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Note

Maillard Reaction Products from 3-Deoxyglucosone and Butylamine under Physiological Conditions

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Acetic acid, *N*-butylacetamide, *N*-butyl-2-formyl-5-(hydroxymethyl)pyrrole, and *N*-butylformamide were formed as major compounds in a butylamine and 3-deoxyglucosone (3DG) reaction system under physiological conditions of 50°C at pH 7.4. *N*-Butylformamide is postulated to be formed by the cleavage of the C-C bond in α-dicarbonyl groups with the addition of amino compounds. Carbon at the 6 position in 3DG is speculated to be principally converted into methyl carbons of *N*-butylacetamide and acetic acid during the Maillard reaction.

Keywords: Maillard reaction, 3-deoxyglucosone, pyrraline, amide, acetic acid

In an advanced stage of the Maillard reaction of proteins, the intermediate dicarbonyls result in damage to proteins in biological systems as well as foods. Our group (Kato *et al.*, 1987a, b) revealed that 3-deoxyglucosone (3DG) liberated through the degradation of ε-deoxyfructosyllysine residues, attacks reactive amino acids such as arginine, lysine and tryptophan in secondary reactions to crosslink proteins in the solid state at 50°C and 75% relative humidity and under physiological conditions.

In this study, we identified volatile compounds formed between 3DG and butylamine as a model of the reaction of lysine residues under physiological conditions.

Materials and Methods

3DG was synthesized according to the method previously described (Kato *et al.*, 1990). Five hundred millimoles of *n*-butylamine adjusted to pH 7.4 with phosphoric acid and 0.25 M of 3DG were incubated in 0.2 M sodium phosphate buffer at pH 7.4 and 50°C up to 1 week. Two hundred fifty millimoles of 3DG or 0.25 M D-glucose was also incubated in the same buffer for 1 week. These reaction mixtures with an added 1 ml of an ether solution of *n*-tridecane (1 mg/4 ml) were thoroughly extracted with diethyl ether after saturation with sodium chloride. The ether extracts were concentrated at 36–38°C under atmospheric pressure. The reaction products in each resulting concentrate were analyzed and measured by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Each ether-soluble fraction was analyzed with a Shimadzu Model 7A Gas Chromatograph equipped with a flame ionization detector (FID). A chemically bonded fused silica capillary column (50 m×0.25 mm i.d.) coated with PEG-20M was used. The column oven temperature was programmed from 50 to 190°C at a rate of 4°C/min. The injection port and detector temperature were kept at 200°C. Nitrogen was used as the carrier gas at a flow rate of 1 ml/min with a split ratio of 1 : 50. Measurement of the gas chromatographic peak area was performed with Shimadzu Chromatopac C-R6A integrator connected to the gas chromatograph. GC-MS spectra were recorded with a JEOL Automass-50 Mass Spectrometer. The ionization voltage was 70 eV. Helium was used as the carrier gas. The other GC conditions were the same as described above.

Results and Discussion

The gas chromatogram of ether extracts obtained from reaction mixtures of 3DG with butylamine at pH 7.4 for 1 week shows 6 major peaks and 2 minor peaks except for tridecane used as an internal standard and butylated hydroxy toluene as a contaminant originating from the ether used as the solvent. Table 1 shows the relative amount of each peak detected on GC of ether extracts obtained from reaction mixtures of 3DG with butylamine, 3DG, and glucose in phosphate buffer at pH 7.4. No volatile compounds were detected in ether extracts on incubation of glucose alone. However, acetic acid was formed as the only detectable compound from incubation system of 3DG alone. In the 3DG-butylamine reaction system, acetic acid, 5-methyl-2-furfuryl alcohol, *N*-butylacetamide, and *N*-butyl-2-formyl-5-(hydroxymethyl)pyrrole (named butyl-pyrraline) increased markedly with the reaction time. On the other hand, the amount of *N*-butylformamide appears to be maximum at 3 days.

Our reaction system at 50°C is considered to simulate the reaction under physiological conditions, because the difference in the reaction between 37°C and 50°C was only that of the reaction rate (Hayase *et al.*, 1995).

