Examination of Aflatoxin B₁ in Leaf Tobacco and in Cigarette Smoke Condensate*

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Aflatoxin B₁, the most toxic of the four known aflatoxins, is produced by *Aspergillus flavus*. The binding of aflatoxin B₁ to both native and denatured DNA (deoxyribose nucleic acid) partially explains its extreme toxicity and carcinogenicity (1). Aflatoxins have been reported to occur in many commodities (2), but its presence in leaf tobacco was never positively confirmed, although *A. flavus* was found in various grades of air-cured Burley tobacco (3).

The amount of certain phenolic compounds in tobacco, especially scopoletin (6-methoxy-7-hydroxy coumarin), was found to be associated with the levels of phenylalanine and tyrosine present (4). These two amino acids are also reported to be incorporated into aflatoxins B and G produced by *A. flavus* in a defined medium (5). These results suggest that the coumarin nucleus of scopoletin and aflatoxin may be derived from the same biosynthetic pathways.

Certain types of tobacco contain higher populations of fungi than other types (6). These differences probably are the result of culture, curing, and handling practices as well as chemical composition of tobacco leaf. Seven samples of good-grade cured tobacco from flue-cured, Burley, and Maryland types, and four samples of moldy tobaccos from flue-cured type, were used for aflatoxin studies following methods described by Pons et al. (7), with minor modifications. n-Hexane was used to wash the column before eluting with chloroform. The thickness of the thin layer chromatogram was reduced to 250 microns. This method was designed specially for determination of aflatoxins, and not for scopoletin which is not the main object of this present study. Most of the scopoletin came off the column when it was washed with n-hexane. The chloroform fraction contains all aflatoxins and the remaining portion of scopoletin. Aflatoxin B₁ has a R_f value very close to scopoletin in several of the solvent systems tested. Using silica gel H thin layer chromatograms, the R_f values of aflatoxin B₁ and scopoletin are, respectively, 0.55 and 0.45, with a solvent system containing 3% MeOH in chloroform; and 0.66 and 0.56 when 5% MeOH was used. The identity of aflatoxin B₁ was further confirmed by another method (8).

Results in Table 1 show the absence of aflatoxin B₁ in these good and moldy tobacco samples tested within the sensitivity of the present method, which is 0.3 parts per billion (7). These findings are from two determinations of normal tobacco and three determinations of molded tobacco samples. The results, however, should not be interpreted to indicate that aflatoxins or other mycotoxins are absolutely free from all leaf tobaccos. A better understanding of the environmental conditions for growth of microorganisms in tobacco, and more specified techniques for chemical detection of individual mycotoxins in tobacco are needed.

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Aflatoxin B₁ has a melting point of 268–269° C, at which it decomposes (9). This temperature is much lower than that at the combustion zone of a cigarette, which averages around 884° C (10). Aflatoxin B₁, if present, may possibly enter smoke via dry distillation immediately ahead of the burning area. To examine this possibility, samples of cigarette smoke condensates were studied. Regular, non-filter cigarettes, with or without the addition of aflatoxin B₁** , were smoked by a generally accepted method (11), and the smoke condensate was collected on a Cambridge filter.

TABLE 1

Aflatoxin B₁ and scopoletin in leaf tobacco

Samples	Aflatoxin B ₁	Scopo- letin* (p.p.m.)
Regular good-grade tobaccos:		
Flue-cured: Maleic Hydrazide treated	Not detected	5
Without maleic hydrazide	Not detected	4
Burley: Lower leaf	Not detected	3
Middle leaf	Not detected	3
Upper leaf	Not detected	4
Maryland: Suckers not removed	Not detected	10
Suckers removed	Not detected	4
Moldy tobaccos:		
Flue-cured: Eastern, N. C.	Not detected	5
Wilson, N. C.	Not detected	7
Old Belt	Not detected	7
Tifton, Ga.	Not detected	7
Aflatoxin B₁ recovery tests:		
Flue-cured samples with aflatoxin B ₁ added		
A. 50 ppb	95-100% recovered	4
B. 100 ppb	95-100% recovered	4
C. 200 ppb	95-100% recovered	4

