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REDUCTION OF TOBACCO SMOKE MUTAGENICITY:  
THE INFLUENCE OF NITROGENOUS COMPOUNDS

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Interest in this field is almost exclusively Japanese with a programme of work well under way by 1977. Initial experiments included an investigation of the effect of growing tobacco in soils with differing levels of nitrate fertiliser (1). As fertiliser levels were increased so the mutagenic potency of the smoke condensate was seen to rise (1). Thus the concept of nitrogenous compounds contributing to smoke mutagenicity was established.

Tobacco Nitrate Content

In the initial experiments, by utilising plants grown with different levels of nitrogenous fertiliser and by examining the mutagenicity of smoke from different cigarette types, a positive correlation between tobacco nitrate content and mutagenicity was noted (1). However, later studies (2) which utilised more samples did not confirm this correlation between nitrate and mutagenicity. The situation regarding nitrate is further complicated by the work of Kier et al. (5), who added nitrate to the final cured tobacco and found increased mutagenic activity of the condensate. The role of nitrate and smoke mutagenicity clearly requires further investigation.

Tobacco Protein

Polycyclic aromatic hydrocarbons (PAH) have been regarded as the main compounds in tobacco smoke with tumour initiating activity. However, following the condensate fractionation work of Kier et al. (5) and Hutton et al. (11), the mutagenic agents seem to be in the basic fractions whereas the PAHs are in the neutral fractions. In addition,

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the mutagenicity of condensate from one cigarette is more than 10,000 x greater than the mutagenicity of the benzo(a)pyrene content of the condensate (12). So thoughts have turned away from the PAH fraction of condensates and towards identifying the agents responsible for the bulk of mutagenic activity in smoke condensate.

Considering that the mutagenic activity was in the basic fraction of condensate, nitrogenous compounds were rapidly put under consideration and, as the bulk of tobacco compounds are proteins and amino acids, then these as a particular class of compounds came under close scrutiny. Additionally at this time (late 1970s), the interest in mutagenicity of compounds inherent in man's lifestyle was gaining momentum and many studies investigated the mutagenicity of cooked food. These studies have examined the mutagenicity of pyrolysed: amino acids (3, 8, 13), peptides (6) and whole proteins (4, 7). The majority of pyrolysed amino acids/proteins are mutagenic, requiring metabolic activation to express their activity and include some of the most powerful known mutagens (4, 8). Thus, pyrolysis products of amino acids and proteins were identified as clear candidates to contribute significantly to the mutagenicity of cigarette smoke condensate.

Experiments to investigate the influence of protein content of tobacco leaf on smoke mutagenicity have been carried out by the Japan Tobacco and Salt Public Corporation (J.T.S.) (2, 6). Using experimental cigarettes made from leaf of different stalk positions, lowest mutagenicity was found in condensate from leaves from the lowest part of the plant (Table 1)(2). This mutagenicity was also linked to the leaf level of total nitrogen, protein nitrogen and nicotine. The influence of nicotine was not considered important by the Japanese as it is known to be a non-mutagen and the nicotine pyrolysis in smoke is not thought to be extensive (2, 15). In a second series of experiments, pyrolysed tobacco from plants of different maturities and therefore different leaf protein levels was examined. Again the plants with lowest amino acid levels had the lowest mutagenic activity (Table 2) (6). From both these papers of the J.T.S. (2, 6), use of tobacco with low protein levels is considered important in a programme to reduce the mutagenic activity of cigarette smoke condensate. However, from the literature search, J.T.S. have not carried out any published work on the mutagenic activity of deproteinised tobacco condensate.

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Chemical Identity of Some of the Mutagenic Products of Protein Pyrolysis

The majority of work has centred on identifying the mutagens from the pyrolysed amino acid tryptophan, which represent some of the most powerful mutagens known to date (3, 4). From tryptophan pyrolysate, the mutagens 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole and 3-amino-1-methyl-5H-pyrido(4,3-b)indole have been isolated (4). These compounds are both  $\gamma$ -carbolines (Figure 1) and have also been found in pyrolysis products of whole proteins (7). Additionally, mutagenic pyrolysis products from glutamic acid have been identified as the amino-dipyridoimidazoles 2-amino-6-methyldipyrido(1,2-a:3',2'-d)imidazole and 2-amino-dipyrido(1,2-a:3',2'-d)imidazole (Figure 1)(4).

