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Volatile compounds generated from thermal interaction of glucose and alliin or deoxyalliin in propylene glycol

Tung-Hsi Yu,[a] Chung-May Wu[b] & Chi-Tang Ho[a,*]

[a] *Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08803, USA*

[b] *Food Industry Research and Development Institute, PO Box 246, Hsinchu, Taiwan*

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Alliin and deoxyalliin, the two important nonvolatile flavor precursors of garlic, were reacted with and without glucose in propylene glycol, respectively. The reactions were accomplished in a closed sample cylinder at 180°C for 1 h. The volatile compounds generated were isolated and analyzed by GC and GC-MS. A considerable amount of allylthio-containing and cyclic sulfur-containing compounds were found in deoxyalliin systems. No allylthio-containing compounds and only a small amount of cyclic sulfur-containing compounds were found in alliin systems. Some pyrazines were identified from both alliin and deoxyalliin model systems even in the absence of glucose. Propylene glycol was proposed to participate in the formation of some pyrazines. More thiophenes were generated in deoxyalliin model systems than in alliin model systems, while more thiazoles were generated in alliin model systems than in deoxyalliin model systems.

INTRODUCTION

γ-Glutamyl alk(en)yl cysteine dipeptides and alk(en)yl cysteine sulfoxides were found to be two important groups of nonvolatile flavor precursors of garlic in intact garlic cloves. Three γ-glutamyl alk(en)yl cysteines, i.e. γ-glutamyl allyl, cysteine, γ-glutamyl (*E*)-1-propenyl cysteine, and γ-glutamyl methyl cysteine have been found in garlic with the first two compounds predominant. Three alk(en)yl cysteine sulfoxides, i.e. allyl cysteine sulfoxide, (*E*)-1-propenyl cysteine sulfoxide, and methyl cysteine sulfoxide, have also been found in intact garlic cloves with the first being the predominant compound. After being heated in boiling water, γ-glutamyl allyl cysteine was converted to *S*-allylcysteine (deoxyalliin); γ-glutamyl (*E*)-1-propenylcysteine was converted to (*E*)-1- and (*Z*)-1-propenylcysteines, and alliin was completely lost in eight hours to unknown compounds (Fenwick & Hanley, 1985; Lawson, 1991*a,b,c*; Ueda *et al.*, 1991; Block, 1992; Block *et al.*, 1992*a,b*, 1993).

In the study of fried garlic flavor, Yu *et al.* (1993) found that both alkenyl cysteine sulfoxides and γ-glutamyl alk(en)yl cysteines could be important contributors to the flavor of fried garlic. In model systems, Yu *et al.* (1994*a,b,c*) reported that synthesized alliin and deoxyalliin could self-degrade or interact with 2,4-decadienal at 180°C and generate volatile flavor compounds in an aqueous solution. Yu *et al.* (1994*d,e*) also found that the synthesized alliin and deoxyalliin could react with glucose or inosine-5'-monophosphate and generate Maillard-type flavor compounds which contributed to the roasted meaty or fried garlic flavor of the model reaction systems in an aqueous solution.

Many factors, such as pH, temperature, water activity and water content, have been reported to have significant effects on the formation of Maillard-type flavor (Shibamoto & Bernhard, 1976; Lingnert, 1990; Reineccius, 1990). Propylene glycol (PG) has previously been used to control the water activity or water content in the study of Maillard flavor generation (Hartman *et al.*, 1984; Güntert *et al.*, 1990). In the present study, the authors also used PG as a reaction medium to simulate the flavor generation of garlic in nonaqueous processing conditions such as frying or baking.

EXPERIMENTAL

Synthesis and purification of deoxyalliin and alliin

Deoxyalliin and alliin were synthesized according to the procedures of Iberl *et al.* (1990) with slight modifi-

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*To whom correspondence should be addressed.

Table 1. Final appearances and flavor descriptions of the model systems

Model system	Final appearance	Flavor Description
Alliin	Slightly dark brown	Sulfury, nutty, burnt and pungent
Alliin + glucose	Dark brown	Fried garlic note with burnt and pungent character
Deoxyalliin	Brown	Pungent garlic with burnt note
Deoxyalliin + glucose	Slightly dark brown	Pungent garlic with fried garlic note

cation as shown in our previous reports (Yu *et al.*, 1994*a,b,c*).

