfer of the samples from the glc traps to a KBr micro pellet or into a Barnes micro cavity cell have been described by Copier (1968) and Copier and van der Maas (1967).

Mass Spectra. Volatile components were introduced directly from the glc traps into the ion source of an AEI MS-9 mass spectrometer. A component was transferred by heating it and flushing it with a stream of helium through a capillary allowing a flow rate of 0.1 ml/min.

Nuclear Magnetic Resonance (nmr). Nmr spectra were recorded on a Varian HA-100 spectrometer of the Unilever Research Laboratory, Vlaardingen, The Netherlands. Chemical shifts were measured as δ values relative to the internal standard, tetramethylsilane. Spectra were recorded from carbon tetrachloride solutions in a spherical microcell (Frost *et al.*, 1967). Spectrum accumulation was performed either on a Northern Scientific accumulator or with an IBM 1800 computer.

Synthesis of Reference Compounds. Reference compounds necessary for identification were synthesized at this laboratory, 1-methylthio-ethanethiol, and its analogs by Peer *et al.* (1971), and 3,5-dimethyl-1,2,4-trithiolane and its homologs by Haakman and Tjan (1972). The latter also elucidated the stereoisomerism of these compounds. Thialdine was synthesized by the method of Wöhler and Liebig (1847).

RESULTS

The gas chromatogram of the pentane extract of the content of trap R is given in Figure 2. The numbered peaks in the chromatogram correspond to interesting odors, detected upon elution at the column exhaust. The compounds corresponding to peaks 1, 2, 4, and 5 were readily identified by mass and infrared spectrometry and glc of reference compounds. The identification of the compounds corresponding to peaks 3, 6, 7, and 8 (hereafter referred to as compounds 3, 6, 7, and 8) required further spectroscopic investigation.

Compound 3. The exact molecular weight is 108.0065, which corresponds with a molecular formula $C_3H_8S_2$ (calculated mass 108.0067). The presence of two sulfur atoms was also suggested by the height of the $M + 2$ peak (9% of parent) in the mass spectrum. In addition to those at m/e

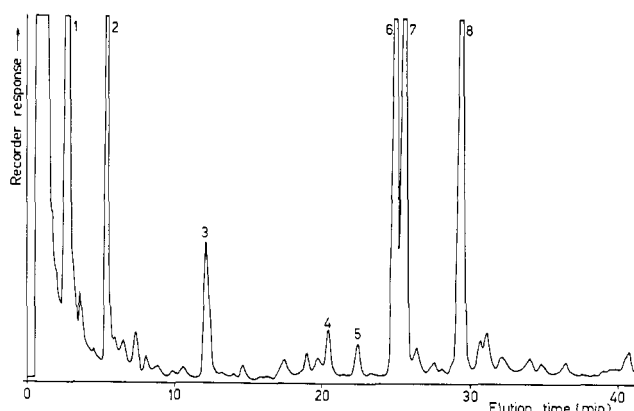
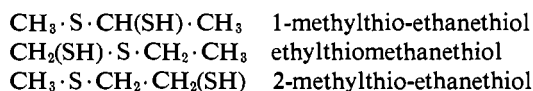


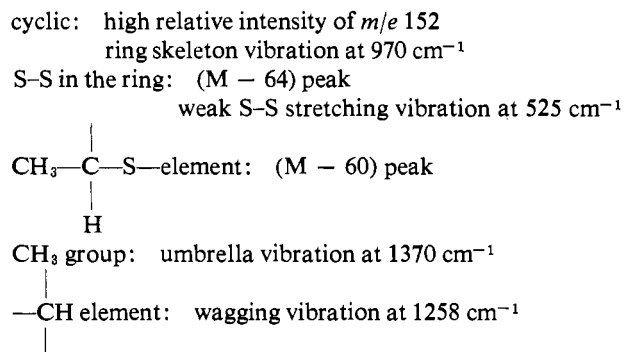
Figure 2. Gas chromatogram of the concentrated pentane extract of the condensate from trap R. Odors corresponding to numbered peaks: 1, faint cabbage, cheese (3-methylbutanal); 2, diacetyl-like (2,3-pentanedione); 3, fresh onion; 4, faint boiled potato (furfural, trace of methional); 5, faint onion (benzaldehyde, contaminant); 6, fresh onion; 7, fresh onion; 8, dry, camphor