N-Butylacetamide, *N*-butylformamide, and butyl-pyrraline were also identified in butylamine-glucose reaction system (Hayase & Kato, 1985), suggesting that 3DG is an important intermediate of these products. Lysyl-pyrraline has been reported to increase in samples *in vivo* with diabetes and aging (Hayase *et al.*, 1989; Miyata & Monnier, 1992). We found that *N*-butylacetamide and *N*-butylformamide were

Table 1. The relative amount[a] of volatile components formed by the reaction of 3-deoxyglucosone (3DG) with and without butylamine, and glucose (Glc) alone at pH 7.4 and 50°C.

Peak No.	R.T. (min)	Compounds	Reaction system (reaction time[b])				
			Glc (7)	3DG (7)	3DG-butylamine (1)	(3)	(7)
1	18.7	Acetic acid	—[c]	0.27	—	1.12	1.66
2	21.7	Unknown	—	—	—	0.07	0.07
3	24.8	Butanoic acid	—	—	—	t[d]	t
4	27.7	5-Methyl-2-furfuryl alcohol	—	—	—	t	0.04
5	30.6	*N*-Butylacetamide	—	—	—	0.15	0.15
6	31.5	*N*-Butylformamide	—	—	0.01	1.37	0.87
7	37.1	1-Butyl-2,5-pyrrolidinedione	—	—	—	t	t
8	71.3	1-Butyl-2-formyl-5-(hydroxymethyl)-pyrrole	—	—	0.15	5.58	7.93

[a] The relative amount of each component is expressed as the peak area taking the values for the internal standard ($C_{13}H_{28}$: 1.0 μg/μl) to be 1.0.
[b] Reaction time: day.
[c] —: not detected.
[d] t: trace amount.

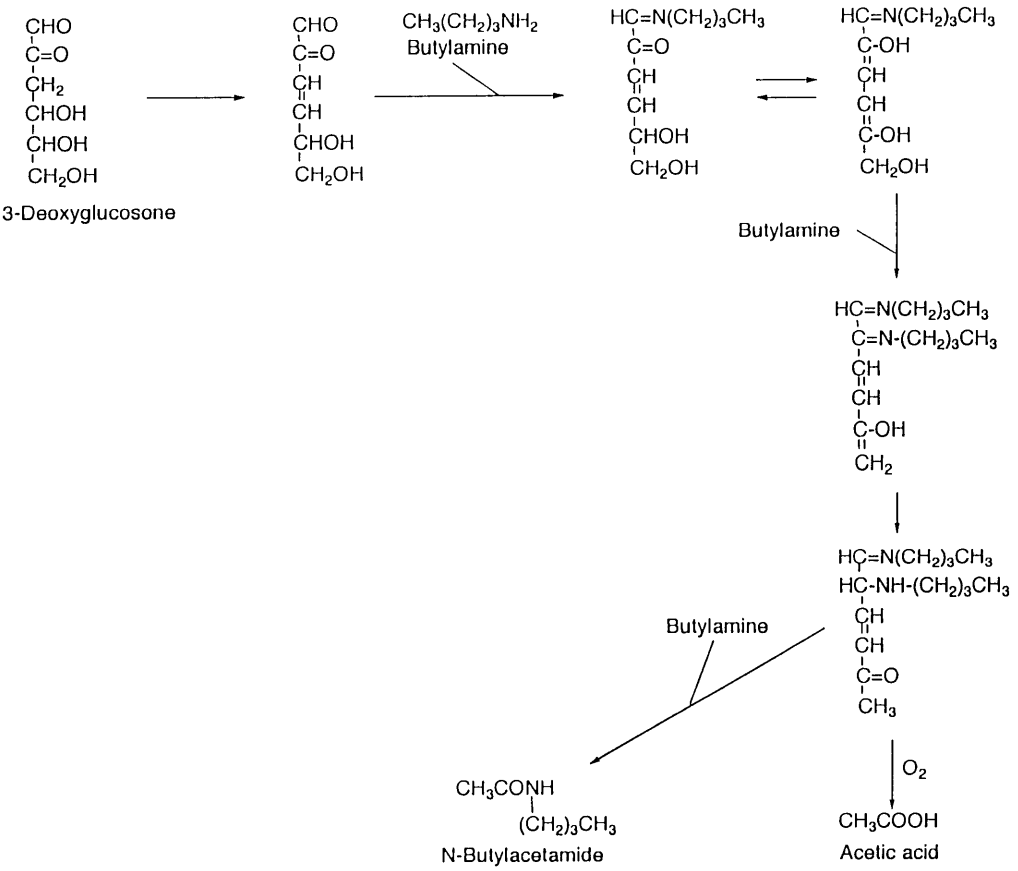

Fig. 1. Proposed schemes for the formation of *N*-butylacetamide and acetic acid by the reaction of 3-deoxyglucosone with butylamine.