Thermal interactions of glucose and alliin or deoxyalliin in propylene glycol (PG)

Synthesized alliin or deoxyalliin (0·005 mol) was mixed with 0·005 mol of α-D-glucose (Aldrich, 96% purity) in 100 ml of propylene glycol (PG or 1,2-propanediol, Aldrich, 99% purity). The solution was added to a 0·3 l Hoke SS-DOT sample cylinder (Hoke Inc., Clifton, NJ) and sealed. This cylinder was then heated at 180°C in a GC oven for 1 h. After being cooled to room temperature, an interaction mass of glucose and alliin or deoxyalliin in PG was obtained.

Isolation of the volatile compounds.

The total reaction mass was washed and mixed with 200 ml of distilled water and 50 g sodium chloride, and then was extracted by 200 ml of redistilled diethyl ether containing 5 ml of heptadecane stock solution (0·0770 g in 200 ml diethyl ether) in a 1 l flask. Using a separatory funnel, the water layer was again extracted with another 200 ml diethyl ether. The ether layers from the two extractions were combined and then extracted twice with 200 ml of distilled water to remove the remaining PG in the ether layer. After being separated from the water layer, the ether layer was concentrated to appropriate volume using a Kuderna–Danish apparatus fitted with a Vigreaux distillation column. The flavor concentrate was then simultaneously distilled for 2 h and extracted into diethyl ether using a Likens–Nickerson (L–N) apparatus to remove the nonvolatile residues. After being dried over anhydrous sodium sulfate and filtered, the distillate was concentrated again to about 5 ml using a Kuderna–Danish apparatus fitted with a Vigreaux distillation column, then slowly concentrated further under a stream of nitrogen in a small sample vial to a final volume of 0·2 ml.

Gas chromatographic analysis

A Varian 3400 gas chromatograph equipped with a fused silica capillary column (60 m × 0·25 mm i.d.; 1 μm thickness, DB-1, J & W Inc., Folsom, CA) and a flame ionization detector was used to analyze the volatile compounds. The operating conditions were as follows: injector temperature, 270°C; detector temperature, 300°C; helium carrier flow rate, 1 ml/min; temperature program, 40°C (5 min), 2°C/min, 260°C (60 min). A split ratio of 50 : 1 was used.

Gas chromatography–mass spectrometry (GC–MS) analysis

The concentrated isolate was analyzed by GC–MS using a Hewlett-Packard 5840A gas chromatograph coupled to a Hewlett-Packard 5985B mass spectrometer equipped with a direct split interface and the same column as used for the gas chromatography. The operating conditions were the same as described above. Mass spectra were obtained by electron ionization at 70 eV and an ion source temperature of 250°C.

Identification of the volatile compounds

Identification of the volatile compounds in the isolate was mostly based on GC–MS, and information from the GC retention index (I_k) used a C_5–C_{25} mixture as a reference standard. The structural assignment of volatile compounds was accomplished by comparing the mass spectral data with those of authentic compounds available from the Browser–Wiley computer library, NBS computer library or previously published literature (Yu *et al.*, 1989; 1993, 1994*c*). The retention indices were used for the confirmation of structural assignments.

RESULTS AND DISCUSSION

The final appearances and flavor descriptions of the reaction mass of alliin, alliin with glucose, deoxyalliin, and deoxyalliin with glucose in PG are listed in Table 1. The color development in the reaction systems clearly shows that the degradation and/or the interaction of reactant(s) did proceed drastically. It is very interesting to note that the isolate from the alliin model system possessed sulfury, nutty, burnt and pungent flavor; the isolate from the alliin + glucose model system possessed a fried garlic note with burnt and pungent character; the isolate from the deoxyalliin model system possessed a pungent garlic odor with burnt and pungent note; and the isolate from the deoxyalliin + glucose model system possessed a pungent garlic odor with a fried garlic note.