110 and 108, there were peaks at m/e 96, 95, 75, 61, 60, 59, 47, 45, and 39. The peaks at m/e 96, 95, and 39 were almost certainly due to traces of furfural. The prominent peak at m/e 75 was ascribed to an $(M - SH)$ ion; the presence of a thiol group was also indicated by an infrared absorption at 2540 cm^{-1} . The peak at m/e 61, which was ascribed to an $(M - CH_3S)$ ion, is common in sulfides and thiols; the ion may have either of the structures $CH_3-\overset{+}{S}=\overset{-}{C}H_2$ or $CH_3-\overset{+}{C}H=\overset{-}{S}H$. Compound 3 is therefore one of the three alkylthio-alkanethiols shown below, and most probably 1-methylthio-ethanethiol:

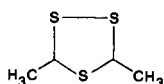


The three alkanethiols were synthesized at our laboratory by Peer *et al.* (1971) and their mass and infrared spectra were recorded for comparison with those of compound 3. The spectra of compound 3 most resembled those of 1-methylthio-ethanethiol; the close match of the infrared spectra is shown in Figure 3. Comparison of glc retention times and of odors confirmed that compound 3 was indeed 1-methylthio-ethanethiol, which was not mentioned in the literature before the article by Peer *et al.*

Compounds 6 and 7. The exact molecular weights of compounds 6 and 7 are 151.9788 and 151.9785, respectively, both corresponding to a molecular formula $C_4H_8S_3$ (calculated mass: 151.9788). The spectral data of compounds 6 and 7 were very similar. The mass spectra of the two compounds had the same peaks (m/e 154, 152, 92, 88, 64, 61, 60, 59, and 45), while the similarity of the infrared spectra is evident from Figure 4. The only differences were small variations in relative intensities and slight shifts in the infrared absorption bands. The following deductions were made about the structure of the two compounds:



The most probable structure for compounds 6 and 7 is therefore:



3,5-dimethyl-1,2,4-trithiolane

Additional evidence was obtained from a 100 MHz nmr spectrum of a 300 μg mixture of the two compounds. (Isolation of the individual compounds for nmr spectroscopy was impracticable in view of the poor glc separation and the limited amounts available.) Absorptions were found at $\delta = 1.66$ and 1.75 ppm (d, 3, $J = 7.2$ Hz, $>CHCH_3$), 4.80 and 4.98 ppm (q, 1, $J = 7.2$ Hz, $>CHCH_3$). Assuming that the small differences in the δ values are due to differences between

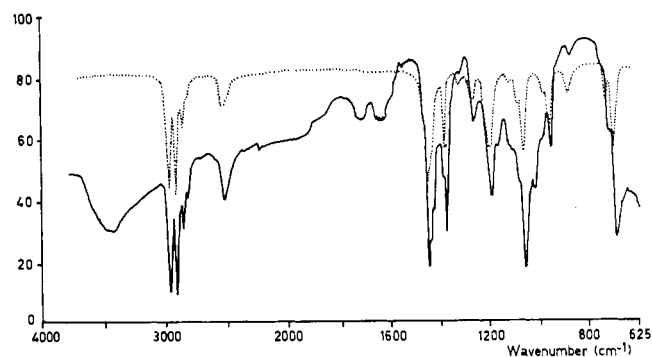


Figure 3. Comparison between the infrared spectra of compound 3 (—) and a synthetic sample of 1-methylthio-ethanethiol (.....)

