Volatile Compounds Formed from Thermal Degradation of Glucosamine in a Dry System

Jianhong Chen and Chi-Tang Ho*

Department of Food Science, Cook College, Rutgers, The State University, 65 Dudley Road, New Brunswick, New Jersey 08901-8520

Glucosamine was pyrolyzed at 200 °C for 30 min under dry conditions. A roasty aroma was obtained. Volatile compounds generated included pyrazines, pyridines, pyrroles, and furans. The most interesting compounds were pyrazines with one or two furyl substituents. They are 2-(2-furyl)pyrazines, 2-(2-furyl)methylpyrazines, 2-(2-furyl)dimethylpyrazines, 2-(2-furyl)acetylpyrazines, and di-2-furylpyrazines. Some of these furyl pyrazines are being reported for the first time. The mechanism of the furan ring formation in polycyclic pyrazine compounds was studied. The furan ring was suggested as a result of the dehydration reaction from the intermediate polyhydroxypyrazines, which were dimerization products of glucosamine. Model reactions were designed to test the proposed mechanism. When fructose and ammonium acetate were heated under the same conditions as the glucosamine pyrolysis, those furyl pyrazines were also generated.

Keywords: *Glucosamine; 2-amino-2-deoxyglucose; Maillard reaction; volatile compound; pyrazines; furylpyrazines*

INTRODUCTION

Glucosamine, which is also called 2-amino-2-deoxyglucose, is an amino sugar. It is widely distributed in nature and can be found in various plant tissue and animal muscle (Balazs and Jeanloz, 1965; Anderson et al., 1972; Roberts et al., 1972, Alabran and Mabrouk, 1973; Basha, 1992). Glucosamine is the monomer form of chitosan, while *N*-acetylglucosamine is the monomer of chitin. Next to cellulose, chitin is the second most abundant biopolymers in nature, and chitosan is a processing product of chitin. Both chitin and chitosan have been found in a wide range of applications (Knorr, 1984).

Chitin can be used as a tobacco extender and cigarette filter. The comparison of volatile generation between tobacco leaves and chitin was studied at 900 °C (Schlotzhauer et al., 1976). When chitin was pyrolyzed at over 300 °C, acetamide and a series of methyl-substituted pyrazines were generated (Koell and Metzger, 1979; Knorr et al., 1985). Only a few studies on thermal degradation of glucosamine have been reported. When glucosamine was heated at 200 °C for 4 h, 2-methylpyrazine and 2,5-dimethylpyrazine were found to be the major degradation products among others, such as pyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, 2-ethyl-5-methylpyrazine, and pyridine (Wang and Odell, 1973). Glucosamine is relatively stable in strong acid solution. But in less acidic solutions (<0.5 M hydrochloric acid), 2,5-bis(tetrahydroxybutyl)pyrazine and 2-(tetrahydroxybutyl)-5-(3,4-dihydroxy-1-butenyl)pyrazine were found to be the major degradation products of glucosamine. Thermal degradation of glucosamine in an aqueous solution at 150 °C was recently reported (Shu, 1997), and the generation of pyrazines, 3-hydroxypyridine, 1*H*-pyrrole-2-carboxaldehyde, furanones, and hydroxyketones was observed.

The present work studied thermal degradation of glucosamine at 200 °C in a dry system and compared its volatile generation with the pyrolysis of a mixture of fructose and ammonium acetate. The formation mechanism of the furyl pyrazines identified in the systems was also discussed.

MATERIALS AND METHODS

Materials. D-Glucosamine hydrochloride, xylitol, fructose, ammonium acetate, and anhydrous sodium phosphate were purchased from Aldrich Chemical Co. (Milwaukee, WI); white quartz sand (50−70 mesh) was purchased from Sigma Chemical Co. (St. Louis, MO).

