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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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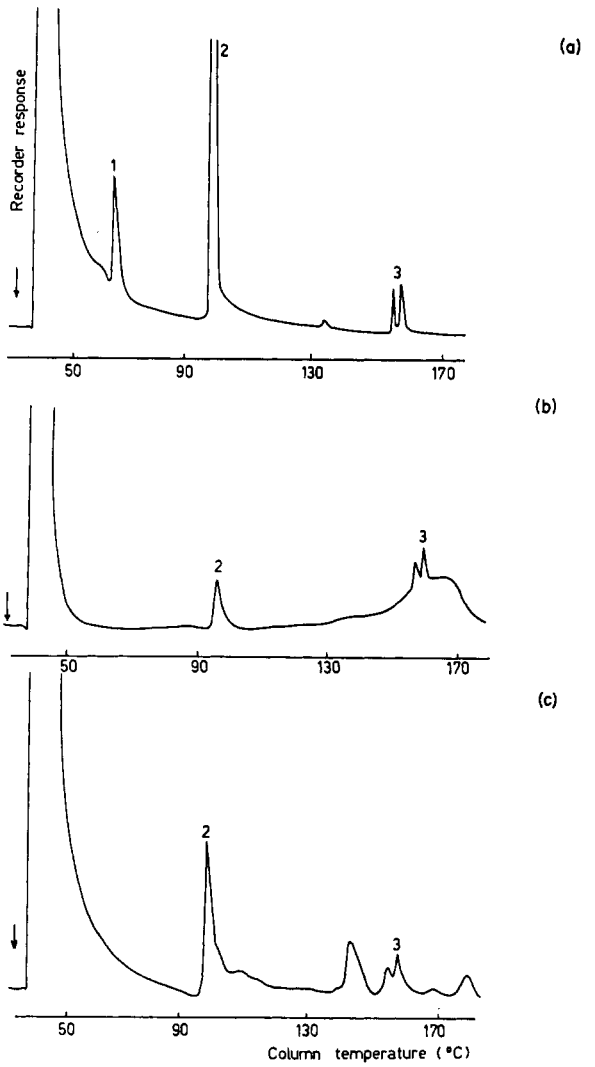


Figure 1. Gas-chromatographic separations on a 3 m × 2 mm (i.d.) glass column packed with 10% Carbowax 20M on 80–100 mesh Diatoport S in a Hewlett-Packard 5750 instrument. Temperature programmed from 50 to 200° C at 4° C/min. a. Condensate in the −196° C trap, after reaction between ethanal, methanethiol, and hydrogen sulfide in aqueous solution (pH 5.9) at 95° C. b. Dichloromethane layer, after reaction between ethanal, methanethiol, and hydrogen sulfide in the two-phase system water (pH 7.5)/dichloromethane at room temperature. c. Condensate in the −196° C trap, after reaction between alanine, cysteine, methionine, and ninhydrin in aqueous solution (pH 6.0) at 100° C. Compounds identified by mass spectrometry are: 1, dimethyl disulfide; 2, 1-methylthio-ethanethiol; 3, *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolane

were detected in the −196° C trap, whatever the combination of vapors. It is therefore improbable that the thiol is formed from the proposed immediate precursors in the vapor phase or in the −196° C trap.

In another experiment 40 mg 1-methylthio-ethanethiol, prepared by the method of Schutte (1971), was mixed with 20 mg (0.5 μCi) 1,2-^{14}C-ethanal in 1 l. of water, buffered by phosphate at pH 5.8. The solution was treated in the same way as the broth in the headspace analysis. An aliquot of the condensate of the −196° C trap was subjected to gas chromatography and the eluent was trapped fractionwise in vials filled with 10 ml of scintillator solution (5 g of PPO and 0.3 g of POPOP per liter of toluene). Radioactivity was measured in a Packard Tricarb Model 3380 Liquid Scintillation Spectrometer. The fraction corresponding to the 1-methylthio-ethanethiol peak showed no radioactivity above the background (about 60 cpm), whereas the net counting rate of the ethanal fraction, eluted at 50–90° C, was 330 cpm. Since there was no transfer of label, we may conclude that the 1-methylthio-ethanethiol is not dissociated into, and subsequently reformed from, its immediate precursors during the isolation of headspace volatiles. It was estimated that about 15 mg (38%) of the thiol had been transferred to the −196° C trap.