formed as major components from diacetyl-butylamine and glyoxal-butylamine reaction systems, respectively (Hayase *et al.*, 1985). These observations suggest that *N*-butylformamide identified in the present study would be formed by the cleavage of the C-C bond in α-dicarbonyl groups with the addition of amino compounds to an aldehyde group.

Surprisingly, *N*-butylacetamide and acetic acid were formed from the butylamine-3DG reaction system under physiological conditions. Tressl *et al.* (1994) suggested a reaction pathway in which carbon at the 6 position in 3DG may be converted into methyl carbon by the Maillard reaction (Fig. 1). Therefore, the acetamide and acetic acid are demonstrated to be formed through the reaction route as shown in Fig. 1 by the reaction between butylamine and 3DG. Some acetic acid would be formed from 3DG without amino compounds, suggesting the formation of an acetyl group by retro aldol scission of 3DG. Indeed, alkylpyrazine has been reported to be formed from 3DG-asparagine reaction system (Weenen & Tjan, 1992). We would be able to verify these speculations using 1-^{13}C-3DG and 6-^{13}C-3DG. Studies on the physiologi-

cal properties of such amides will be necessary to address the question of whether amides play a role in the pathology of diabetes and aging.

References

Hayase, F. and Kato, H. (1985). Maillard reaction products from D-glucose and butylamine. *Agric. Biol. Chem.*, **49**, 467-473.

Hayase, F., Kim, S.B. and Kato, H. (1985). Maillard reaction products formed from D-glucose and glycine and the formation mechanisms of amides as major components. *Agric. Biol. Chem.*, **49**, 2337-2341.

Hayase, F., Nagaraj, R.H., Miyata, S., Njoroge, F.G. and Monnier, V. M. (1989). Aging of proteins: Immunological detection of a glucose-derived pyrrole formed during Maillard reaction *in vivo*. *J. Biol. Chem.*, **264**, 3758-3764.

Hayase, F., Konishi, Y. and Kato, H. (1995). Identification of the modified structure of arginine residues in proteins with 3-deoxyglucosone, a Maillard reaction intermediate. *Biosci. Biotech. Biochem.*, **59**, 1407-1411.

Kato, H., Cho, R.K., Okitani, A. and Hayase, F. (1987a). Responsibility of 3-deoxyglucosone for the glucose-induced polymerization of proteins. *Agric. Biol. Chem.*, **51**, 683-689.

Kato, H., Shin, D.B. and Hayase, F. (1987b). 3-Deoxyglucosone crosslinks proteins under physiological conditions. *Agric. Biol. Chem.*, **51**, 2009-2011.

Kato, H., Chuyen, N.V., Shinoda, T., Sekiya, F. and Hayase, F. (1990). Metabolism of 3-deoxyglucosone, an intermediate compound in the Maillard reaction, administered orally or intravenously to rats. *Biochim. Biophys. Acta*, **1035**, 71-76.

Miyata, S. and Monnier, V.M. (1992). Immunohistochemical detection of advanced glycosylation end products in diabetic tissues using monoclonal antibody to pyrraline. *J. Clin. Invest.*, **89**, 1102-1112.

Tressl, R., Kersten, E., Nittka, C. and Rewicki, D. (1994). Mechanistic studies on the formation of pyrroles and pyridines from [1-^{13}C]-D-glucose and [1-^{13}C]-D-arabinose. In "Maillard Reaction in Chemistry, Food, and Health," ed. by T.P. Labuza, G.A. Reineccius, V.M. Monnier and J.W. Baynes. The Royal Society of Chemistry, Cambridge, pp. 51-68.

Weenen, H. and Tjan, S.B. (1992). Analysis, structure, and reactivity of 3-deoxyglucosone. In "Flavor Precursors," ed. by R. Teranishi, G.R. Takeoka and M. Guntert. ACS Symp. Ser. 490, pp. 217-231.