

Formation of Meatlike Aroma Compounds from Thermal Reaction of Inosine 5'-Monophosphate with Cysteine and Glutathione

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Sulfur-containing amino acid cysteine (Cys) and tripeptide glutathione (γ -Glu-Cys-Gly) in its reduced form (GSH) were reacted with the flavor enhancer inosine 5'-monophosphate (IMP) in an aqueous medium. After mixing, the dissolved solution was heated for 1 h at 180 °C. A roasted coffee and cooked meatlike aroma with strong sulfur notes was observed for both reaction masses. The volatile compounds produced in model systems were analyzed by GC and GC/MS. A total of 47 compounds were identified, only 6 of which were non-sulfur-containing compounds. They were mainly thiophenes, thiazoles, sulfur-substituted furans, and polysulfides. The characteristic meatlike compound, 2-methyl-3-furanthiol, was only detected in the reaction between inosine 5'-monophosphate and cysteine. Fewer polysulfides were identified in the model system of IMP-GSH than the model system of IMP-Cys.

INTRODUCTION

Since the first isolation of inosine 5'-monophosphate by Kuninaka, nucleotides, such as inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP), have been found to possess the synergistic flavor enhancer property. Due to low dosage and high efficiency, IMP partially replaced the monosodium glutamate in some foodstuff (Duff, 1980). However, IMP or GMP was found unstable under certain food-processing conditions, such as in canning. The low pH and high temperature treatment caused the hydrolysis of both phosphate and glycosidic bonds of the nucleotides (Shaoul and Sporns, 1987; Nguyen and Sporns, 1985), and the degradation mechanism was investigated by Matoba et al. (1988). The liberated components such as reducing sugar ribose must be very active to be involved in the formation of aroma compounds, but there is no literature available on volatile aroma compound formations from the nucleotide degradation or the interaction with other food components. In 1969, Tonsbeek isolated a cold-water-soluble nucleotide from beef and reported that it was the precursor for the formation of meatlike aroma compounds.

Sulfur-containing amino acids, such as cysteine and cysteine, are indispensable components for generating meatlike aromas through a reaction with reducing sugars (MacLeod, 1986; Werkhoff et al., 1990; Grosch et al., 1990). In a previous study, we have learned that cysteine was a good sulfur and nitrogen source for the formation of meatlike aroma compounds and glutathione was better able to supply the sulfur source to form the polysulfur-containing aroma compounds (Zhang et al., 1988; Zhang and Ho, 1989). When cysteine and glutathione both reacted with glucose respectively, more sugar-amino acid interaction products were produced in cysteine than in glutathione (Zhang and Ho, 1991). This paper presents an example of the thermal generation of aroma compounds by the further reaction of hydrolyzed IMP products under acidic aqueous conditions.

EXPERIMENTAL PROCEDURES

Sample Preparation. A total of 1.741 g (0.005 mol) of inosine 5'-monophosphate (free acid, grade V, from Torula yeast, 98-100% pure, Sigma Chemical Co., St. Louis, MO) was mixed with 0.606 g (0.005 mol) of cysteine (reagent grade, Sigma) or 1.537 g (0.005 mol) of glutathione (reduced form, reagent grade,

Sigma) respectively. The mixture was dissolved in 100 mL of distilled water, and the pH value of the solution of each mixture was measured as pH 2.3 and pH 2.2 with a pH meter. The mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder, and the cylinder was sealed and heated at 180 °C in an oil bath for 1 h. The reaction mass then was simultaneously solvent-extracted and steam-distilled by using redistilled dichloromethane in a Likens-Nickerson apparatus. Internal standards for the quantitation were added before distillation and extraction. A portion of 0.5 mL of tridecane solution in dichloromethane (1.886 mg/mL) was added to each sample as an internal standard. The distillates or extracts were dried over anhydrous sodium sulfate and concentrated with a Kuderna-Danish apparatus to a final volume of 0.5 mL.

Volatile Separation by Gas Chromatography. A Varian 3400 gas chromatograph equipped with an FID and a nonpolar fused silica capillary column [60 m \times 0.25 mm (i.d.), 0.25- μ m thickness, DB-1; J&W] was used to analyze the volatile compounds isolated from the thermal reaction systems. For each sample, 0.2 μ L was injected with a split ratio of 60:1. The GC was run with an injector temperature of 270 °C, a detector temperature of 300 °C, and the helium carrier flow rate of 0.7 mL/min. The temperature program included an initial column temperature of 40 °C, a temperature increase of 2 °C/min from 40 to 220 °C, and a 10-min isothermal period at the final column temperature.