More specific to tobacco smoke, mutagenic amino- $\alpha$ -carbolines (300 ng/cigarette) have recently been isolated from cigarette smoke condensate (Figure 1)(9, 10). Furthermore, it is well known that the co-mutagens harman and noharman ( $\beta$ -carbolines) from tryptophan pyrolysis are also present in condensate (Figure 1). All these compounds have complex mutagenic activities. The amino- $\alpha$ -carbolines are themselves powerful mutagens and also act synergistically with other mutagens (9) whilst the  $\beta$ -carbolines are well established co-mutagens\*(4). Hence deproteinisation of tobacco may not only reduce the mutagenic activity of smoke by removal of products capable of conversion to mutagens during pyrolysis but also remove synergistic and co-mutagenic activity caused by interactions between protein-derived mutagens and non-protein-derived mutagens.

Sugar-Induced Modulation of Tobacco Smoke Mutagenicity

The studies of J.T.S. to examine the effect of tobacco protein levels on condensate mutagenicity have utilised naturally available tobaccos, not deproteinised tobacco. Thus the experiments have the disadvantage that, in addition to the leaf protein level, other chemical constituents vary, which may have an influence on smoke mutagenicity. Indeed, the sugar content of tobacco has been found to correlate with mutagenicity: as the leaf sugar levels increase, so condensate mutagenicity decreases (2).

\*Co-mutagens are not themselves mutagenic, but are able to enhance the mutagenicity of other compounds.

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However, recent experiments have added different sugars to cigarettes (0.25 - 0.5g/cigarette) and found a substantial reduction (60-70%) in smoke condensate mutagenicity (14). Though these latter experiments use high levels of sugars, the studies indicate that reducing mutagenicity by addition of sugars may substantially reduce the genotoxicity of cigarettes.

Current and Proposed Work in Biological Sciences on Smoke Mutagenicity and Deproteinised Tobacco

Preliminary experiments from cigarettes with different protein levels (POET 2) show that the claims of the J.T.S. will be substantiated. Cigarettes P332 and P333 have the highest protein content and the highest mutagenicity (Figure 2, Table 3). However, as in the J.T.S. experiments, the most mutagenic cigarettes also have the highest levels of total nitrogen, ammonia nitrogen and nitrate nitrogen (Table 3). Furthermore, the sugar content is highest in the cigarettes with the lowest mutagenic activity (P328, P329 and P338) and may act to reduce the mutagenicity independently of the protein content. Therefore the net contribution of protein pyrolysates to the mutagenicity of condensate cannot currently be accurately assessed. For the future, a number of co-ordinated approaches seem to be available:

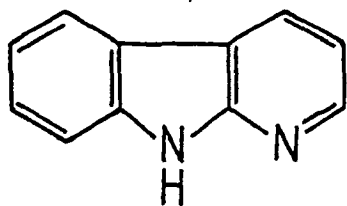
- (a) Use of further natural products - are tobaccos available such that the different nitrogenous and carbohydrate components can be varied in relation to each other?
- (b) Addition of components to tobacco - e.g. amino acid mixtures.
- (c) Use of deproteinised tobacco.

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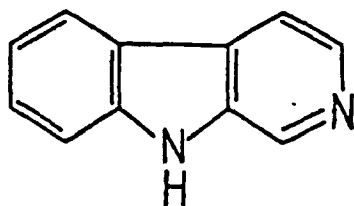
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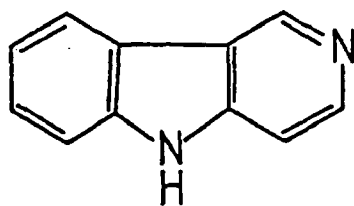
FIGURE 1



