Formation of *N*-Nitrosamines by the Maillard Reaction

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N-Nitrosamines have recently attracted much attention because of their carcinogenic and mutagenic action [1] and because their presence has been demonstrated in tobacco smoke,[2] in tobacco,[3] in fish meals [4,5] and in wheat kernels.[6]

In the case of tobacco, the occurrence of *N*-nitrosamines is readily explained by the presence in tobacco of various secondary amines and of nitrate. In fish meal, the occurrence of dimethyl-*N*-nitrosamine may in the majority of cases be explained by the occurrence of dimethylamine and the use of sodium nitrite as a preservative. However, the demonstration of dimethyl-*N*-nitrosamine in meal from fish where no nitrite has been added, raises the problem of the nature of the nitrite precursor, whether it is nitrate or some other compound. In the case of the diethyl-*N*-nitrosamine in wheat, nitrite has been shown to be present, but here the problem is to identify the source of the diethylamine.

Utilizing an improved polarographic technique,[9] presence of *N*-nitrosamines has been indicated in various types of roasted foods.

In the search for possible precursors, our attention was turned to the possibility that the necessary precursors for *N*-nitrosamines might be formed by side reactions occurring during the condensation of amino acids and aldoses (the Maillard reaction). This condensation is assumed to be an initial step for the subsequent formation of pigments responsible for the browning of various food products. In the proposed reaction mechanism for the pigment formation [7,8] the initial condensations are followed by complicated rearrangements involving deaminations, Strecker degradations, decarboxylations etc. Noteworthy is that the various reaction schemes operate with coupled oxido-reductions between reduced nitrogen and the aldehyde functions. At the same time, decarboxylation of amino acids might lead to formation of amines, and it was therefore thought of interest to see whether *N*-nitrosamines could be detected in mixtures of hexoses and amino acids.

In typical experiments lysine, glutamic acid, and alanine, respectively, were dissolved in water with or without neutralization to pH up to 10. The solutions were adsorbed onto potato starch, on which previously D-glucose had been

Table 1. Yields of volatile *N*-nitrosamines from reaction mixtures of various amino acids with D-glucose, adsorbed onto potato starch, determined polarographically.[9] Reaction mixture: Amino acid 0.010 mol, D-glucose 0.005 mol, starch 20 g.

Mixture	pH	Temp. °C	Time, h	Total *N*-nitrosamine as dimethyl-*N*-nitrosamine, μg
L-Glutamic acid/D-Glucose	7	104	18	60
Same	9.7	104	16	290
L-Glutamic acid alone	5	104	17	0
Same	8	104	17	traces
D-Glucose alone	8	104	16	0
L-Alanine/D-glucose	6.6	104	16	240[a]
L-Alanine/D-glucose	9.6	104	16	1600
L-Lysine/D-glucose	7	105	45	176
Same, nitrogen-atmosphere	7	105	45	119
L-Lysine/D-glucose	7	20	55	0
L-Lysine alone	7	105	45	0

[a] *Cf.* Fig. 1.

adsorbed. Usually 0.010 mol of amino acid and 0.005 mol of D-glucose were adsorbed onto 20 g of potato starch, the slurries dried in air at room temperature and ground. The mixtures were kept 20—45 h at 100°C, usually exposed to air; in some experiments, however, an atmosphere of N_2 was used.

After completion of the experiment, the *N*-nitrosamines were distilled over in vacuum after adjusting the reaction mixture with NaOH to 3 N, and the amounts formed were registered by polarographic analysis.[9] Another portion of the distillate was extracted with CH_2Cl_2, the solvent evaporated off, and aliquots of the residue subjected to repeated polarographic analysis, to gas chromatography, and to thin layer chromatography on silica gel with hexane: CH_2Cl_2:ethyl ether (4:3:2) as eluting system.[10]

Table 1 summarizes the yields from the various amino acids so far tried, and should be indicative of the behaviour of acidic, basic, and neutral amino acids.

Fig. 1 shows a typical polarogram, obtained from a reaction mixture of L-alanine and D-glucose. Similar polarograms were obtained in all cases. There are several half waves apparent, one at a voltage corresponding to dimethyl- or diethyl-*N*-nitrosamine, another at a voltage corresponding to the dibutyl, diamyl, or dipropyl homologues, together with other half waves as yet unidentified. The corresponding gas chromatograms show among other peaks one with the same retention time as diethyl-*N*-nitrosamine, while the thin layer chromatograms upon irradiation with ultraviolet light and sprayed with a diphenyl amine-Pd-HCl reagent show spots with R_F-values similar to or greater than the R_F-value of dibutyl-*N*-nitrosamine.[10]

Work is in progress for further identification of the *N*-nitrosamines, and conditions of formation of *N*-nitrosamines in various aldose-amino acid systems are being studied.

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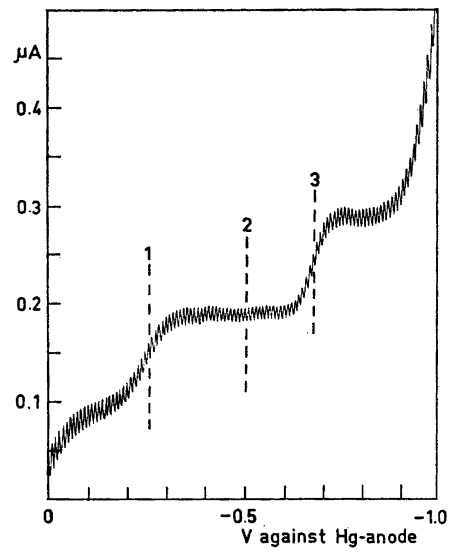

Fig. 1. Polarogram from distillate of reaction mixture α-alanine/D-glucose 2:1 on potato starch. Total volume 85 ml, diluted 1:10, buffer system: 2.5 M H_2SO_4, 2.5 M $(NH_4)_2SO_4$, 1 M KBr.
Half waves: 1: Unknown, 2: Corresponding to dibutyl-*N*-nitrosamine etc. 3: Corresponding to dimethyl-*N*-nitrosamine *etc.*

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