lations Extérieures, Paris, for a postdoctoral fellowship attributed to one of us (N.K.).

Registry No. Gluconic acid, 526-95-4; melibionic acid, 21675-38-7.

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Characterization of Bacon Odor and Other Flavor Components from the Reaction of Isovaleraldehyde and Ammonium Sulfide

Chi-Kuen Shu,* Braja D. Mookherjee, Henry A. Bondarovich, and Myrna L. Hagedorn

Characterization of the reaction of isovaleraldehyde and ammonium sulfide by gas chromatography–mass spectrometry revealed several components including the characteristic fried bacon compounds 2,4,6-triisobutyl-4*H*-1,3,5-dithiazine and 3,5-diisobutyl-1,2,4-trithiolane. A novel pyridine, 2-isobutyl-3,5-diisopropylpyridine, has also been identified by additional proton NMR analysis. Mechanisms of formation of those components are described.

It has been well-known that Maillard reaction and Strecker degradation play a very important role in food systems to provide flavor components using amino acids and sugars. The products from such reactions can further react with each other or react with the degradation products to form additional flavor components. Usually, amino acid generates the corresponding aldehyde with one carbon less through Strecker degradations (Schonberg and Moubacher, 1952), and sulfur-containing amino acid is degraded to different components including ammonia and hydrogen sulfide (Mulders, 1973).

Wiener (1972) reported the reaction between isovaleraldehyde and ammonium sulfide producing fried bacon odor but did not mention which components were responsible for such flavor. Now we are reporting the characterizations of the components of the reaction mixture including the ones that possess the fried bacon aroma.

EXPERIMENTAL SECTION

Preparation of the Reaction Mixture. Isovaleraldehyde was mixed with aqueous 22% ammonium sulfide (Mallinckrodt Inc., St. Louis, MO) in a 1:1 molar ratio at 15 °C for 2 h. The reaction mass was then extracted with hexane. The hexane extract was washed with water, dried, and concentrated.

Analysis. The concentrated material was analyzed by gas chromatography–mass spectrometry (GC–MS) on a 3% SE-30 stainless steel column (10 ft × $^1/_8$ in.) programmed from 70 to 210 °C at 4 °C/min. The mass spectrometer was a Kratos MS-50 operated at 70 eV. The peaks were trapped from GC for organoleptic evaluation and for nuclear magnetic resonance (NMR) analysis. The proton NMR spectrum was obtained from Varian XL-100 operated at 100 MHz in trichlorofluoromethane with tetramethylsilane as an internal standard.

RESULTS AND DISCUSSION

Figure 1 represents the GC chromatogram of the reaction mixture. Table I shows the components identified along with the major mass spectral fragments.

Peaks 12 and 13 were organoleptically evaluated to possess the fried bacon aroma and were identified as 3,5-diisobutyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine respectively. The detailed information regarding the identification, the synthesis, and the organoleptic properties of these two compounds has already been published (Shu et al., 1980, 1981).

The mechanism of the formation of these two components can be described by the mechanism shown in Figure 2. Hydrogen sulfide and ammonia were derived from ammonium sulfide. Isovaleraldehyde reacted either with H_2S to form the intermediate compound I or with NH_3 to form the intermediate compound II. When 2 mol of the intermediate compound I were involved via oxidation and a loss of H_2S, 3,5-diisobutyl-1,2,4-trithiolane was generated. When both intermediate compounds were interacted with a loss of 3 mol of H_2S, 5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine was generated. In the literature, similar

International Flavors and Fragrances (R&D), Union Beach, New Jersey 07735.

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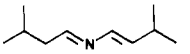
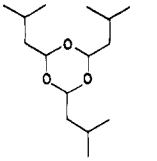
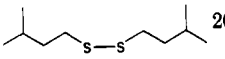
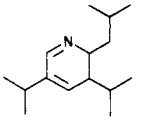
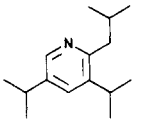
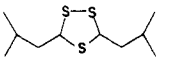
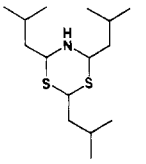
Table I. Volatile Components Identified from the Reaction Mixture and Their Major Mass Spectral Fragments

peak no.	name of component	structure	M_r	major MS fragments
1	2-methyl-2-propanol			
2	trimethylacetaldehyde			
3	isovaleraldehyde			
4	isoamyl alcohol			
5, 6	3-methyl-*N*-(3-methylbutylidene)butenylamine (2 isomers)		153	96 (100), 41 (89), 43 (63), 54 (53), 82 (48), 138 (48), 110 (46), 60 (50)
7, 9	2,4,6-triisobutyl-1,3,5-trioxane (2 isomers)		258	87 (100), 69 (69), 173 (41), 43 (35), 85 (27), 57 (20)
8	diisobutyl disulfide		206	43 (100), 71 (55), 41 (34), 206 (26), 55 (23)
10	2,3-dihydro-2-isobutyl-3,5-diisopropylpyridine		221	43 (100), 71 (65), 41 (57), 85 (52), 178 (42), 122 (31), 136 (29), 164 (23), 206 (19)
11	2-isobutyl-3,5-diisopropylpyridine		219	149 (100), 162 (60), 204 (50), 177 (37), 219 (19)
12	3,5-diisobutyl-1,2,4-trithiolane		236	69 (100), 41 (80), 43 (62), 45 (41), 60 (33), 87 (33), 102 (30), 101 (22)
13	5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine		289	43 (100), 41 (79), 102 (54), 69 (50), 96 (40), 60 (40), 45 (40)