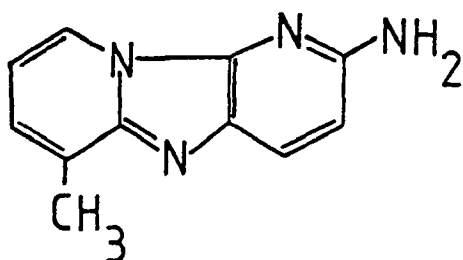
$\alpha$ -carboline



$\beta$ -carboline

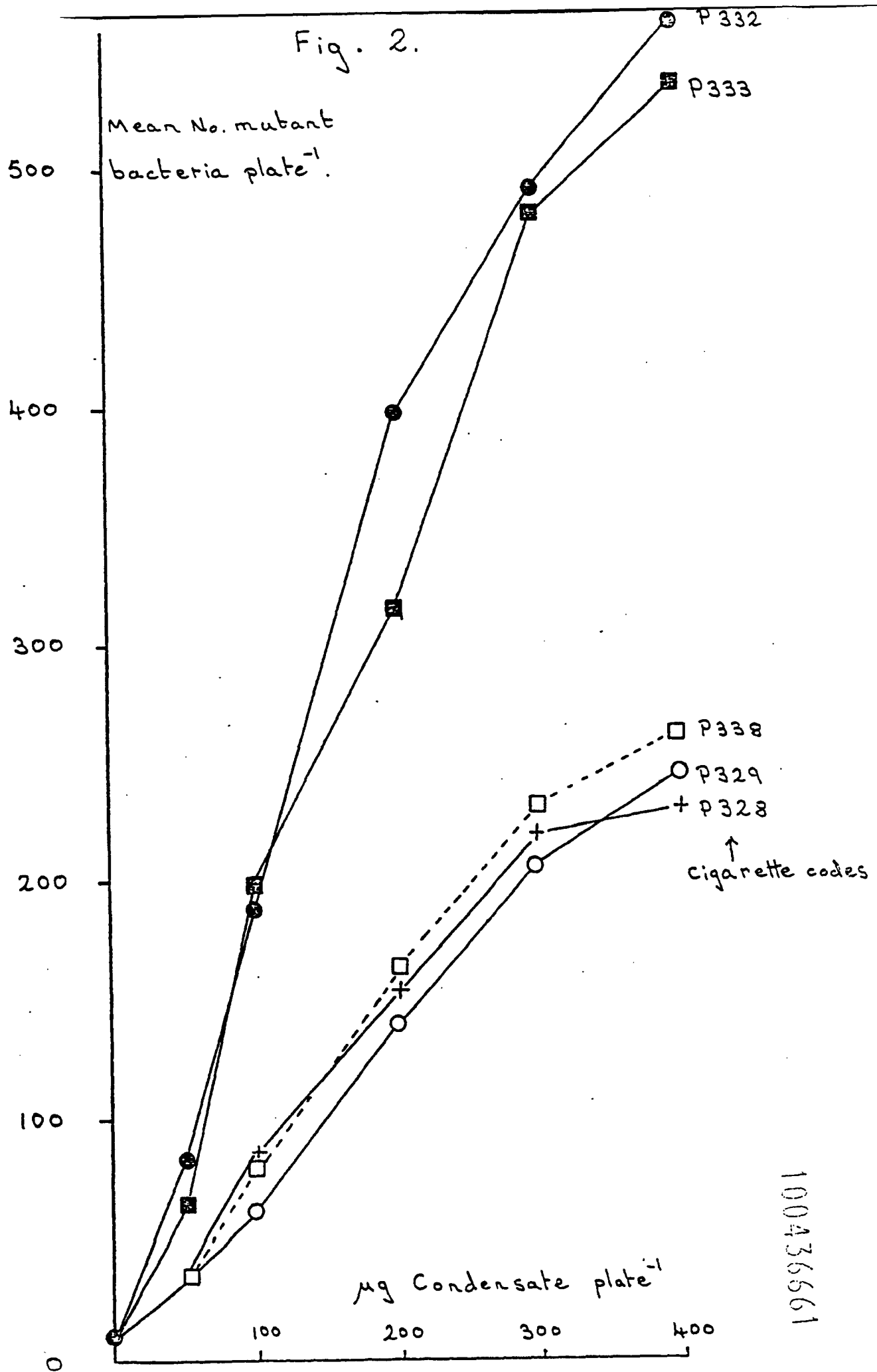


$\gamma$ -carboline



2-amino-6-methyldipyrido-  
(1,2-a:3',2'-d)imidazole

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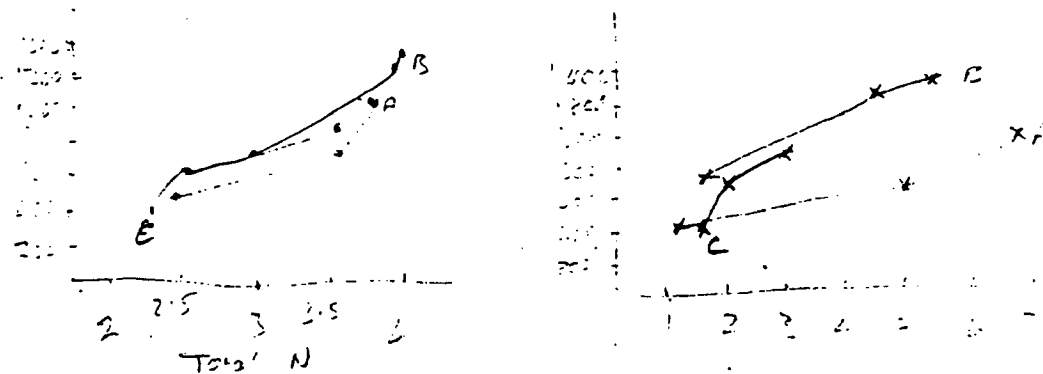
TABLE 1

CONTENT OF NITROGENOUS CONSTITUENTS IN TOBACCO LEAVES  
AND MUTAGENIC ACTIVITY OF SMOKE CONDENSATE

Tobacco leaves from different stalk positions were obtained from Japanese domestic tobacco. The mutagenic activity of smoke condensate was assayed with Salmonella typhimurium TA98 in the presence of S-9 Mix. The number of spontaneous revertants (30 per plate) was subtracted from the number of revertants induced by 0.5mg of the condensate

Tobacco Sample	Leaf Position	Mutagenic Activity of Smoke Condensate (Revertants/Plate)	Nitrogenous Constituents (%)			
			Total Nitrogen	Protein Nitrogen	Nicotine	Nitrate
A	Upper	1069	3.76	1.20	6.63	0.05
	Middle	693	3.51	1.11	5.01	0.30