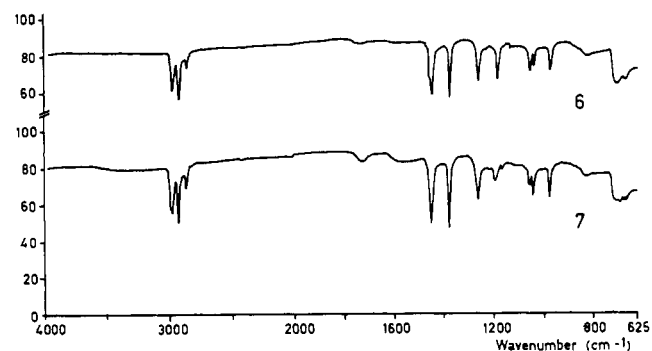


Figure 4. Infrared spectra of compounds 6 and 7

compounds 6 and 7 with respect to the shielding of the $>CHCH_3$ groups, we deduced that these groups are isolated. By comparison with the nmr spectra of some substituted bis-1,3-dithiolanes published by Shapiro *et al.* (1968), we concluded that the $>C(-)H$ elements are contained in five-membered rings. So the only possible structure for both compounds is that of 3,5-dimethyl-1,2,4-trithiolane; stereoisomerism can readily account for the two compounds 6 and 7. The synthesis of 3,5-dimethyl-1,2,4-trithiolane by Haakman and Tjan (1972) yielded two main reaction products, which were shown by mass, infrared, and nmr spectroscopy and by glc to be identical with compounds 6 and 7. In a more detailed study of the stereoisomerism with the aid of the nuclear Overhauser effect, Haakman and Tjan (1972) showed compound 6 to be the trans isomer and compound 7 to be the cis isomer. The two 3,5-dimethyl-1,2,4-trithiolanes have been identified as flavor components of boiled beef by Hirai *et al.* (1968), Chang *et al.* (1968), and Herz (1968).

Compound 8. The exact molecular weight of compound 8 is 163.0484, suggesting the molecular formula $C_6H_9NO_5$ (calculated mass 163.0481) or $C_6H_{13}NS_2$ (calculated mass 163.0489). The isotope abundance ratio $(M + 2):M$ of about 9% strongly favored the latter alternative. In addition to those at m/e 165 and 163, the mass spectrum has peaks at m/e 103, 71, 70, 69, 61, 60, 59, 58, 56, 54, 45, 43, and 42. The nmr parameters of compound 8 were $\delta = 1.42$ ppm (d, 6, $J = 6$ Hz, $>CHCH_3$), 1.44 ppm (d, 3, $J = 7$ Hz, $>CHCH_3$), 3.95 ppm (q, 2, $J = 6$ Hz, $>CHCH_3$), and 4.12 ppm (q, 1, $J = 7$ Hz, $>CHCH_3$). This indicates that compound 8 is very similar to compounds 6 and 7. Evidence for a $CH_3-C(-)H-S-$ element was provided by the prominence of the peak at m/e 103 ($M - C_2H_4S$) in the mass spectrum. The 2:1 ratio of the relative intensities of the two doublets and of the two quartets in the nmr spectrum show that the molecule contains at least three isolated $CHCH_3$ groups. The presence

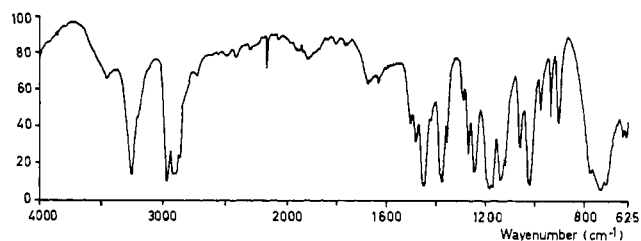
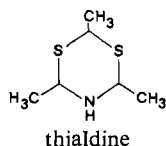


Figure 5. Infrared spectrum of compound 8

of these groups, two of which are equivalent, is corroborated by absorptions at 1380 and 1270 cm^{-1} in the infrared spectrum (Figure 5). The infrared absorption near 3280 cm^{-1} indicates the presence of an $>\text{NH}$ group, while the high parent peak intensity in the mass spectrum and the vibrations at 1020 and 980 cm^{-1} infer a cyclic structure. The latter inference is in agreement with the chemical shift for the proton resonances of a $>\text{CHCH}_3$ group in a six-membered ring observed with bis-1,3-dithianes by Shapiro *et al.* (1968).

The spectroscopic evidence suggests that compound 8 is 2,4,6-trimethyl-perhydro-1,3,5-dithiazine. This compound was described by Wöhler and Liebig as early as 1847 and named thialdine. The mass spectral data of compound 8 were compatible with those of synthesized thialdine, while its infrared and nmr spectra were almost identical with those of the synthetic sample. Comparison of glc retention times and odors confirmed that compound 8 was thialdine.