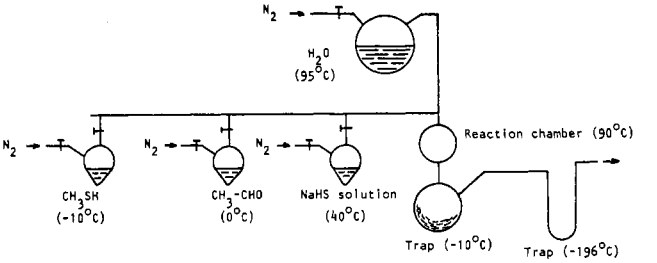


Figure 2. Apparatus in which vapors of ethanal, methanethiol, hydrogen sulfide, and water are led either separately or simultaneously into a −196° C trap

Table I. Products of the Reaction Between Equimolar Amounts of Ethanal, Methanethiol, and Sodium Sulfide in Water/Dichloromethane (2:1)

	Reaction products		
pH	1-methyl-thio-ethane-thiol	Others	Remarks
0.0[a]	−	Thioacetal	Fast reaction
1.0	+	Mixture, mainly thioacetal	Fast reaction
4.4	+	Single byproduct	Possibly from buffer (acetate)
5.3	+		
7.5	+	Mixture, including trithiolanes	See Figure 1b
10.6	−	Mixture	No 1-methylthio-ethanethiol upon acidification

[a] Na_2S omitted.

These experiments together indicate that any 1-methylthio-ethanethiol in the −196° C trap originates from the broth.

Formation of 1-Methylthio-ethanethiol in the Aqueous Phase. If the 1-methylthio-ethanethiol is indeed formed in the aqueous phase and subsequently steam distilled from the reaction mixture, it should be possible to demonstrate this with the aid of a two-phase system, comprising an aqueous phase as a polar reaction medium and an organic phase to remove the thiol as it is formed. A number of vials were filled with 1 ml of dichloromethane and 2 ml of one of a number of buffer solutions (pH 1.0–10.6). To each vial was added 50 μl of ethanal, 50 μl of methanethiol and (except in run 2) 300 mg of sodium sulfide nonahydrate (*i.e.*, about 1 mmol of each material). The vials, which were kept at room temperature, were shaken regularly and the extent of reaction was monitored by gas chromatography of 2-μl aliquots of the dichloromethane layer. The reactions proceeded very slowly, except in highly acidic media. It usually took about 24 hr before any change could be detected. Table I summarizes the results of these experiments.

The thioacetal, di(methylthio)methane, is known to be a product of the reaction between ethanal and methanethiol in acidic media. This was the sole product at pH 0.0. One of the eight components formed in the reaction at pH 1.0

was identified as the thioacetal by comparison of retention times. 1-Methylthio-ethanethiol was formed most selectively at pH 4.4 and 5.3 (acetate buffer). The gas chromatogram of the product of the reaction at 7.5 (phosphate buffer) was characterized by a hump, upon which a twin peak was superimposed (Figure 1b). The twin peak was shown by mass spectrometry to be due to 3,5-dimethyl-1,2,4-trithiolane. The hump itself may be caused by compound(s) reacting on the column during gas chromatography.

These findings were the basis for a facile synthesis of 1-methylthio-ethanethiol and related compounds in water/-dichloromethane at a pH of about 5 (Schutte, 1971).

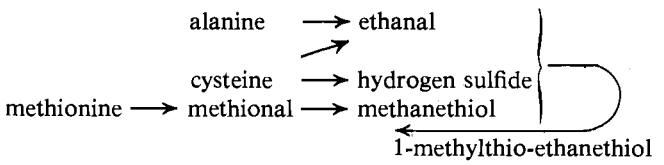
Formation of 1-Methylthio-ethanethiol by the Strecker Degradation of Amino Acids. In this experiment we again used the apparatus designed by Brinkman *et al.* (1971). A mixture of 0.5 g (5.5 mmol) of alanine, 0.5 g (3.4 mmol) of methionine, and 0.75 g (4.8 mmol) of cysteine hydrochloride was boiled in 200 ml of water, buffered at pH 6 by phosphate and containing 1.5 g of ninhydrin and 2 g of hardened fat. Although ninhydrin is not a food ingredient, it was used in this experiment as a model Strecker degrading agent because of its high efficacy. After 1 hr a gas chromatogram (Figure 1c) of the contents of the −196° C trap demonstrated that about 0.05 mg (0.01 %) of 1-methylthio-ethanethiol had been collected.