	Lower	511	2.44	0.92	1.19	1.65
B	Upper	1355	3.92	1.35	5.21	0.14
	Middle	1281	3.89	1.21	4.32	0.14
	Lower	721	2.93	0.99	1.52	3.20
C	Upper	893	3.50	1.13	2.98	0.63
	Middle	619	2.48	0.91	2.01	0.45
	Lower	370	2.34	0.85	1.58	0.83

From Mizusaki et al. (Ref. 2).



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TABLE 2

MUTAGENIC ACTIVITY OF PYROLYZATE AND TOTAL AMINO ACID  
RESIDUES OF TOBACCO LEAVES

Bright tobacco powders were pyrolyzed at 600°C and the pyrolyzates were tested for mutagenic activity in Salmonella typhimurium TA98 in the presence of S-9 Mix. The number of revertants per pyrolyzate of 100 µg tobacco powder is presented after subtraction of the average number of spontaneous revertants (34). The dried tobacco was hydrolyzed and the total amino acid residues were determined in an amino acid analyser.

	Mutagenic Activity (Revertants/Pyrolyzate of 100 µg Tobacco)	Total Amino Acid Residues (µ mol/g Tobacco Dry Wt.)
Young	478	1324
Matured	47	287

From Matsumoto et al. (Ref. 6).

