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STEROLS AND FATTY ACIDS OF AFLATOXIN AND NON-AFLATOXIN PRODUCING ISOLATES OF *ASPERGILLUS**

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Abstract—The fatty acids and sterols present in 5 isolates of *Aspergillus flavus* and 3 isolates of *A parasiticus* were determined, 2 isolates within each species were aflatoxin producers The 4 major fatty acids were 16 0, 18 0, 18 1 and 18 2 with a trace of 15 0 in one isolate and traces of 17 0 in 3 other isolates Cholesterol, ergosterol and 5, 7-ergostadienol were present in all isolates, the 5 isolates of *A flavus* could be identified on the basis of retention times of minor sterols present There was no correlation of total lipids, fatty acids or sterols with the production of aflatoxins Water soluble complexes of sterols were not detected

INTRODUCTION

DIENER and DAVIS[1] calculated that 40% of the *Aspergillus flavus* isolates collected throughout the world did not produce aflatoxins Although many workers have investigated the factors affecting the level of aflatoxin production, only one study has been reported that compares aflatoxin and non-aflatoxin producing isolates Gupta *et al* [2] reported that on aflatoxin producing strain had a higher total lipid level than a non-aflatoxin producing strain, indicating a relation between aflatoxin and lipid biosynthesis Only 2 isolates were included in their study and ergosterol was the only sterol investigated We have compared the fatty acids and sterols present in 8 isolates of *Aspergillus* including both aflatoxin and non-aflatoxin producing strains to investigate further the proposed relationship between lipid metabolism and aflatoxin synthesis

RESULTS

Lipid production

The amounts of total lipids for *Aspergillus flavus* and *A parasiticus* varied with each species (Table 1) The two isolates that produced the highest amounts of total lipids, i e ATCC 15517 produces aflatoxins whereas ATCC 10124 is a non-producer The lowest

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[1] DIENER, V L and DAVIS, N D (1969) *Aflatoxins* (GOLDBLATT, L A , ed), p 13, Academic Press, New York

[2] GUPTA, S R , VISWANATHAN, L and VENKITASUBRAMANINA, I A (1970) *Ind J Biochem* 7, 108

amount of total lipid was produced by isolate ATCC 13697, a non-aflatoxigenic strain of *A flavus*, and the remaining isolates produced intermediate amounts of lipids

TABLE 1 TOTAL LIPID, PERCENTAGE FATTY ACIDS AND PERCENTAGE STEROLS IN EIGHT ISOLATES OF *Aspergillus*

Isolate No	Species	Total lipid (mg)	% Fatty acids of total lipid	% Sterols of total lipid
15517	*parasiticus**	359 0	3 31	0 33
11906	*parasiticus*	198 0	3 87	1 10
20 245	*parasiticus*	355 0	4 98	1 40
2221	*flavus**	298 5†	0 75	0 36
18 166	*flavus**	200 4	1 29	1 30
15 546	*flavus**	294 5	8 76	2 82
10 124	*flavus**	359 0	9 14	1 91
13 697	*flavus*	94 3	6 33	0 36

* Indicates aflatoxin producer
† 5 g mycelium of each isolate were extracted

Our results indicate that *A flavus* produces higher percentages of fatty acids and sterols than *A parasiticus* (Table 1), but there was no relationship between aflatoxin production and the percentages of either sterols or fatty acids present within the different strains of *Aspergillus* However, the aflatoxin producing isolate of *A parasiticus*, had lower percentage of total sterols than the non-aflatoxin producing isolates

TABLE 2 MAJOR FATTY ACIDS FOUND IN THE EIGHT *Aspergillus* ISOLATES

Isolate No	Species	Fatty acids 15 0	16 0	17 0	18 0	18 1	18 2
15 517	*parasiticus**		16 3		6 6	74 8	2 3
11 906	*parasiticus*		10 4		4 9	24 4	60 3
20 245	*parasiticus*		7 6	T	4 0	38 6	49 2
2221	*flavus**		10 7		T	39 8	48 8
18 166	*flavus**		17 8		2 6	14 5	65 1
15 546	*flavus**	T†	6 1		T	20 0	73 1
10 124	*flavus*		5 5	T	3 2	29 1	62 0
13 697	*flavus*		16 0	1 2	4 6	23 9	54 3

* Indicates aflatoxin producer
† Fatty acids occurring in amounts less than 1% are denoted by T