The identification and quantification of the volatile compounds generated from alliin and alliin + glucose model systems are shown in Table 2. The identification and quantification of the volatile compounds generated from deoxyalliin and deoxyalliin + glucose model systems are shown in Table 3. Comparisons of the yields of volatile compounds generated in the model systems

Table 2. Volatile compounds identified from (A) alliin in PG, and (B) alliin + glucose in PG model reaction systems

Compound[a]	MW	RI[b]	mg/mole of alliin[c]	
			A	B
Aldehydes				
Acetaldehyde	44	<500	21·0	155·5
Tolyl aldehyde	178	1068	4·0	nd
2-Methylbenzaldehyde	120	1085	nd	29·0
Pyridines				
2-Methylpyridine	93	799	49·5	14·9
4-Methylpyridine	93	848	4·9	1·0
Dimethylpyridine	107	905	4·5	nd
3-Ethylpyridine	107	935	nd	2·7
2-Propylpyridine	121	980	2·3	nd
Ethylpyridine	107	989	2·2	nd
Methyl ethylpyridine (isomer)	121	1010	79·9	25·0
Methyl ethylpyridine (isomer)	121	1025	17·1	nd
Methyl vinylpyridine (isomer)	119	1032	6·1	32·6
Methyl vinylpyridine (isomer)	119	1065	6·0	22·9
Methyl vinylpyridine (isomer)	119	1073	26·2	nd
3-Ethyl-2,5-dimethylpyridine	136	1084	9·5	29·0
4-Isopropenylpyridine	119	1095	nd	25·1
Thiazoles				
Methylthiazole	99	786	15·3	5·0
4,5-Dihydro-2-methylthiazole	101	859	2·7	nd
2,4- or 2,5-Dimethylthiazole	113	868	2·9	1·0
2-Ethylthiazole	113	887	0·8	1·1
2-Ethyl-4,5-dihydrothiazole	115	958	4·5	nd
2-Propylthiazole	127	969	1·5	1·8
2-Acetylthiazole	127	991	75·0	65·3
Dimethylthiazole	113	1142	nd	34·6
4-Isopropyl-2-methylthiazole	141	1230	4·7	37·0
Pyrazines				
2,5-Dimethylpyrazine	108	891	54·1	19·7
Ethylpyrazine	108	898	15·7	18·3
2,3-Dimethylpyrazine	108	900	nd	2·0
Vinylpyrazine	106	908	nd	17·4
2-Ethyl-3-methylpyrazine	122	981	nd	13·1
2-Ethyl-5-methylpyrazine	122	990	nd	5·8
2-(2'-Furyl)pyrazine	146	1288	nd	16·8
Thiophenes				
5-Methyl-2-formylthiophene	110	929	nd	2·1
2-Formyl-5-methylthiophene	126	1132	1·1	nd
Cyclic sulfur-containing compounds				
1,2-Dithiacyclopentane	106	963	9·4	24·4
1,2,3-Trithiacyclohexane	138	1195	10·9	46·2
Methyl-1,2,3,4-tetrathiane (isomer)	170	1270	8·3	13·0
Methyl-1,2,3,4-tetrathiane (isomer)	170	1369	10·2	50·2
3,6-Dimethyl-1,2,5-trithiepane	180	1444	7·2	37·8
4-Ethyl-6-methyl-1,2,3,5-tetrathiane	198	1565	29·1	14·5
Others				
1-Propene	42	<500	10·7	15·0
Ethyl acetate	88	602	6·8	24·3
2,3-Dihydo-1H-indole	119	1070	19·5	32·6
Methyl-1,2,3,4-tetrahydroquinoline	147	1277	14·0	25·8
5,6,7,8-Tetrahydro-3-methyl-quinoline	147	1301	nd	7·4
1-Ethyl-1H-indole	145	1310	0·6	14·0
Tolyaldehyde propylene glycol acetal	178	1376	14·8	8·9
6-Methyllilolidine	173	1498	3·6	6·7
Total			556·6	899·5

[a] All identifications are based on the comparisons of GC and MS properties with those of literature data and should be considered tentative.

[b] Calculated Kovat's retention indices.

[c] These quantities are based on an equivalent amount of internal standard.

MW, Molecular weight.

nd, Not detected.