DISCUSSION

The possible introduction of artefacts with our apparatus was checked with the blank experiment. Glc of the contents of the traps revealed no such artefacts. This suggests that all the peaks in Figure 2 correspond with compounds isolated from the headspace of beef broth. The presence of relatively large amounts of the 3,5-dimethyl-1,2,4-trithiolanes and thialdine in trap R (cooled at -196°C) is nevertheless rather surprising. In view of their high boiling points, one would expect them to condense mainly in the first trap P. Of these compounds only thialdine was in fact found in trap P. In a subsequent run we therefore decided to chromatograph the condensate in trap R as soon as a small amount had been collected, thus avoiding delays due to the collection (and storage) of sufficient material for subsequent trapping. The chromatogram of 30 μl of thawed condensate (Figure 6) revealed the presence of only very small amounts of the 3,5-dimethyl-1,2,4-trithiolanes, but of relatively large amounts of 1-methylthio-ethanethiol and thialdine. Moreover, when the condensate in trap R was collected, stored, and accumulated as in the routine isolation procedure, its smell became progressively more onion-like. The concentration of the 3,5-dimethyl-1,2,4-trithiolanes was found to increase, while that of thialdine decreased. This suggests that, although they may be present in fresh beef broth, the trithiolanes are formed mainly during storage of the condensate. Chang *et al.* (1968) and Hirai *et al.* (1968) were the first to isolate and identify the 3,5-dimethyl-1,2,4-trithiolanes from beef broth. Their isolation procedure, however, does not exclude the formation of these compounds in the condensate. The same

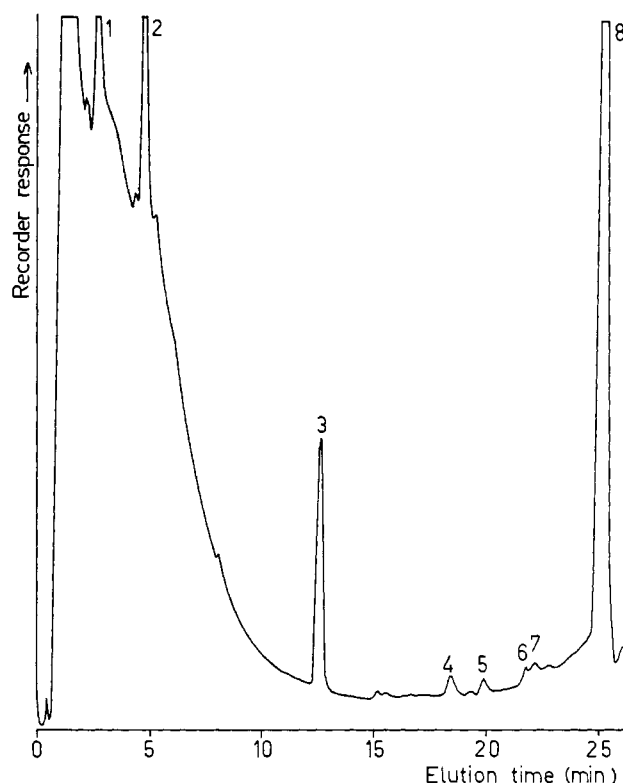


Figure 6. Gas chromatogram of 30 μl of the condensate of trap R. Oven temperature programmed linearly at $6^\circ\text{C}/\text{min}$. 1, 3-methylbutanal; 2, 2,3-pentanedione; 3, 1-methylthio-ethanethiol; 4, furfural, trace of methional; 5, benzaldehyde, contaminant; 6, *trans*-3,5-dimethyl-1,2,4-trithiolane; 7, *cis*-3,5-dimethyl-1,2,4-trithiolane; 8, thialdine

may be true for the procedure of Buttery *et al.* (1970), who describe the isolation of the trithiolanes from potatoes.

The synthesis of thialdine from ammonia, hydrogen sulfide, and acetaldehyde, as described by Wöhler and Liebig (1847), is very simple. These three volatile compounds are also present in the headspace of beef broth (Hornstein *et al.*, 1960; Kramlich and Pearson, 1960; Yueh and Strong, 1960) and they will condense mainly in trap R. They may therefore act as precursors for the thialdine in trap R, which may be formed before or during thawing of the condensate. The thialdine in the first trap P may originate from the beef broth or be formed either in the headspace of the broth or in the trap itself. In view of the ease of its formation from NH_3 , H_2S , and CH_3CHO , thialdine is almost certainly present in the broth or the headspace and can be considered as a contributor to beef broth flavor. The thialdine in trap R, however, seems to have been formed there from the same natural precursors. Our thoughts about the formation of the trithiolanes and thialdine in trap R were confirmed by the following experiment. When, instead of beef broth, an aqueous solution of thialdine and the two trithiolanes was subjected to the isolation procedure described, the trithiolanes condensed in trap R and, to a lesser extent, in trap P. Thialdine, on the other hand, condensed solely in trap P.