Quantitative determination was accomplished by internal standards. The quantity of each component was finally converted into milligrams of volatiles generated by either 1 mol of cysteine or 1 mol of glutathione. Linear retention indices for the volatile compounds were calculated by using *n*-paraffin standards (C_6 - C_{26} , Alltech Associates) as references (Majlat et al., 1974).

GC/MS Analysis. The concentrated samples were analyzed by GC/MS using a Varian 3400 gas chromatograph coupled to a Finnigan MAT 8230 high-resolution mass spectrometer, using the same GC program as for the separation. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and spectra were recorded on a Finnigan MAT SS 300 data system.

RESULTS AND DISCUSSION

Although some research work has been done on the thermal stability of nucleotides, such as inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP) in aqueous solution (Nguyen and Sporns, 1985; Shaoul and Sporns, 1987; Matoba et al., 1988), literature information on aroma compounds generated by thermal decomposition and reaction of nucleotides is lacking.

Table I. Volatile Compounds Identified from the Interaction of IMP and Cysteine or Glutathione

compound	MW	Ik (DB-1)	amount, mg/mol		I.D.
			IMP-Cys ^a	IMP-GSH ^b	
diacetyl	86	561	41.49	15.54	c
2-butenal	70	578	55.40		c
mercaptopropane	76	624	7.23		c
thiophene	84	650		20.23	c
2,3-pentanedione	100	650	50.25		c
thiazole	85	707	8.70		c, d
1-mercapto-2-propanone	90	743	7.00		c
2-methylthiophene	98	754		21.32	c, d
2,4-pentanedione	100	756	22.99		c
3-mercapto-2-butanone	104	780	4.43	1.24	c
furfural	96	804	1619.40	1070.08	c
2-methyl-3-furanthiol	114	848	9.60		c
furfurylthiol	114	887	384.43	180.49	c
tetrahydrothiophen-3-one	102	897	2.87	32.99	c
1-(2-furyl)-2-propanone	124	919	12.59		t, c
5-methyltetrahydrothiophen-3-one	116	945	69.96	66.05	c
2-methyltetrahydrothiophen-3-one	116	951	16.04	17.26	c
thiophene-2-carboxaldehyde	112	952	1.82		c, d
thiophene-3-carboxaldehyde	112	968	49.40	34.56	c, d
2-acetylthiazole	127	989	3.64	4.76	c
thiopyran-3(2H)-one	114	1003	3.83	3.52	c
3-methyl-2-oxo-2,5-dihydrothiophene	114	1004	16.57		t, c
tetrahydrothiopyran-4-one	116	1011	2.89		t, c
2-acetylthiophene	126	1049	24.56	1.51	c, e
methylformylthiophene	126	1053	28.00	27.21	c, e
3-acetylthiophene	126	1055	3.35		c, d
3-methyl-2-formylthiophene	126	1090	2.62		c, d
anti-3,5-dimethyl-1,2,4-trithiolane	152	1103		1.83	c
syn-3,5-dimethyl-1,2,4-trithiolane	152	1110		0.81	c
2-furfuryl hydrodisulfide	146	1121	2.42		e
3-methyl-5-oxo-1,2-dithiane	148	1164	24.71	50.79	e
3-methyl-4-oxo-1,2-dithiane	148	1175	10.21		c
thieno[3,2-b]thiophene	140	1185	48.00	3.02	c
2-furfuryl methyl disulfide	160	1196		0.41	e
3-acetyl-1,2-dithiolane	148	1208	2.43		c
2-(2-furyl)thiazole	151	1219	2.04		e
5-methylthieno[2,3-d]-thiophene	154	1289	8.00		c
1,2,3-trithia-5-cycloheptene	150	1319	55.60	5.86	c
5-ethylthieno[2,3-d]thiophene	168	1353	2.65		c
6-methyl-1,2,3,4,5-pentathiane	188	1397	2.40		c
2-difurfuryl sulfide	194	1421		t	e
2-furfuryl 2-methyl-3-furyl disulfide	226	1608	3.11		e
di-2-furfuryl disulfide	226	1658	15.29	32.81	e
2-furfuryl 2-thienyl disulfide	228	1736	6.52		e
2-furfuryl 2-thienylmethyl disulfide	242	1820		t	e
di-2-furfuryl trisulfide	258	1901	3.22		e
2-furfuryl 2-thienyl trisulfide	260	1964	22.82		e

^a IMP-Cys, inosine 5'-monophosphate with cysteine. ^b IMP-GSH, inosine 5'-monophosphate with glutathione. ^c Identifications were done by searching with computer or following published sources: Stoll et al. (1967); Heller and Milne (1980); Ten Noever de Brauw et al. (1983); Hartment et al. (1984); Shu (1984); Werkhoff et al. (1990); Farmer and Mottram (1990). ^d Identification confirmed by GC retention index of authentic compound. ^e Tentative identified for the first time or for the substitution position of analogue isomers.