Pyrolysis of Glucosamine, Xylitol, or a Mixture of Fructose and Ammonium Acetate. A 20 mmol amount of D-glucosamine hydrochloride, 20 mmol of xylitol, or 20 mmol of fructose with 20 mmol ammonium acetate was ground and mixed with anhydrous sodium phosphate (Na_2HPO_4) and 50 g of quartz sand. Sodium phosphate was added to neutralize hydrochloride in the reactants. The mixture was sealed in a 100 mL stainless steel vessel (Taiatsu, Japan) and then put in a 200 °C oil bath and heated for 30 min. After cooling to room temperature under tap water, the heated mixture was ground and then extracted with 3 × 50 mL of 0.1 M HCl. The pH value of the aqueous solution was adjusted to 11.0 with a 1 M NaOH solution. The solution was then extracted with 3 × 30 mL CH_2Cl_2 after spiking with tridecane as the internal standard. The organic phase was dried over anhydrous sodium sulfate and concentrated to approximately 0.5 mL under a gentle stream of nitrogen gas. The concentrated sample was ready for GC and GC/MS analysis.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. The gas chromatograph was performed on a Varian Model 3400 equipped with a flame ionization detector (FID) and a nonpolar fused silica capillary column (DB-1, 60 m × 0.32 mm (i.d.), 1.0 μm film thickness: J&W Scientific, Folsom, CA). The column temperature was programmed from 40 °C to 260 °C at a rate of 3 °C

* Corresponding author [fax (732) 932-8004; e-mail ho@aesop.rutgers.edu].

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Table 1. Volatile Compounds Identified in the Pyrolysis of Glucosamine (GLU) and Fructose and Ammonium Acetate (FRU−N) at 200 °C

compd	RI[a]	concn[b] (mg/mol) GLU	concn[b] (mg/mol) FRU−N	ref	*m/z* (abundance)
2,3-pentanedione	672	nd[c]	0.7	Lib[d]	
pyrazine	714	0.6	0.6	Lib	
pyridine	728	4.7	1.3	Lib	
dihydro-2-methyl-3(2*H*)-furanone	779	nd	4.6	Lib	
2-methylpyridine	799	1.1	1.4	Lib	
methylpyrazine	802	9.6	21.5	Lib	
2-furfural	808	1.2	16.6	Lib	
2-acetylfuran	888	11.3	19.7	Lib	
2,5-dimethylpyrazine	891	5.2	1.8	Lib	
ethylpyrazine	897	nd	2.3	Lib	
2,3-dimethylpyrazine	900	nd	1.2	Lib	
5-methylfurfural	937	1.0	15.6	Lib	
2-ethyl-5-methylpyrazine	981	nd	1.1	Lib	
trimethylpyrazine	986	nd	1.6	Lib	
2-ethyl-3-methylpyrazine	989	nd	0.7	Lib	
2-acetylpyridine	1018	1.0	0.4	Lib	
2-acetylpyrrole	1048	0.4	1.2	Lib	
3-ethyl-2,5-dimethylpyrazine	1098	nd	0.5	Lib	
5-methyl-2-pyrrolecarboxaldehyde	1105	1.6	nd	Lib	
2-acetyl-3-methylpyrazine	1112	0.3	nd	Lib	
2-(2-furyl)pyrazine	1255	3.4	1.2	Friedel et al. (1971)	147 (10), 146 (100), 145 (10), 118 (17)
				Baltes et al. (1987)	117 (12), 93 (60), 92 (20), 64 (28), 63 (32), 53 (15), 39 (26)
2-(2-furyl)-5(6)-methylpyrazine	1346	1.6	1.3	Friedel et al. (1971)	161 (10), 160 (100), 159 (11), 131 (16), 93
				Baltes et al. (1987)	(30), 92 (86), 64 (36), 63 (42), 52 (5), 40 (18), 39 (44), 38 (24)
2-(2-furyl)-5(6)-methylpyrazine	1350	11.3	3.3	Friedel et al. (1971)	161 (11), 160 (100), 159 (7), 131 (18), 93
				Baltes et al. (1987)	(34), 92 (79), 64 (32), 63 (39), 52 (11), 40 (11), 39 (44), 38 (22)
2,2′-bipyridine	1444	0.5	nd	Lib	157 (16), 156 (100), 155 (42), 130 (16), 129 (24), 128 (33), 78 (27), 52 (21), 51 (46), 50 (25)
2-(2-furyl)dimethylpyrazine	1452	0.4	trace	N/A[e]	175 (10), 174 (78), 173 (21), 159 (12), 145 (5), 93 (14), 92 (100), 64 (30), 63 (40), 53 (11), 52 (15), 39 (36)
2-(2-furyl)-5(6)-acetylpyrazine	1573	0.5	nd	N/A	189 (9), 188 (66), 187 (6), 173 (19), 160 (34), 159 (6), 146 (26), 145 (43), 119 (12), 118 (58), 93 (13), 92 (23), 64 (28), 63 (55), 53 (12), 43 (100), 39 (27)
1,2-di(2-pyrazyl)ethane	1626	0.9	nd	Lib	187 (8), 186 (59), 185 (100), 171 (16), 158 (20), 132 (36), 131 (46), 118 (12), 107 (86), 93 (10), 80 (22), 79 (24), 66 (24), 53 (42), 52 (48), 51 (25), 39 (75)
2,5-di-(2-furyl)pyrazine	1764	0.3	trace	N/A	213 (17), 212 (100), 211 (15), 183 (19), 155 (13), 106 (6), 93 (26), 92 (63), 64 (35), 63 (56), 51 (15), 39 (29)
2,5-di(2-furyl)pyrazine	1780	3.9	trace	N/A	213 (14), 212 (100), 183 (15), 156 (10), 155 (17), 106 (6), 93 (9), 92 (42), 64 (35), 63 (48), 51 (7), 39 (20)

