

## 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<sup>1,2</sup> and trimethylamine oxide<sup>1</sup> in connexion with the possible formation of N-nitrosamines in fish products cured with nitrite. The nitrosative cleavage of tertiary amines is not new and has been described before<sup>3,4</sup>.

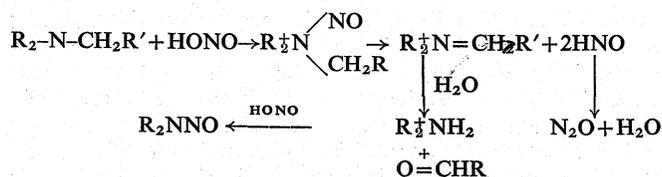
We are reporting the formation of dimethylnitrosamine (DMNA) from several quaternary ammonium compounds and some of their related tertiary amines in conditions simulating those found in comminuted meat products. The N-containing compounds (4.56 mmol) were reacted with  $\text{NaNO}_2$  (22.8 mmol) in pH 5.6 buffer for 4 h at 78° C, then extracted with  $\text{CH}_2\text{Cl}_2$ ; the extracts were dried with anhydrous  $\text{Na}_2\text{SO}_4$  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  $\times$  1/8 inch ~~Calsorbane~~ o.d. 100–120 mesh 'Chromosorb 101'; flow rates ( $\text{cm}^3 \text{min}^{-1}$ )—He 102, air 429,  $\text{H}_2$  57; temperatures (°C)—detector 210°; 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

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

| Structure   | Name                                | mg DMNA/mole amine* |
|---|-------------------------------------|---------------------|
| $(\text{CH}_3)_2\text{NH}\cdot\text{HCl}$   | Dimethylamine . HCl                 | 7,108               |
| $(\text{CH}_3)_3\text{N}\cdot\text{HCl}$  | Trimethylamine . HCl                | 643                 |
| $(\text{CH}_3)_4\text{N}^+ \text{Cl}^-$   | Tetramethylammonium chloride        | 433                 |
| $(\text{CH}_3)_3\text{N}^+-\text{CH}=\text{CH}_2 \text{Cl}^-$   | Neurine chloride                    | 133                 |
| $(\text{CH}_3)_3\text{N}^+-\text{CH}_2\text{CH}_2\text{OAc} \text{Cl}^-$                              | Acetylcholine chloride              | 0.897               |
| $(\text{CH}_3)_3\text{N}^+-\text{CH}_2\text{CH}_2\text{OH} \text{Cl}^-$                               | Choline chloride                    | 0.160               |
| $(\text{CH}_3)_3\text{N}^+-\text{CH}_2\text{CO}_2^-$  | Betaine                             | 0.159               |
| $(\text{CH}_3)_3\text{N}^+-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{CO}_2\text{H} \text{Cl}^-$ | Carnitine chloride                  | 0.044               |
| $(\text{CH}_3)_2\text{N}-\text{CH}_2\text{CH}_2\text{OAc}$  | 2-Dimethylaminoethyl acetate        | 3,524               |
| $(\text{CH}_3)_2\text{N}-\text{CH}_2\text{CH}_2\text{OH}$   | 2-Dimethylamino-ethanol             | 1,179               |
| $(\text{CH}_3)_2\text{N}-\text{CH}_2\text{CO}_2\text{Me}$   | Methyl ester of N,N-dimethylglycine | 3,004               |
| $(\text{CH}_3)_2\text{N}-\text{CH}_2\text{CO}_2\text{H}$  | N,N-Dimethylglycine                 | 1,405               |

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

trimethylamine in the absence of nitrite. Smith<sup>4</sup> has proposed a pathway for the N-nitrosation of tertiary amines that appears to be applicable in this case:



The naturally occurring quaternary ammonium compounds, neurine, carnitine, betaine, choline and acetylcholine, also yield DMNA when reacted with  $\text{NaNO}_2$  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  $\text{Me}_2\text{N}-$  group and another substituent. N,N-Dimethylglycine and its methyl ester are tertiary amines de-

rived 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  $4 \times 10^3$  to  $2 \times 10^4$  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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Received October 18; revised December 14, 1971.

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