Table 3. Volatile compounds identified from (A) deoxyallin in PG, and (B) deoxyalliin + glucose in PG model reaction systems

Compound[a]	MW	RI[b]	mg/mole of alliin[c]	
			A	B
Aldehydes				
Acetaldehyde	44	<500	26·0	12·0
4-Heptenal	112	826	30·4	4·3
Pyridines				
2-Methylpyridine	93	799	0·7	4·3
4-Methylpyridine	93	848	2·8	0·5
3-Ethylpyridine	107	933	14·5	nd
3-Ethyl-4-methylpyridine	121	1011	328·5	1·7
Thiazoles				
2,4- or 2,5-Dimethylthiazole	113	899	16·6	nd
2,4-Dimethyl-5-propylthiazole	155	1230	nd	80·4
Pyrazines				
2,5-Dimethylpyrazine	108	891	38·7	36·0
2-Ethyl-5-methylpyrazine	122	980	nd	39·3
2-Ethyl-6-methylpyrazine	122	984	nd	21·9
2-Ethyl-3-methylpyrazine	122	990	2·6	24·4
Isopropenylpyrazine	120	1045	nd	15·6
3-Ethyl-2,5-dimethylpyrazine	136	1067	13·4	80·5
2-Methyl-5-(1-propenyl)pyrazine	134	1077	nd	9·4
Thiophenes				
2-Formylthiophene	112	936	11·5	15·0
3-Formylthiophene	112	970	nd	1·6
2-Methyl-3-methyoxythiophene	128	981	4·6	nd
3-Formyl-2-methylthiophene	126	1086	22·0	50·2
2-(Mercaptoethyl)tetrahydrothiophene	148	1225	120·1	58·4
Allyl sulfur-containing compounds				
Allyl mercaptan	74	579	21·4	18·5
Diallyl sulfide	114	849	159·5	31·3
(Allylthio)acetaldehyde	116	897	6·5	143·3
Diallyl disulfide	146	1073	419·0	500·3
Diallyl trisulfide	178	1292	nd	10·8
Cyclic sulfur-containing compounds				
Mercaptomethylcyclopentane	116	955	37·0	233·6
1,2-Dithiacyclopentane	106	963	8·5	109·7
4-Methyl-1,3-dithiacyclopentane	120	1004	11·5	6·8
2-Methyl-1,3-dithiane	134	1023	117·7	3·9
1,5-Dithiacyclooctane	148	1046	10·0	nd
1,4-Dithiepane	134	1092	81·4	60·0
2-Ethyl-1,3-dithiane	148	1129	146·7	49·0
3,6-Dimethyl-1,4-dithiane	148	1133	114·1	21·6
2-Methyl-1,4-dithiane	148	1158	456·2	87·6
4-Methyl-1,2-dithiepane	148	1169	31·6	46·3
Methyl-1,2,3-trithiacyclopentane	138	1190	15·0	11·9
3,5-Dimethyl-1,2,6-trithiane	166	1262	38·2	nd
3,5-Dimethyl-1,2,6-trithiane	166	1271	88·8	6·1
4-Methyl-1,2,5-trithiepane	166	1293	95·2	6·5
1,2,5-Trithiacyclooctane	166	1297	39·6	8·7
1,2,3,4-Tetrathiepane	170	1352	27·2	8·9
3-Ethyl-5-methyl-1,2,4-trithiolane	166	1401	57·2	26·3
3,6-Dimethyl-1,2,5-trithiepane	180	1428	172·6	78·6
3,6-Dimethyl-1,2,5-trithiepane	180	1443	447·9	89·9
4-Ethyl-6-methyl-1,2,3,5-tetrathiane	198	1563	19·5	21·4
Others				
1-Propene	42	<500	85·8	121·2
5-Methyl-5-hexen-2-one	112	813	13·7	0·8
Unknown [135(100), 81(30), 144(19), 101(10), 145(1·5), 146(0·9)][d]	—	1095	84·6	23·8
Allyl-*N*-methylaniline	147	1214	12·2	9·3
Unknown [68(100), 41(84), 154(81), 136(67), 74(48), 169(40), 97(39), 45(30)][d]	—	1239	195·4	nd
Total			3646·4	2191·6

[a] All identifications are based on the comparisons of GC and MS properties with those of literature data and should be considered tentative.

[b] Calculated Kovat's retention indices.

[c] These quantities are based on an equivalent amount of internal standard.

MW, Molecular weight.

nd, Not detected.