Qualitative examination by GC/MS of volatile compounds generated from the reaction of IMP with cysteine (IMP-Cys) and of IMP and glutathione (IMP-GSH) demonstrated that 47 compounds were identified. Their identification, retention indices, and quantitation data are listed in Table I. Among the compounds identified, 41 compounds were sulfur-containing compounds mostly of the cyclic sulfur-containing class. The major classes of compounds are thiophenes, sulfur-substituted furans, and polysulfides. The identification was accomplished by

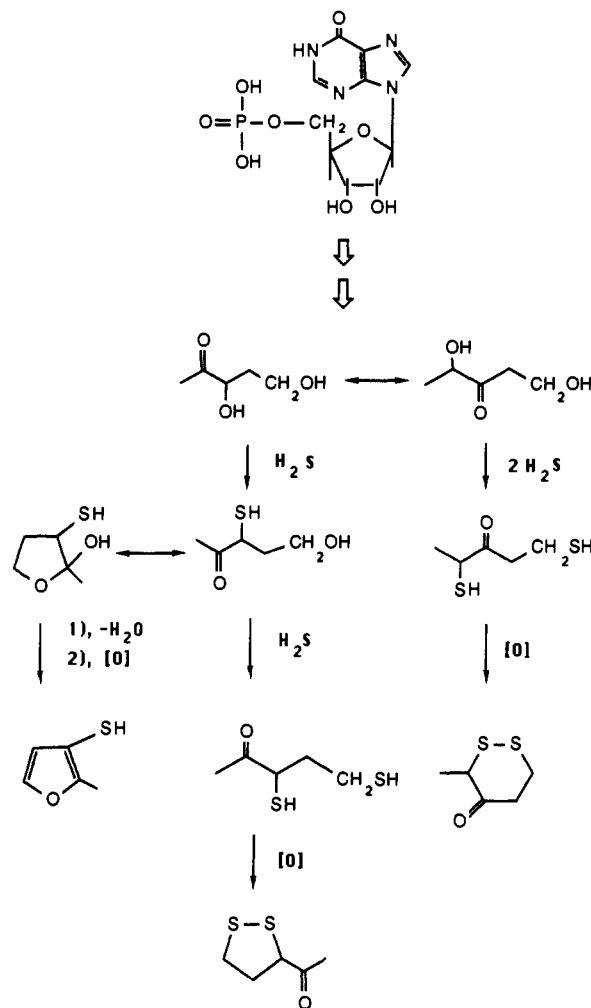


Figure 1. Possible mechanism for the formation of 2-methyl-3-furanthiol and related sulfur-containing compounds from thermal reaction of IMP with cysteine and glutathione.

comparing the mass spectra with those of authentic compounds available in either the computer library (NBS) or the literature (Stoll et al., 1967; Heller and Milne, 1980; Ten Noever de Brauw et al., 1983; Hartman et al., 1984; Shu, 1984; Werkhoff et al., 1990; Farmer and Mottram, 1990).

As shown in Table I, fewer volatile compounds were observed from the reaction of IMP with GSH than from the reaction of IMP with cysteine. This suggests that cysteine or its degradation products favor the hydrolysis of IMP and further subsequential interactions. However, for both systems, there was no pyrazine detected. Except for thiazole and acetylthiazole, there were no other nitrogen-containing volatile compounds identified. This was probably because the carbonyl-amino interaction, as well as the related Strecker degradation and deamination, is not favorable under acidic pH. The two major compounds obtained from both model reaction systems were furfural and furfurylthiol. This clearly indicated that the major pathway for the IMP-Cys and IMP-GSH systems is the liberation of ribose from IMP; thus, the acidic condition favors the dehydration and oxidation of the sugar to form furfural and the further reaction product with hydrogen sulfide, furfurylthiol. Furfurylthiol was found at very high concentrations in the aroma of roasted coffee (Tressl, 1989) and was described as extremely powerful and diffusive and penetrating, and it possessed coffeelike, caramellic burnt, and sweet aromas (Fors, 1983). The high concentration of furfurylthiol detected in both