TABLE 3

CIGARETTE ANALYSIS - DRY WEIGHT BASIS

Cigarette Code	% Total Nitrogen	% Protein Nitrogen	% Ammonia Nitrogen	% Nitrate Nitrogen	% Total Sugars
P328	2.65	1.10	0.030	0.034	14.1
P329	2.54	1.21	0.013	0.027	7.9
P332	4.51	1.69	0.17	0.49	1.0
P333	4.23	1.80	0.11	0.50	0.8
P338	2.15	1.07	0.031	0.025	15.0

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REFERENCES

1. Mizusaki, S., Takashima, T. and Tomaru, K.  
Factors affecting mutagenic activity of cigarette smoke condensate in Salmonella typhimurium TA1538.  
Mut. Res. 48, 29-36, 1977.
2. Mizusaki, S., Okamoto, H., Akiyama, A. and Fukuhara, Y.  
Relation between chemical constituents of tobacco and mutagenic activity of cigarette smoke condensate.  
Mut. Res. 48, 319-326, 1977.
3. Matsumoto, T., Yoshida, D., Mizusaki, S. and Okamoto, H.  
Mutagenic activity of amino acid pyrolysates in Salmonella typhimurium TA98.  
Mut. Res. 48, 279-286, 1977.
4. Sugimura, T. and Naigo, M.  
Mutagenic factors in cooked foods.  
CRC Critical Reviews in Toxicology 6, 189-209, 1979.
5. Kier, L.D. and Yamasaki, E.  
Detection of mutagenic activity in cigarette smoke condensates.  
Proc. Nat. Acad. Sci. USA 71, 4159-4163, 1974.
6. Matsumoto, T., Yoshida, D., Mizusaki, S. and Okamoto, H.  
Mutagenicities of the pyrolysates of peptides and proteins.  
Mut. Res. 56, 281-288, 1978.
7. Yoshida, D., Matsumoto, T. and Nishigata, H.  
Effect of heating methods on mutagenic activity and yield of mutagenic compounds in pyrolysis products of protein.  
Agric. Biol. Chem. 44, 253-255, 1980.
8. Sugimura, T., Nagao, M., Matsushima, T., Yahagi, T. and Hayashi, K.  
Recent findings on the relation between mutagenicity and carcinogenicity.  
Nucleic Acids Res., Special Publ. No.3, S41-S44, 1977.
9. Yoshida, D. and Matsumoto, T.  
Amino- $\alpha$ -carbolines as mutagenic agents in cigarette smoke condensate.  
Cancer Letters 10, 141-149, 1980.
10. Matsumoto, T., Yoshida, D. and Tomita, H.  
Determination of mutagens, amino- $\alpha$ -carbolines in grilled foods and cigarette smoke condensates.  
Cancer Letters 12, 105-110, 1981.
11. Hutton, J.J. and Hackney, C.  
Metabolism of cigarette smoke condensates by human and rat homogenates to form mutagens detectable by Salmonella typhimurium TA1538.  
Cancer Res. 35, 2461-2468, 1975.
12. Sugimura, T., Saito, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M. and Kawachi, T.  
Overlapping of carcinogens and mutagens.  
In Fundamentals in Cancer Prevention, P.N. Magee et al. (Eds.), Univ. Tokyo Press, pp.191-215, 1976.

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13. Kosuge, T., Tsuji, K., Wakabayashi, K., Okamoto, T., Shudo, K., Iitaka, Y., Itai, A., Sugimura, T., Kawachi, T., Nagao, M., Yahagi, T. and Seino, Y.  
Isolation and structure studies of mutagenic principles of amino acid pyrolysates.  
Chem. Pharm. Bull. 26, 611-619, 1978.
14. Saito, S., Ohka, T., Nagao, M., Tsuji, K. and Kosuge, T.  
Reduction in mutagenicity of cigarette smoke condensate by added sugars.  
Mut. Res. 60, 155-161, 1979.
15. Schmeltz, I., Wenger, A., Hoffmann, D. and Tso, T.C.  
Chemical studies on tobacco smoke, Pt.13: on the fate of nicotine during pyrolysis and in a burning cigarette.  
J. Agric. Food Chem. 27, 602-608, 1979.

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