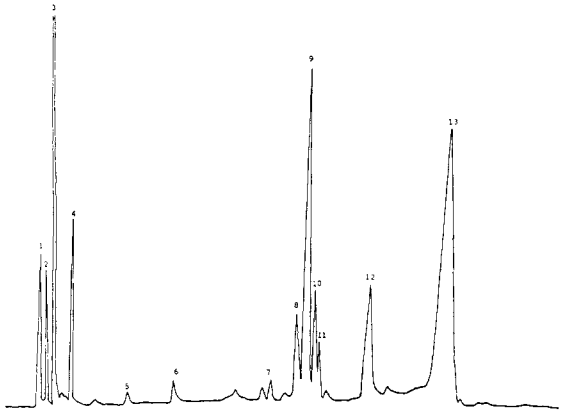


Figure 1. GC chromatogram of the reaction mixture. Column: 3% SE-30 stainless steel (10 ft × $^1/_8$ in.). Temperature: 70 to 210 °C programmed at 4 °C/min. Detector: thermal conductivity.

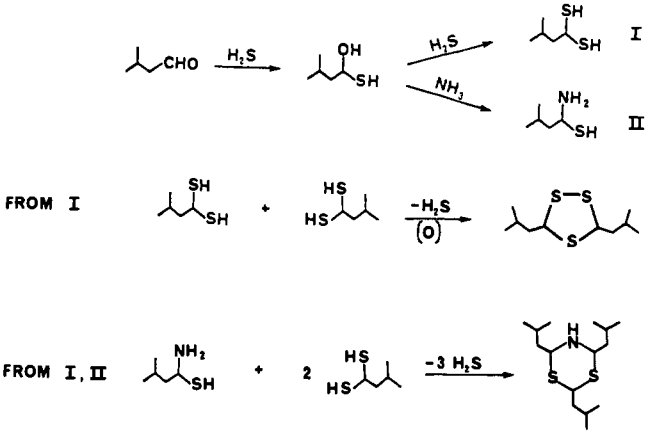


Figure 2. Possible mechanism of the formation of 3,5-diisobutyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine.

mechanisms were already postulated to the formation of these types of compounds (Boelens et al., 1975).

3,5-Diisobutyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine are expected to be present in the processed meat products, because leucine is the precursor of isovaleric aldehyde and cystine and cysteine are the precursors of H_2S. Ammonia can be provided by any amino acids.

Table II shows the proton NMR spectral data of peaks 10 and 11. Combination of the NMR spectra and the mass spectra (Table I) provided the identification of peak 10 as 2,3-dihydro-2-isobutyl-3,5-diisopropylpyridine and peak 11 as 2-isobutyl-3,5-diisopropylpyridine, which is a novel pyridine.

The mechanism of the formation of this pyridine is postulated as shown in Figure 3.

Table II. Proton NMR Spectra (δ): Peaks 10 and 11

peak 10: 7.52 (s, olefinic H), 5.62 (br s, olefinic H), 3.72 (br s, 1 H), 3.37 (m, 1 H), 2.6–1.0 (m, 5 H), 1.08 (d, 2 CH_3), 0.94 (d, CH_3), 0.90 (d, CH_3), 0.89 (d, CH_3), 0.82 (d, CH_3)

peak 11: 8.14 (d, J_{46} = 2 Hz, pyridine H), 7.24 (d, J_{46} = 2 Hz, pyridine H), 3.0–3.4 (m, 1 H), 2.7–3.05 (m, 1 H), 2.65 (d, 2 H), 1.9–2.4 (m, 1 H), 1.3 (d, 2 CH_3), 1.2 (d, 2 CH_3), 0.9 (d, 2 CH_3)

Chichibabin (1924) synthesized 2,3,5-trialkylpyridine from aldehyde and ammonia but did not provide the detailed information for the formation mechanism. Virnin and Parkanyi (1982) described the formation mechanism in three steps: (i) aldol condensation to 2,4-dienal, (ii) imine formation from ammonia, and then (iii) cyclization and oxidation to pyridine. However, if based on that mechanism, cyclization of the imine from isovaleraldehyde

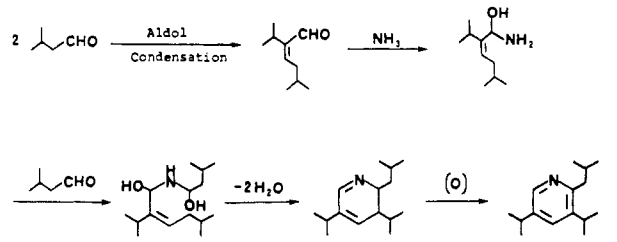


Figure 3. Possible mechanism of the formation of 2-isobutyl-3,5-diisopropylpyridine.