Table II. Mass Spectral Data of Some Tentatively Identified Sulfur-Containing Compounds from IMP-Cys and IMP-GSH

compound	Ik (DB-1)	mass spectral data m/z (relative intensity)
2-furfuryl hydrodisulfide	1121	146 (2), 114 (5), 82 (10), 81 (100), 53 (47), 51 (11), 45 (21), 39 (2); $M_r = 146$
3-methyl-4-oxo-1,2-dithiane	1164	150 (5), 148 (66), 115 (9), 78 (100), 64 (36), 60 (49), 45 (46), 42 (83), 41 (61), 39 (49); $M_r = 148$
2-furfuryl methyl disulfide	1194	160 (4), 81 (100), 79 (13), 64 (12), 53 (36), 45 (36), 39 (38); $M_r = 160$
2-(2-furyl)thiazole	1219	151 (58), 69 (2), 58 (100), 57 (6), 51 (4), 39 (13); $M_r = 151$
6-methyl-1,2,3,4,5-pentathiane	1397	188 (32), 124 (8), 123 (5), 92 (2), 64 (35), 60 (41), 59 (100), 45 (22); $M_r = 188$
di-2-furfuryl sulfide	1421	194 (3), 113 (9), 85 (8), 82 (9), 81 (100), 53 (31), 45 (26), 39 (10); $M_r = 194$
2-furfuryl 2-methyl-3-furyl disulfide	1608	226 (2), 113 (9), 85 (5), 82 (6), 81 (100), 69 (4), 53 (20), 45 (17), 39 (5); $M_r = 226$
di-2-furfuryl disulfide	1658	226 (2), 161 (1), 113 (0.5), 85 (3), 82 (5), 81 (100), 69 (4), 53 (26), 45 (10), 39 (4); $M_r = 226$
2-furfuryl 2-thienyl disulfide	1736	228 (4), 115 (4), 103 (3), 85 (3), 82 (6), 81 (100), 71 (6), 53 (20), 45 (20), 39 (9); $M_r = 228$
2-furfuryl 2-thienyl-methyl disulfide	1820	113 (3), 99 (4), 97 (54), 82 (5), 81 (100), 69 (4), 53 (34), 45 (25), 39 (17); $M_r = 242$
di-2-furfuryl trisulfide	1901	258 (<0.5), 161 (5), 113 (20), 85 (3), 82 (5), 81 (100), 69 (2), 53 (25), 45 (13), 39 (5); $M_r = 258$
2-furfuryl 2-thienyl trisulfide	1964	196 (5), 178 (1), 115 (7), 85 (3), 82 (5), 81 (100), 71 (9), 53 (23), 45 (22), 39 (11); $M_r = 260$

systems could be the reason the reaction mass possessed a strong roasted coffee, caramellic burnt aroma.

The volatile compounds generated by the thermal reaction of IMP with cysteine and of IMP with glutathione were unique. From previous studies, we have learned that cysteine and glutathione are both good sources of hydrogen sulfide, which are important for the formation of meatlike aroma compounds, such as thialdine (2,4,6-trimethylperhydro-1,3,5-dithiazine), which was commonly found in a wide range of different meats such as cooked beef (Brinkman et al., 1975), mutton (Nixon et al., 1979), and Antarctic krill (Kubota et al., 1980). However, thialdine was not detected at all in both reaction systems. Polysulfides, such as 3,5-dimethyl-1,2,4-trithiolane which was first identified in boiled beef (Chang et al., 1968), are the components associated with meatlike aromas. They are formed from the interaction of aldehydes and hydrogen sulfide (Boelens et al., 1974). Although they are the major volatile compounds formed by cysteine and glutathione degradation, in the presence of IMP, their formations were significantly inhibited. Only a small amount of trithiolane was formed in the IMP-GSH system, and none were formed in the system IMP-Cys. An interesting polysulfide found in the present study is 3-methyl-4-oxo-1,2-dithiane, which was identified in the thermal degradation of thiamine (Hartman et al., 1984). Its presence in the IMP-Cys system shows that a common pathway must exist between the two systems. A postulated pathway is shown by Figure 1. Under high-temperature and acidic condi-

