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A new type of N-nitrosation inhibitor

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Beside our work on nitrosamines in meat products (Groenen et al., This symposium), we are also studying the occurrence of nitrosamines in tobacco and tobacco smoke. Some of this work has been published in *Beiträge zur Tabakforschung* (Groenen & ten Noever de Brauw 1975). I now report in preliminary form that we have detected in tobacco smoke a compound which has the ability to trap NO_x vapours and to inhibit the formation of *N*-nitrosamines from secondary amines by competing for nitrite.

Though tobacco smoke and cured meat products have little in common, we consider it appropriate to describe some of the observations and conclusions from this work at this symposium, as it is conceivable that this new *N*-nitrosation inhibitor is active also in cured meat and other food products, and in vivo.

We will describe elsewhere how we became aware of this compound. We determined its identity and it was found to be pyrrole.

We then carried out a series of model experiments. The results were as follows:

1. Pyrrole in acidified aqueous solution (pH 1 or 3) is consumed extremely rapidly after addition of nitrite. A very fine black precipitate is formed.
2. New volatile or semivolatile nitroso compounds or other compounds are not formed in this reaction.
3. Experiments with an equimolar aqueous system of morpholine, pyrrole and nitrite at pH 3 showed that pyrrole can *completely inhibit* formation of *N*-nitrosomorpholine. A black carbon-like precipitate is again formed.
4. The extent of inhibition by pyrrole of the nitrosation of morpholine was compared with that of a series of acknowledged or potential nitrosation inhibitors under identical circumstances. The extent of inhibition was found to increase steadily in the order ammonium chloride $\leqslant$ phenol $<$ *iso*-amylmercaptan $<$ indole $<$ ascorbic acid $<$ guaiacol $<$ 4-methyl catechol $\leqslant$ pyrrole.

According to Challis & Bartlett (1975), 4-methylcatechol must be one of the best known scavengers of HNO_2. We now can add that pyrrole is at least as effective. Under the conditions of our experiment, 4-methylcatechol was also a highly effective nitrosation inhibitor. This is in contrast with the remarkable observations of Challis & Bartlett (1975), who report a 1 000-fold increase in the rate of nitrosamine formation, in spite of the rapid HNO_2 consumption of 4-methylcatechol.

There is suprisingly little literature on the nitrosation of pyrrole. *N*-Nitrosopyrrole does not exist (Stevens 1957a); 3-nitrosopyrroles, however, do exist (Stevens 1957b). Indole compounds also are known to be *C*-nitrosated at the 3-position (Challis 1973). Pyrrole has the structure of a secondary amine, but it does not behave like one. The *N*-hydrogen atom can be replaced by potassium, so that the compound behaves rather like a weak acid (Karrer 1950), and has nothing in common with, for instance, pyrrolidine or piperazine. As early as 1917, it was known that a black polymeric precipitate is formed, called nitrosopyrrole black, on nitrosation of pyrrole (Angeli & Cusmano 1917).

Pyrrole and its derivatives occur in food products and in vivo (for pyrrole see, for instance, van Straten & de Vrijer 1973 and 1976) and it would certainly be worth while to study their inhibitory properties in more detail. It is conceivable that the type of reaction we have described plays a role in the partially unexplained loss of nitrite in cured meat products. Whether nitrosopyrrole black or similar products are really formed in small amounts in cured meat or tobacco smoke remains to be seen; of course other competitive compounds are at work too. If polymers like nitrosopyrrole black *are* formed, their toxicological properties should be elucidated.

Acknowledgment

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Discussion on the session

Inhibition of nitrosation

In Dr Castegnaro's laboratory, the action of phenol on nitrosation of diethylamine at moderate phenol concentrations had been examined. A catalytic effect was found, increasing with the amount of phenol. Since phenol reacts with nitrite to give nitrosophenol, the effect of nitrosophenol was also tested. At concentrations of nitrosophenol a hundredth of those of phenol in the first experiments, an equivalent catalytic effect was observed. Although high concentrations of phenol were not used, it might well be that high levels compete with amine to give an inhibitory effect. Recents results obtained by Dr Davies confirm the findings of Castegnaro. He found that at pH 5.0, the only pH studied, *p*-cresol catalysed the reaction between nitrite and pyrrolidine to give nitrosopyrrolidine. The same effect was shown by some nitrosophenols at that pH.

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General discussion on formation of nitrosamines

Contents of amines in bacon

Dr Roberts said that Patterson & Mottram (1974) give measurements of the contents of several amines (MA, DMA, TMA, *n*PA, *i*PA and DEA in decreasing order of 1 000 to 10 μg/kg), at different stages from slaughter of pigs through curing and storage as vacpacked bacon. Amounts changed before curing, conceivably by autolytic changes, arrested by salting. During storage, there was little change, despite rising contents of nitrite and increases in number contents of bacteria from perhaps $10^3 - 10^9$ g^{-1}. These bacteria were mostly lactic acid bacteria (not causing proteolysis) because of the vacuum packing. But even in uncured pork kept in air to the point of spoilage, with large production of NH_3, the levels of MA, DMA and TMA were similar in magnitude (Patterson & Edwards 1975).