TABLE 2

Aflatoxin B₁ and scopoletin in cigarette smoke condensate

Samples	Aflatoxin B ₁	Scopo- letin* (p.p.m.)
Control (regular, non-filter)	Not detected	0.2
0.5 microgram B ₁ added to each cigarette (approx. 500 ppb) before smoke	Not detected	0.2
0.5 microgram each of B ₁ and NaNO ₃ added to each cigarette (approx. 500 ppb) before smoke	Not detected	0.2
5.0 microgram B ₁ added to each cigarette (approx. 5,000 ppb)	Not detected	0.2
5.0 microgram each of B ₁ and NaNO ₃ added to each cigarette (approx. 5,000 ppb)	Not detected	0.2
0.5 microgram B ₁ added to smoke condensate before assay (approx. 500 ppb of tobacco weight)	90-95% Recovered	0.2
1.0 microgram B ₁ added to smoke condensate before assay (approx. 1000 ppb of tobacco weight)	90-95% Recovered	0.2

* These data represent only the portion of scopoletin which was eluted from column together with aflatoxin.

In another test, NaNO₃ was injected together with aflatoxin B₁ into cigarettes. The presence of NaNO₃ would provide in the smoke an additional source of nitrogen dioxide which detoxifies aflatoxins (12). Each test consisted of three replications of smoke condensates from three cigarettes. Smoke condensates collected on Cambridge filters and chloroform traps were assayed for aflatoxin B₁ in the manner used for tobacco samples. Results in Table 2 show the absence of aflatoxin B₁ in all smoke condensates. Aflatoxin B₁ which was injected in the cigarettes before smoking also was not recovered in the condensate. It is evident that aflatoxin B₁, if present, is decomposed during the combustion process. In addition, although the chemical reactions involved and the decomposition products are not yet known, the presence of the lactone ring and the olefinic double bond of the terminal furan ring make aflatoxins labile for oxidation, hydrolysis and partial re-cyclization (9, 12).

The biosynthesis of aflatoxins in a defined medium is approximately 90% B₁ and 10% G₁ (5). Aflatoxin B₂ (dihydroaflatoxin B₁) usually was not detected in agricultural products when aflatoxin B₁ was absent. It appears that aflatoxins are either not present in the tobacco samples examined, or, if present, are decomposed during combustion.

** Aflatoxin B₁ was kindly supplied by Dr. G. N. Wogan of the Mass. Institute of Technology.

SUMMARY

An examination of several samples of leaf tobacco, including three types of good-grade tobaccos and heavily molded flue-cured tobacco, and of cigarette smoke condensate, failed to detect aflatoxin B₁. Pure aflatoxin B₁ added to cigarettes was not recovered in the smoke condensate indicating that aflatoxin B₁, when present, was changed or decomposed during the smoking process.

ZUSAMMENFASSUNG

Die Autoren untersuchten mehrere Stichproben von Blattabak (einschließlich dreier Arten von normalem Tabak ohne Schimmelbefall und stark verschimmelten Virginia-Tabaks) sowie Cigarettenrauchkondensat auf das Vorkommen von Aflatoxin B₁. Die Verbindung konnte weder in den genannten Proben noch im Rauchkondensat von Cigaretten nachgewiesen werden, denen reines Aflatoxin B₁ zugesetzt worden war. Aus diesem Befund wird gefolgert, daß möglicherweise vorhandenes Aflatoxin B₁ während des Rauchvorganges umgewandelt oder abgebaut wird.

RÉSUMÉ

On a étudié la teneur en aflatoxine B₁ de plusieurs échantillons de tabac brut (y compris trois types de tabac normal sans moisissure et du tabac Virginia fortement moisi) ainsi que du condensat de fumée de cigarette. Le composé ne pouvait être identifié ni dans les échantillons mentionnés de feuilles de tabac ni dans le condensat de la fumée de cigarettes au tabac desquelles de l'aflatoxine B₁ pure avait été ajoutée. Ces résultats indiquent que l'aflatoxine B₁ possiblement présente dans le tabac se modifie ou se décompose pendant le processus de la combustion de la cigarette.

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