BIOLOGICAL SCIENCES

Formation of N-Nitrosodimethylamine from Naturally Occurring Quaternary Ammonium Compounds and Tertiary Amines

THE potential for the formation of N-nitrosamines in the human environment, either during the processing of foods or *in vivo* from nitrate and/or nitrite, and precursor amines has received considerable attention in recent years. Traditionally only secondary amines were thought to undergo N-nitrosation reactions. While secondary amines *per se* are not common in biological systems, tertiary amines and quaternary ammonium compounds do occur in plant and animal tissue. The possible formation of N-nitrosamines from these compounds must be considered because recent reports have appeared on the nitrosation of trimethylamine[1,2] and trimethylamine oxide[1] in connexion with the possible formation of N-nitrosamines in fish products cured with nitrate. The nitrosative cleavage of tertiary amines is not new and has been described before[3,4].

We are reporting the formation of dimethylnitrosamine (DMNA) from several quaternary ammonium compounds and some of their related tertiary amines in conditions simulating those found in comminuted meat products. The N-containing compounds (4.56 mmol) were reacted with $NaNO_2$ (22.8 mmol) in *p*H 5.6 buffer for 4 h at 78° C, then extracted with CH_2Cl_2; the extracts were dried with anhydrous Na_2SO_4 and concentrated in a Kuderna-Danish concentrator. DMNA was determined by a Varian aerograph model 1520 gas-liquid chromatograph using FID. The GLC conditions are as follows: column—2 feet × 1/8 inch absorbance 100–120 mesh 'Chromosorb 101'; flow rates ($cm^3\ min^{-1}$)—He 102, air 429, H_2 57; temperatures (°C)—detector 218; injector port 185°, column isothermal 150°. A gas-liquid chromatography–mass spectroscopy (GLC-MS) system with a DuPont model 492 mass spectrometer was used for all confirmation of identification. The results are shown in Table 1. DMNA is produced from tetramethylammonium chloride at almost the same level as from trimethylamine. The quaternary ammonium compound is demethylated, as shown by the accumulation of trimethylamine in the absence of nitrite. Smith[4] has proposed a pathway for the N-nitrosation of tertiary amines that appears to be applicable in this case:

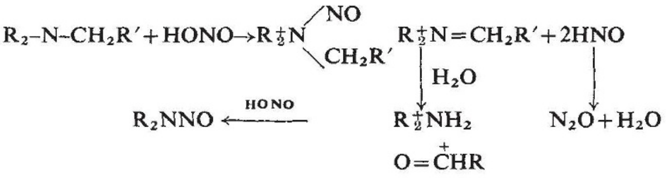

Table 1 Formation of N-Nitrosodimethylamine from Naturally Occurring Quaternary Ammonium Compounds and Tertiary Amines

Structure	Name	mg DMNA mol amine*
$(CH_3)_2NH.HCl$	Dimethylamine . HCl	7,108
$(CH_3)_3N.HCl$	Trimethylamine . HCl	643
$(CH_3)_4\overset{+}{N}Cl^-$	Tetramethylammonium chloride	433
$(CH_3)_3\overset{+}{N}-CH=CH_2Cl$	Neurine chloride	133
$(CH_3)_3\overset{+}{N}-CH_2CH_2OAcCl^-$	Acetylcholine chloride	0.897
$(CH_3)_3\overset{+}{N}-CH_2CH_2OHCl^-$	Choline chloride	0.160
$(CH_3)_3\overset{+}{N}-CH_2CO_2^-$	Betaine	0.159
$(CH_3)_3\overset{+}{N}-CH_2\underset{OH}{CH}\ CH_2CO_2HCl^-$	Carnitine chloride	0.044
$(CH_3)_2N-CH_2CH_2OAc$	2-Dimethylaminoethyl acetate	3,524
$(CH_3)_2N-CH_2CH_2OH$	2-Dimethylamino-ethanol	1,179
$(CH_3)_2N-CH_2CO_2Me$	Methyl ester of N,N-dimethylglycine	3,004
$(CH_3)_2N-CH_2CO_2H$	N,N-Dimethylglycine	1,405

* Quantification based on the use of cyclohexanone as an internal standard.

The naturally occurring quaternary ammonium compounds, neurine, carnitine, betaine, choline and acetylcholine, also yield DMNA when reacted with $NaNO_2$ although at a considerably lower level than the tetramethylammonium compound. The results reflect the ability of the quaternary compound to dealkylate to trimethylamine or a mixed amine containing the Me_2N– group and another substituent. N,N-Dimethylglycine and its methyl ester are tertiary amines derived from the rearrangement of betaine in acidic conditions; 2-dimethylaminoethanol and its acetate derivative are similarly related to choline and acetylcholine respectively. The DMNA yield from the mixed alkyl tertiary amines is two to five times that obtained from trimethylamine and 4×10^3 to 2×10^4 times the DMNA formed from the parent quaternary ammonium compounds. In fact, 2-dimethylaminoethyl acetate forms DMNA almost one-half that formed by dimethylamine. It is interesting to note that larger quantities of DMNA are produced from the esters of the quaternary and tertiary compounds than from the parent compounds.

It is significant that DMNA can be formed from quaternary ammonium compounds, albeit at low levels, in the conditions described. We can therefore conclude that although quaternary ammonium compounds may represent a potential source of nitrosamine, the tertiary amines and free secondary amines present in food products are of greater concern.

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N-Dimethylnitrosamine in Tobacco Smoke Condensate

THE possible presence of N-nitrosamines in cigarette smoke and/or condensate has been of interest for some years. Druckrey and Preussmann[1] postulated that N-nitrosamines could be produced in burning tobacco, but the difficulty of measuring these compounds prevented the testing of this postulate. We have developed[2] a gas chromatographic method for the selective detection of N-nitrosamines, and now report its application to the analysis of cigarette smoke condensate. We have identified N-dimethylnitrosamine (DMN) in cigarette smoke condensate, and shown that certain tobaccos grown in different conditions vary widely in their content of DMN.