[a] Retention index, calculated according to the retention time of *n*-alkanes on DB-1 column. [b] Milligrams of volatile per mole of reactant(s). [c] Not detected. [d] Identification refers to Wiley mass spectra library. [e] Reference not available.

/min. The injector and detector temperatures were maintained at 270 and 300 °C, respectively. The flow rate of helium carrier gas was 1 mL/min. The volume of the injected sample was 1 μL, and the spilt ratio was 25:1. GC/MS analysis was performed using an HP Model 5790 GC coupled with an HP 5970A mass-selective detector. The capillary column and temperature program were the same as in the GC analysis. Mass spectra were obtained by electron ionization at 70 eV and a mass scan from 33 to 300. Quantification was based on GC/FID data, and compound identification was based on mass spectra obtained from the GC/MS.

RESULTS AND DISCUSSION

Roasty, smoky, and acidic flavors were generated from the pyrolysis of glucosamine, while roasty, caramel aroma was observed in the pyrolysis of fructose with ammonium acetate. The composition of isolated volatiles in both reaction mixtures is listed in Table 1. The compounds were listed according to their retention indices. A total of 29 compounds were identified in these two reactions. The identified compounds included furans, pyridines, pyrroles, pyrazines, and others.

The major compounds generated when glucosamine was pyrolyzed at 200 °C for 30 min were 2-(2-furyl)-6-methylpyrazine, 2-acetylfuran, methylpyrazine, 2,5-dimethylpyrazine, pyridine, 2,6-di(2-furyl)pyrazine, 2-(2-furyl)pyrazine, 5-methyl-2-pyrrolecarboxaldehyde, and 5-methylfurfural in decreasing order.

Pyrazine compounds are the most important degradation products among the volatiles identified by pyrolysis of glucosamine. These compounds are widely distributed in food systems, especially when foods are processed at high temperatures and under dry conditions. A series of extensive reviews on pyrazines in food were published (Maga, 1982, 1992). There are several precursors and pathways for pyrazine compound generation. The α-aminocarbonyls, which can be formed from the reaction between dicarbonyl compounds and amino acids during Strecker degradation, were generally considered to be the precursors of pyrazine compounds. An alternative pyrazine formation pathway is also recognized in the pyrolysis of such β-hydroxy amino acids as serine and threonine (Reese and Baltes, 1992). Those β-hydroxy amino acids may react with each other to