Table 4. Comparison of the yields of volatile compounds generated in the model system

Compounds	Concentration (mg/mole of alliin or deoxyalliin)[a]			
	Alliin	Alliin + glucose	Deoxyalliin	Deoxyalliin + glucose
Aldehydes	25·0	184·5	56·4	16·3
Pyridines	208·2	153·2	346·5	6·5
Thiazoles	107·4	145·8	16·6	80·4
Pyrazines	69·8	93·1	54·7	227·1
Thiophenes	1·1	2·1	158·2	125·2
Allyl sulfur-containing compounds	0·0	0·0	606·4	704·2
Cyclic sulfur-containing compounds	75·1	186·1	2015·9	876·8
Others	70·0	134·7	391·7	155·1
Total	556·6	899·5	3646·4	2191·6

[a] These quantities are based on an equivalent amount of internal standard.

of this study are shown in Table 4. As shown in these tables, the volatile compounds generated from the model systems were aldehydes, pyridines, thiazoles, pyrazines, thiophenes, allylthio-containing compounds, cyclic sulfur-containing compounds, and others.

Aldehydes

Acetaldehyde, tolyl aldehyde, 2-methylbenzaldehyde, and 4-heptanal were identified from the model systems. Acetaldehyde has been proposed to be released from both of alliin and deoxyalliin through thermal degradation (Yu *et al.*, 1994*a,b,c*). The acetaldehyde molecules released may interact with each other or react with other reactive degradation products of alliin or deoxyalliin to form other volatile flavor compounds identified in the model systems. Acetaldehyde could also be generated from the degradation of glucose through retro-aldol condensation. This may be the reason that higher amounts of acetaldehyde were produced in alliin + glucose system.

Pyridines

A significant amount of pyridine compounds, especially 2-methylpyridine, 3-ethyl-4-methylpyridine, and methyl vinylpyridines, was identified in the model systems. These pyridines are proposed to be mainly generated from the interactions of ammonia, one of the thermal degradation products of alliin or deoxyalliin and aldehydes, especially acetaldehyde. These pyridines identified are considered to be important contributors of the pyridine note detected in the model systems. None of these pyridines was identified from alliin + glucose and deoxyalliin + glucose aqueous model reaction systems (Yu *et al.*, 1994*d*). This strongly suggested that the nonaqueous condition of propylene glycol favored the formation of these pyridines in the model systems in this study.

Thiazoles

Several thiazoles were identified in the model reaction systems. 2-Acetylthiazole was found to be the predominant thiazole in both of alliin and alliin + glucose model systems and was not detected in deoxyalliin and deoxyalliin + glucose systems. The differences in the yields of 2-acetylthiazole between alliin and deoxyalliin systems could result in the different thermal degradation patterns of alliin and deoxyalliin in PG. It is proposed that the bond between the sulfur atom and the allyl group in alliin breaks down thermally and more easily than that between the sulfur atom and the amino end of the alliin molecule. The fragment containing sulfur and amino groups degraded from alliin could then generate 2-acetylthiazole through the thermal interactions of its further degradation products. Upon heating, the bond between the sulfur atom and the amino end in deoxyalliin should break down more easily than the bond between the sulfur atom and the allyl group in deoxyalliin and hence favor the formation of allyl mercaptan. 2,3-Sigmatropic rearrangement of alliin in aqueous solution has been proposed to generate allyl alcohol and cysteine. The interactions of the degradation products of cysteine which released from alliin could favor the formation of thiazoles (Yu *et al.*, 1993, 1994*a,b,c,d,e*). However, the 2,3-sigmatropic rearrangement was probably not favorable in the PG solution used in this study. This was supported by the absence of allyl alcohol in alliin systems in this study.

Pyrazines

Several pyrazines have been identified in the model systems in this research. It was interesting to find some pyrazines, i.e. 2,5-dimethyl-, ethyl-, 2-ethyl-3-methyl-, and 3-ethyl-2,5-dimethylpyrazines were formed from alliin in PG and deoxyalliin in the PG systems. These pyrazines are, therefore, thought to be generated from the interactions of oxidized propylene glycol and the amino group in alliin or deoxyalliin or ammonia released from alliin or deoxyalliin.