The findings about the origin of thialdine and the trithiolanes in trap R prompted an investigation into the origin of the 1-methylthio-ethanethiol. This compound has particularly interesting organoleptic properties; in very dilute aqueous solutions (1–5 $\mu\text{g}/\text{l}$) it has a meaty rather than an onion-like odor. Schutte and Koenders (1972) found that the 1-methylthio-ethanethiol is formed in the broth from hydrogen sulfide, methanethiol, and acetaldehyde.

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Components Contributing to Beef Flavor.

Natural Precursors of 1-Methylthio-ethanethiol

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A system of reactions is proposed for the formation of the flavor compound 1-methylthio-ethanethiol in beef broth from known beef components. The proposed system has been substantiated experimentally. 1-Methylthio-ethanethiol is formed when ethanal, methanethiol, and hydrogen sulfide are heated in aqueous solution at pH 6. These immediate precursors are in turn generated under the same

conditions from alanine, methionine, and cysteine in the presence of a Strecker degradation agent such as pyruvaldehyde. Mechanisms are proposed for the reactions involved. The formation of methanethiol from methional, the initial degradation product of methionine, proved to be the weakest link in the reaction sequence and was therefore studied in some detail.

A novel sulfur compound, 1-methylthio-ethanethiol, was isolated from the headspace of beef broth by Brinkman *et al.* (1971). We were interested in identifying its precursors, and in determining whether it is formed in the aqueous broth itself, in the headspace of the broth, or as an artifact in the -196°C trap from which it was isolated.

Of the many substances known to be present in beef broth during its preparation, ethanal, methanethiol, and hydrogen sulfide are the most probable immediate precursors of 1-methylthio-ethanethiol. A simple combination reaction can be held responsible for the formation of this dithiohemiacetal (Schutte, 1971). These three immediate precursors are, in turn, known to be formed by Strecker degradation of alanine (*e.g.*, Schönberg and Moubacher, 1952), methionine (Ballance, 1961), and cysteine (Kbayash and Fujimahi, 1965), respectively. The presence of these amino acids in beef is well established (Wasserman and Spinelli, 1970). We have investigated firstly whether 1-methylthio-ethanethiol indeed can be formed from the proposed immediate precursors, secondly where the formation takes place, and thirdly what the optimal conditions are for its formation. Finally, we investigated whether 1-methylthio-ethanethiol is formed by Strecker degradation of the appropriate amino acids under conditions similar to those prevailing during broth preparation.

EXPERIMENTS AND RESULTS

Formation of 1-Methylthio-ethanethiol from the Immediate Precursors under Conditions of Beef Broth Preparation. To

show that the proposed immediate precursors were capable of yielding 1-methylthio-ethanethiol under the conditions of broth preparation, we used the apparatus designed by Brinkman *et al.* (1971) for the isolation of the headspace volatiles. A solution of 1 g (23 mmol) of ethanal, 1 g (21 mmol) of methanethiol, and 6 g (25 mmol) of sodium sulfide nonahydrate in 1 l. of water, buffered by phosphate at pH 5.8, was added to the reaction flask, which was heated in an oil bath at 95°C . The headspace vapor was led in turn through a condenser, a -10°C trap and a -196°C trap with a stream of pure nitrogen. After 1 hr the contents of last trap were warmed to ambient temperature and subjected to gas chromatography (Figure 1a). The compounds in the effluent were collected (Copier and Schutte, 1970) and identified by mass and infrared spectrometry. The largest peak in the chromatogram corresponded to 1-methylthio-ethanethiol, of which an estimated 10–20 mg (0.5–1%) had condensed in the -196°C trap. The double peak corresponded to the *cis* and *trans* isomers of 3,5-dimethyl-1,2,4-trithiolane (Brinkman *et al.*, 1971). Another peak corresponded to dimethyl disulfide which, like the trithiolane, has been identified in boiled beef (Herz and Chang, 1970).

Nonformation of 1-Methylthio-ethanethiol in the Vapor Phase or in the -196°C Trap. Ethanal, methanethiol, a solution of sodium hydrosulfide and water were placed in separate flasks, kept at appropriate temperatures (Figure 2). The flasks were interconnected so that any combination of the vapors in the flasks could be led with a nitrogen stream *via* a reaction vessel and a trap at -10°C into a trap cooled in liquid nitrogen. Only traces of 1-methylthio-ethanethiol

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