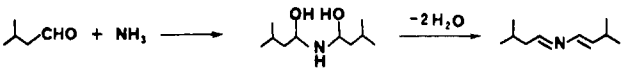


Figure 4. Possible mechanism of the formation of 3-methyl-*N*-(3-methylbutylidene)butenylamine.

should yield 1,2-dihydro-2-isobutyl-3,5-diisopropylpyridine instead of the 2,3-dihydro corresponding pyridine. On the contrary, from the current study, the 2,3-dihydro corresponding pyridine has been found; therefore, an alternate formation mechanism (Figure 3) has been suggested.

Formation of 3-methyl-*n*-(3-methylbutylidene)butenylamine isomers may be via the mechanism shown in Figure 4.

Peaks 7 and 9 were 2,4,6-triisobutyl-1,3,5-trioxanes, which were the isovaleraldehyde trimers formed by oxidation. None of the components from peak 1 to peak 11 showed bacon aroma.

Registry No. Isovaleraldehyde, 590-86-3; 5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine, 74595-94-1; 3,5-diisobutyl-1,2,4-trithiolane, 92900-67-9; 2-isobutyl-3,5-diisopropylpyridine, 7033-68-3; 2-methyl-2-propanol, 75-65-0; trimethylacetaldehyde, 630-19-3; isoamyl alcohol, 123-51-3; 3-methyl-*N*-(3-methylbutylidene)butenylamine, 92900-68-0; 2,4,6-triisobutyl-1,3,5-trioxane, 68165-40-2; diisobutyl disulfide, 1518-72-5; 2,3-dihydro-2-isobutyl-3,5-diisopropylpyridine, 92900-69-1; ammonium sulfide, 12135-76-1.

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Partial Characterization of the Amylase Inhibitor of Black Beans (*Phaseolus vulgaris*), Variety Rico 23

Franco M. Lajolo* and Flavio Finardi Filho

The amylase inhibitor of black (kidney) beans (*Phaseolus vulgaris*) has an apparent molecular weight of 53 000 by gel filtration, an isoelectric point of 4.35, and a sedimentation coefficient ($s^0_{20,w}$) of 4.4 S. It contains mannose, xylose, galactose (5.4%), and glucosamine (3%) and is highly resistant to proteolysis. The N-terminal amino acids are alanine, glutamic acid, and threonine; the C terminals identified were leucine and tyrosine. The inhibitor can be dissociated into three different subunits that can reassociate with complete restoration of activity but it's molecular structure is modified as shown by the fluorescence and circular dichroism spectra. The inhibitor is active against mammalian α-amylases and amyloglucosidase from *Rhizopus genus* and *Aspergillus niger*; it is not active against *Bacillus subtilis* or *Aspergillus orizae* α-amylases. The optimum pH for inhibitor is 4.5 for salivary and 5.5 for pancreatic α-amylase. Several anions, nitrate > chloride > bromide > iodide > thiocyanate, increase the rate of salivary amylase inhibition.

Proteinaceous inhibitors of amylases have been detected in different organs of several vegetable species: in cereal seeds such as wheat (Buonocuore et al., 1977), rye (Granum, 1978), triticale (Finardi Filho and Lajolo, 1982), corn (Blanco-Labra and Iturbe-Chiñas, 1981), and ragi (Shivaraj and Pattabiraman, 1980) and in legumes such as kidney beans (Mancini Filho and Lajolo, 1981), peanuts (Irshad and Sharma, 1981), black grams (Reddy and Salunkhe, 1980), and chickpeas (Singh et al., 1982). They were also found in tubers (Shivaraj et al., 1979) and fruits (Mattoo and Modi, 1970). Their proteinaceous nature differentiates them from the low molecular weight oligosaccharide and peptide amylase inhibitors produced by microorganisms (Frommer et al., 1979), but very little is still known of its chemical properties and physiological functions.

The presence of amylase inhibitors in kidney (navy) beans was reported as early as 1945 (Bowman, 1945) and rediscovered by Jaffé and Lette (1968). Only recently they started to receive a more systematic investigation, contrary to what happened to the inhibitors found in wheat that have been more studied and already have their primary structure established (Kashland and Richardson, 1981). More recently Marshall and Lauda (1975), Pick and Wöber (1978), and Powers and Whitaker (1977a) established techniques for isolation and partially characterized the

Depto. de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas—USP Conj. das Químicas, B. 14—Caixa Postal 30786—São Paulo, SP, Brazil.

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