As shown in Table 4, more pyrazine compounds were generated in the deoxyalliin + glucose system than in the alliin + glucose system. The differences in the yields of pyrazine compounds between the alliin + glucose and deoxyalliin + glucose systems probably resulted from the differences of pHs in these two systems, as discussed in the authors previous report (Yu *et al.*, 1994*d*). The more acidic conditions in the glucose + alliin system may result in a low unprotonated amino group to protonated amino group ratio. The lower unprotonated amino group concentration in glucose + alliin system not only resulted in less pyrazine compound formation, but also resulted in a lower yield of volatile compounds. The protonated amino group may also have a catalytic effect on the degradation of alliin or deoxyalliin, and the interactions of glucose and alliin or deoxyalliin.

The competition for the consumption of ammonia to form pyridines and pyrazines was found in deoxyalliin model systems. The ammonia released from deoxyalliin was found favorable to the formation of pyridine compounds; however, the ammonia released in the deoxyalliin + glucose system was found favorable to the formation of pyrazines. Therefore, the presence or absence of the degradation products of glucose may result in the difference in the yields of pyridines and pyrazines between the deoxyalliin and deoxyalliin + glucose model systems.

Thiophenes

As shown in Table 4, a significant amount of thiophenes, especially 2-(mercaptoethyl)tetrahydrothiophene and 3-formyl-2-methylthiophene, was found in deoxyalliin model systems. However, only small amounts of these thiophene compounds were found in alliin model systems. The thiophenes identified in this study are proposed to be mainly generated from the interactions of hydrogen sulfide and thermal degradation products, especially aldehydes, of alliin or deoxyalliin. The difference in the concentration of hydrogen sulfide between alliin and deoxyalliin model systems could lead to the difference in the yields of the thiophene compounds. Deoxyalliin is proposed to generate more hydrogen sulfide and therefore favor the formation of thiophene compounds.

Allylthio-containing compounds

High amounts of allylthio-containing compounds were generated in deoxyalliin model systems. However, none of these compounds was detected in alliin model systems. As already discussed, the differences in the yields of the allyl sulfur-containing compounds could result from the difference in the thermal degradation patterns between alliin and deoxyalliin. Allyl mercaptan released from deoxyalliin could interact themselves or with other degradation products of deoxyalliin to generate the allylthio-containing compounds identified in deoxyalliin model systems. Most of these allyl sulfur-containing compounds were also generated in large amounts in the aqueous deoxyalliin model systems (Yu *et al.*, 1994*d*). The presence of water in the model systems is therefore not important to the formation of these allylthio-containing compounds.

Cyclic sulfur-containing compounds

A high amount of cyclic sulfur-containing compounds was generated in deoxyalliin model systems and only a small amount of these compounds was detected in alliin model systems. As shown in the authors' previous reports (Yu *et al.*, 1994*a,b,c,d,e*), allyl mercaptan, acetaldehyde, ammonia, and hydrogen sulfide could be released from deoxyalliin and then the interactions of these compounds would lead to the cyclic sulfur-containing compounds identified in deoxyalliin model systems. Most of the formation mechanisms of these cyclic sulfur-containing compounds have been reported before (Yu *et al.*, 1994*a*).

CONCLUSIONS

Volatile compounds identified in the model systems in this study were aldehydes, pyridines, thiazoles, pyrazines, thiophenes, allylthio-containing compounds, cyclic sulfur-containing compounds, and miscellaneous compounds. The formation of thiazoles was favored in alliin model systems; the formation of pyrazines, thiophenes, allylthio-containing compounds, and cyclic sulfur-containing compounds were favored in deoxyalliin model reaction systems.

Several pyrazines, i.e. 2,5-dimethylpyrazine, ethylpyrazine, 2-ethyl-3-methylpyrazine, and 3-ethyl-2,5-dimethyl pyrazine, could be generated from the thermal degradation of alliin or deoxyalliin in PG without glucose.

The degradation pattern of alliin in PG was found to be significantly different from that in water. Allyl alcohol was not detected in the model systems in this study; the 2,3-Sigmatropic rearrangement of alliin was, therefore, unlikely to occur in the PG system.

A significant amount of pyridines was generated from both alliin and deoxyalliin in PG systems. These compounds were not identified in the aqueous model systems of alliin and deoxyalliin. Therefore, the non-aqueous PG solution favored the formation of these pyridine compounds.

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