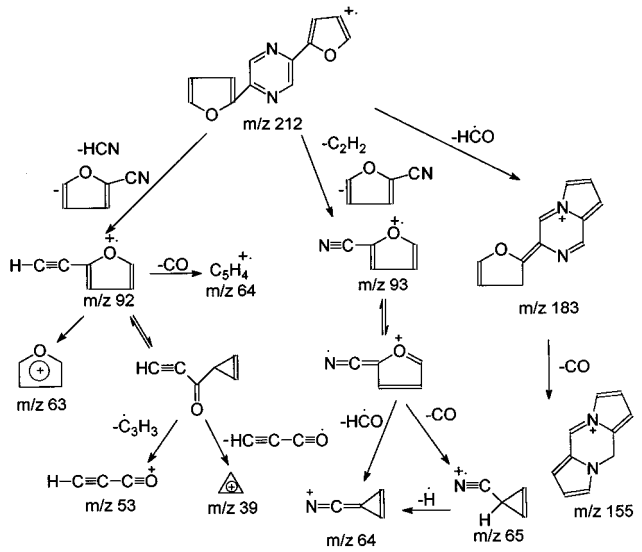

Figure 1. Fragmentation of 2,5-di(2-furyl)pyrazine and the proposed fragment structures.

generate pyrazines under thermal processing, without involving reducing sugar (Baltes and Bochmann, 1987). Some amino sugars such as glucosamine have an α-aminocarbonyl structure; they can interact with each other to form pyrazine compounds without a reducing sugar.

The most interesting compounds identified from the pyrolysis of glucosamine were furyl-substituted pyrazines such as 2-(2-furyl)pyrazine, 2-(2-furyl)-5(and 6)-methylpyrazine, 2-(2-furyl)-dimethylpyrazine, 2-(2-furyl)-5(or 6)-acetylpyrazine, and 2,5(and 2,6)-di(2-furyl)-pyrazine. Some of these have been previously reported. 2-(2-Furyl)pyrazine has been reported in the volatiles of coffee (Friedel et al., 1971), and 2-(2-furyl)pyrazine and 2-(2-furyl)-5(and 6)-methylpyrazine were generated when serine or threonine was pyrolyzed with sucrose (Baltes and Bochmann, 1987). Most recently, furyl-substituted pyrazines were reported in the reaction of glutamine and glucose under simulated deep-fat frying conditions (Chun and Ho, 1997).

Baltes and Bochmann (1987) have proposed the mass fragmentation pattern for the furyl-substituted pyrazines. The common mass spectrometrical behavior of these pyrazines is characterized by the loss of HCO radical from the furan ring and leads to an ion of a bicyclic system which includes a nitrogen atom. Figure 1 shows the proposed mass fragmentation pathway for 2,5-di-2-furylpyrazine.

The suggested formation pathway for 2,5-di-2-furyl-pyrazine identified in this study is shown in Figure 2. As an α-aminocarbonyl compound, two molecules of glucosamine react with each other to form polyhydroxy-dihydropyrazine and then oxidize to polyhydroxypyra-zine. Such polyhydroxypyrazine compounds have been previously reported. 2,5-Bis(tetrahydroxybutyl)pyra-zine (fructosazine) was the major nonvolatile compound found in the reaction mixture of glucosamine and lysine under simulated physiological conditions (Candiano et al., 1990), and fructosazine and deoxyfructosazine were formed when glucosamine was incubated at room temperature in a pH 11 buffered solution for 3 weeks (Sumoto et al., 1991). The ring formation and dehydration of polyhydroxypyrazines will lead to furyl-substituted pyrazines.