tions, hydrolysis of both the phosphate bond and the glycosidic bond of IMP occurred simultaneously (Shaoul and Sporns, 1987), which leads to the formation of the intermediate, 4-oxo-1,3-pentenediol. After tautomerism and further displacement with hydrogen sulfide, the intermediate undergoes oxidative cyclization to form 3-methyl-4-oxo-1,2-dithiane, 2-methyl-3-furanthiol, and 3-acetyl-1,2-dithiolane (Figure 1). On the other hand, many new furan-substituted polysulfide compounds were formed in the reaction system of IMP-Cys. These are 2-furfuryl 2-methyl-3-furyl disulfide, 2-furfuryl thienyl disulfide, difurfuryl disulfide, furfuryl thienylmethyl disulfide, difurfuryl trisulfide, and 2-furfuryl thienyl trisulfide. Their mass spectral data are listed in Table II. 2-Methyl-3-furanthiol recently has been recognized as being an important character impact compound in the aroma of cooked beef (Gasser and Grosch, 1988). It was only detected in the reaction system of IMP-Cys. Also, more sulfur-containing furans were obtained from this system. These results agree, for the most part, with the recent study on meatlike aroma compounds by Farmer and Mottram (1990). It was found that the formation of sulfur-substituted furans is favored by acidic conditions, the amount being dramatically reduced from pH 3 to pH 6, and almost no polysulfur-substituted furans were detected at a higher pH. However, in a very similar model system, when ribose alone reacted with cysteine or glutathione at pH 5.7, the major volatile compound produced was still 2-methyl-3-furanthiol, and glutathione produced a larger amount than cysteine did (Grosch et al., 1990). Therefore, the pH played a very important role in manipulating the volatile compositions in producing meatlike aroma in model systems.

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Registry No. 5'-IMP, 131-99-7; 2-butenal, 4170-30-3; mercaptopropane, 79869-58-2; 2,3-pentanedione, 600-14-6; thiazole, 288-47-1; 1-mercapto-2-propanone, 24653-75-6; 2,4-pentanedione, 123-54-6; 2-methyl-3-furanthiol, 28588-74-1; thiophene-2-carboxaldehyde, 98-03-3; 3-methyl-2-formylthiophene, 5834-16-2; 3-acetylthiophene, 1468-83-3; 2-furfuryl hydrodisulfide, 133447-31-1; 3-methyl-4-oxo-1,2-dithiane, 90238-76-9; 3-acetyl-1,2-dithiolane, 89712-89-0; 2-(2-furyl)thiazole, 42140-91-0; 5-methylthieno[2,3-d]thiophene, 26238-22-2; 5-ethylthieno[2,3-d]thiophene, 5912-01-6; 6-methyl-1,2,3,4,5-pentathiane, 133447-32-2; 2-difurfuryl sulfide, 13678-67-6; 2-furfuryl 2-methyl-3-furyl disulfide, 109537-55-5; 2-furfuryl 2-thienyl disulfide, 133447-33-3; 2-furfuryl 2-thienylmethyl disulfide, 133447-34-4; di-2-furfuryl trisulfide, 71243-23-7; 2-furfuryl 2-thienyl trisulfide, 133447-35-5; thiophene, 110-02-1; 2-methylthiophene, 554-14-3; anti-3,5-dimethyl-1,2,4-trithiolane, 38348-24-2; syn-3,5-dimethyl-1,2,4-trithiolane, 38348-23-1; 2-furfuryl methyl disulfide, 57500-00-2; diacetyl, 431-03-8; 3-mercapto-2-butanone, 40789-98-8; furfural, 98-01-1; furfurylthiol, 98-02-2; tetrahydrothiophen-3-one, 1003-04-9; 1-(2-furyl)-2-propanone, 6975-60-6; 5-methyltetrahydrothiophen-3-one, 50565-24-7; 2-methyltetrahydrothiophen-3-one, 13679-85-1; thiophene-3-carboxaldehyde, 498-62-4; 2-acetylthiazole, 24295-03-2; thiopyran-3(2H)-one, 29431-30-9; 3-methyl-2-oxo-2,5-dihydrothiophene, 33687-85-3; tetrahydrothiopyran-4-one, 1072-72-6; 2-acetylthiophene, 88-15-3; methylformylthiophene, 109578-87-2; 3-methyl-5-oxo-1,2-dithiane, 133447-36-6; thieno[3,2-b]thiophene, 251-41-2; 1,2,3-trithia-5-cycloheptene, 13005-82-8; di-2-furfuryl disulfide, 4437-20-1; cysteine, 52-90-4; glutathione, 70-18-8.