Formation of Methanethiol from Methional. When degrading agents other than ninhydrin were tried, only traces of 1-methylthio-ethanethiol were detected in the −196° C trap. Subsidiary experiments indicated that the weakest link in the reaction sequence was the elimination of methanethiol from methional, the initial Strecker degradation product of methionine. This prompted us to study this step, particularly its catalysis, in more detail. Three pear-shaped three-necked 100-ml flasks, each containing 100 mg of methional in 20 ml of phosphate buffer solution, were heated in the same oil bath at 110° C. One of the flasks served as a blank, while about 50 mg of a substance was added to the other two for determination of its catalytic potential. Nitrogen was swept through each solution and led through a separate reflux condenser to a 4% mercuric cyanide solution. The rate of precipitation in the mercuric cyanide trap was taken as a measure for the ability of the substance to catalyze the formation of methanethiol from methional. The results are summarized in Table II.

Similar experiments were performed with methionine instead of methional. Ninhydrin was again found to be by far the most effective degrading agent, followed by alloxan and pyruvaldehyde. Formation of methanethiol from *S*-methylcysteine proved to be even more difficult. Of the various degrading agents tried, only ninhydrin was effective.

DISCUSSION AND CONCLUSIONS

In boiling aqueous solution at pH 6 and in the presence of a suitable Strecker degradation agent, 1-methylthio-ethanethiol can be formed, from the reaction sequence depicted below



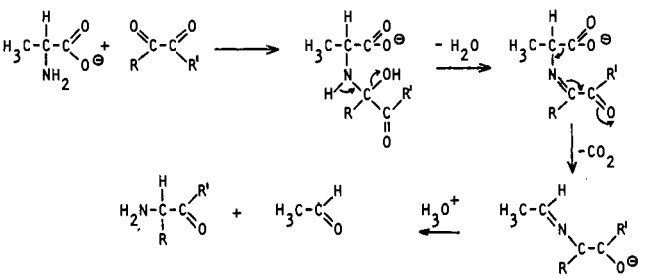
The reaction mechanism for the formation of ethanal by the Strecker degradation of alanine is represented in Scheme 1. Conjugated diketones are suitable degradation agents, ninhydrin being particularly active (Schönberg and Moubacher, 1952).

Table II. Effect of Various Catalysts on the Formation of Methanethiol from Methional in an Aqueous Solution Boiled for 15 min. (Unless otherwise stated the solution was buffered at pH 6 with phosphate)

Catalyst	Methanethiol formation
Ninhydrin	+++
Alloxan	+
Pyruvaldehyde	+
2-Hydroxypyridine	+
Pyridine[a]	−
Isatin	−
Ammonium acetate	−
Fructose	−
Glucosone	−
Glucosamine	−
Ethanal	−
Chloral hydrate	−
Dehydroascorbic acid	−
Borax, pH 9 (no phosphate)	++
HCl, pH 2 (no phosphate)	−−

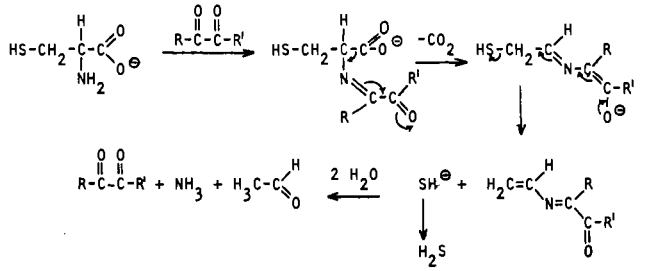
Legend: +++, high; ++, moderate; +, low; −, traces, as in blank; −−, none at all.

[a] Precipitate caused by pyridine and not by methanethiol, as was demonstrated by reaction of the contents of the trap with *N,N*-dimethyl-*p*-phenylene diamine and ferric chloride (Swilinsky and Doty, 1958).



Scheme 1

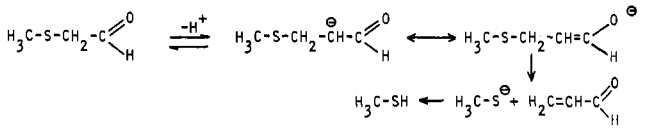
Hydrogen sulfide formation from cysteine by diketones is well established (Kbayash and Fujimahi, 1965). The reaction involves oxidation of cysteine and a subsequent reduction, as represented in Scheme 2. No diketone is consumed in the overall reaction. On account of side reactions, however, more than catalytic amounts of the diketone are needed.