It is of interest to understand the formation mechanism of the furan rings in furyl pyrazines. The well-

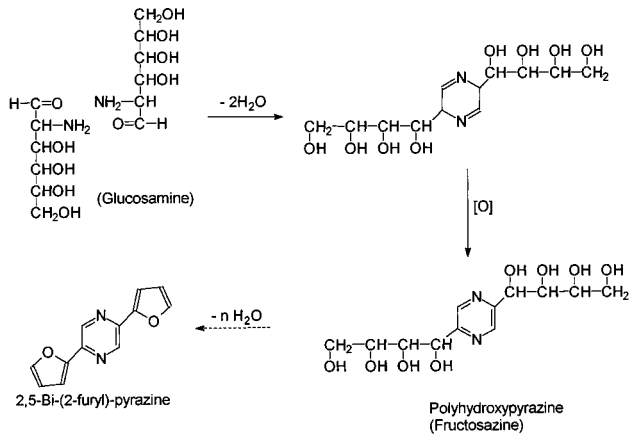

Figure 2. Suggested formation pathway of 2,5-di(2-furyl)pyra-zine from glucosamine.

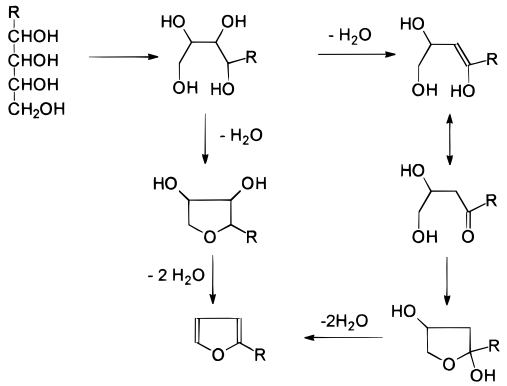

Figure 3. Suggested pathway of furan ring formation from dehydration of polyhydroxyl groups.

known pathway for the formation of a furan or pyran ring is the interaction between a carbonyl group and a hydroxyl group. In the case of furyl-substituted pyra-zines, the furan ring is supposed to be the dehydration product of hydroxyl groups in a polyhydroxylpyrazine, as shown in Figure 3. A high-temperature and dry condition may facilitate such a dehydration process. To prove this hypothesis, xylitol, a five carbon sugar alcohol, was pyrolyzed under the same heating condi-tions as glucosamine. The formation of 2-(hydroxymeth-yl)furan was indeed observed. It provided evidence that the furan ring can be a dehydration product of the hydroxyl groups in polyhydroxypyrazine.

It is thought that glucosamine is an intermediate during the Maillard reaction such as the reaction between the fructose and ammonia source. A mixture of fructose and ammonium acetate was pyrolyzed under the same conditions as the pyrolysis of glucosamine. The major compounds identified in this system were meth-ylpyrazine, 2-acetylfuran, 2,5-dimethylpyrazine, 2-fur-fural, 5-methylfurfural, dihydro-2-methyl-3(2H)-furan-one, 2-(2-furyl)methylpyrazine, ethylpyrazine, trimethyl-pyrazine, 2-methylpyridine, pyridine, 2-(2-furyl)pyra-zine, 2-acetylpyrrole, 2,3-dimethylpyrazine, and 2-ethyl-5-methylpyrazine in decreasing order. More impor-tantly, pyrolysis of fructose and ammonium acetate also generated some furyl-substituted pyrazine compounds that were identified in the pyrolysis of glucosamine. Figure 4 shows the pathway of glucosamine formation during fructose and ammonium acetate reaction. It is therefore assumed that furyl-substituted pyrazines exist in the thermal interaction products of amino acids and sugars.

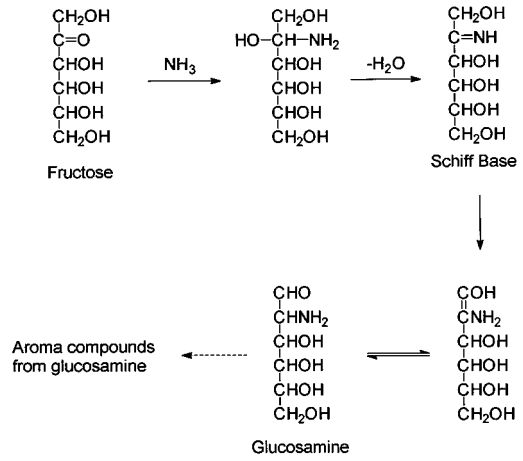

Figure 4. Formation mechanism of glucosamine-specific volatiles in fructose and ammonium acetate reaction.

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