Scheme 2

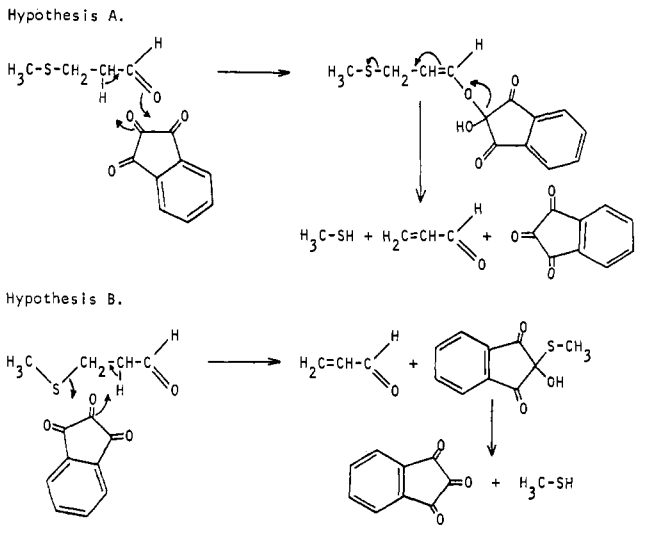
The critical link in the proposed precursor system is the conversion of methionine into methanethiol. The first step is the formation of methional by the Strecker degradation of methionine in a manner analogous to that shown in Scheme 1 (Ballance, 1961). The second step, the elimination of methanethiol, proceeds readily at pH 9 but not at all at pH 6 in the presence of a base like pyridine (Table II). It seems, therefore, that specific base catalysis is involved. This may

be explained if it is assumed that methional behaves as an anion, as depicted in Scheme 3.



Scheme 3

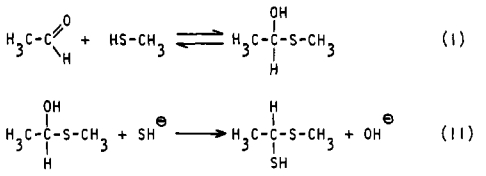
The results summarized in Table II show that, at pH 6, several compounds can serve as catalysts in the elimination of methanethiol. These catalysts have in common a basic and an acidic site adjacent to each other, 2-hydroxypyridine being a classic example in this respect. Ninhydrin, alloxan, and pyruvaldehyde possess strongly polarized carbonyl groups that are easily hydrated. These carbonyl groups may therefore be expected to show a tendency to react with electronegative oxygen or sulfur groups to form hemiketals. These considerations suggested alternative mechanisms for the elimination of methanethiol; both of these mechanisms are concerted, based on the acidity of the α-hydrogen atom of methional. The alternative hypotheses are represented in Scheme 4, for the case when the degrading agent is ninhydrin.



Scheme 4

When the degrading agent is 2-hydroxypyridine, hypothesis A becomes a catalyzed enolization step. If, however, enolization were the rate-determining step, the formation of methanethiol from methional would be generally rather than specifically base-catalyzed, which is not observed. Consequently, hypothesis B is regarded more plausible than hypothesis A, at least for catalysis by 2-hydroxypyridine. Of the compounds shown to be active in Strecker degradation and in the formation of methanethiol, pyruvaldehyde is known to occur in cooked beef (Yamoto *et al.*, 1970).

A mechanism for the formation of 1-methylthio-ethanethiol from the immediate precursors is proposed in Scheme 5.



Scheme 5

Equilibria of the type depicted for step I have been investigated by Jencks (Lienhard and Jencks, 1966; Barnett and Jencks, 1969). The equilibrium constant is about 30, and the addition occurs by specifically base-catalyzed and generally acid-catalyzed pathways. Step II involves nucleophilic displacement of OH^- by SH^-. The SH^- ions are excellent nucleophiles (Swain and Scott, 1953) and, since the pH_a of H_2S is about three units lower than that of CH_3SH, they are present in far higher concentrations between pH 5 and 7 than CH_3S^- ions which might otherwise compete with them. 1-Methylthio-ethanethiol is very volatile and distils readily. The reactants, however, are also very volatile and hence yield is low. For preparative purposes the yield of 1-methylthio-ethanethiol can be increased when the reaction is performed in a two-layer system, the organic solvent serving to extract the product as it is formed and to enhance the solubility of the apolar reactants.

The findings of this investigation are fully compatible with the proposed system of precursors, all of which are known components of beef or beef broth. We may conclude that alanine, methionine, and cysteine react in broth with a diketone such as pyruvaldehyde to yield ethanal, methanethiol, and hydrogen sulfide. These volatile compounds are the immediate precursors of 1-methylthio-ethanethiol. The same three compounds also give rise to formation of dimethyl disulfide and 3,5-dimethyl-1,2,4-trithiolane in the broth.

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