All of the isolates had the same major fatty acids i e 16 0, 18 0, 18 1 and 18 2 (Table 2), however, isolates 2221 and 15546 had only trace amounts of 18 0 There were slight differences in the percentages of minor fatty acids as well For example, 15546 was the only isolate to contain trace amounts of 15 0, isolates 20245, 10124 and 13697 contained relatively small amounts of 17 0 These differences in fatty acid analyses support the findings of a recent study[3] in which the fatty acid analyses could be used to identify the different species of aquatic fungi (Phycomyetes) Although the *Aspergillus* isolates could be identified by this method there was no apparent correlation of fatty acid analyses with either *Aspergillus* species or the production of aflatoxins

[3] BEAN G A, PATTERSON, G W and MOTTA J J (1972) *Comp Biochem Physiol* **43**, 935

DISCUSSION

Since acetate has been demonstrated to be the precursor to aflatoxin synthesis,[4] the assumption by Gupta *et al*[2] that sterol synthesis may influence aflatoxin production is not unreasonable However, a comparison of the different sterols present and their percentages (Table 3) provides no evidence that sterols may be involved in aflatoxin synthesis Cholesterol, ergosterol and 5,7-ergostadienol were the main sterols present and there were also minor sterols present which were not identified For example, isolate 15546 differed from all other isolates in that it contained 3 minor additional sterols

TABLE 3 STEROL CONTENT OF EIGHT ISOLATES OF *Aspergillus*

Isolate No	Species	Individual sterols (as % of total sterols) Cholesterol	Ergosterol	5,7-Ergosta-dienol	Unknown†
15517	*parasiticus**	4	80	16	
11906	*parasiticus*	2	89	5	
20245	*parasiticus*	2	72	26	
2221	*flavus**	13	70	14	4 (1 67)
18166	*flavus**	13	61	26	
15546	*flavus**	3	63	10	10 (1 52) 3 (1 77) 3 (1 97)
10124	*flavus*	3	70	24	3 (1 65)
13697	*flavus*	8	63	24	5 (1 65)

* Indicates aflatoxin producer

† Percentage and retention times of unidentified free sterols relative to cholesterol on an SE30 column

Although water-soluble sterols have been found in some plants, including yeast,[5] and Adding *et al*[6] suggests that their presence should be included in all studies on phytosterols, we were unable to detect water-soluble sterols in the 8 isolates of *Aspergillus* studied

Our studies indicate that aflatoxin and non-aflatoxin producing strains of *Aspergillus* can not be identified on the basis of total lipid, fatty acid or sterols present or their amounts It also demonstrates the weakness in an assumption concerning lipid synthesis that is based on a limited number of isolates

EXPERIMENTAL

Growth of the isolates Isolates of *Aspergillus flavus* Link and *A parasiticus* Speare, were grown in 100 ml SMKY medium for 7 days at 24° on a rotary shaker The SMKY medium contained 200 g sucrose, 7 g yeast extract, 3 g KNO_3 and 0 5 $MgSO_4$ $7H_2O$ in 1 l of dist H_2O Three of the isolates, ATCC 15546, 15517 and 18166 were aflatoxin producers and isolate No 2221 received from Dr R Welty, North Carolina State University, also produced aflatoxins The other isolates, Nos ATCC 13697, 10124, 20245 and 11906 do not produce aflatoxins

Extraction of lipids The mycelia were filtered from the medium, freeze dried, ground in a Wiley mill and stored in a desiccator until extracted 5 g of the mycelium from each isolate was extracted for 24 hr with $CHCl_3$–MeOH (2 1) in a Soxhlet apparatus The solvent was dried and the residue was dissolved in $CHCl_3$, then filtered into a weighed beaker The $CHCl_3$ was evaporated and the total lipid was weighed

[4] AYDE, J and MATELES, R I (1964) *Biochem Biophys Acta* **86,** 418

[5] ADAMS, B G and PARKS, L W (1968) *J Lipid Res* **9,** 8

[6] ANDING, C, BRANDT, R D, OURISSON G, PRYCE, R J and ROHMER, M (1972) *Proc R Soc London* **180B,** 115

The saponified portion was acidified with 6 N HCl then extracted with diethyl ether The Et_2O-soluble material was esterfied with BCl_3–MeOH, then extracted with hexane The hexane-soluble material was fractionated on a column containing Woelm grade II neutral alumina

Identification of lipids The fatty acid methyl esters and the sterol fraction collected from the alumina column were analysed using a Chromalab A 110 gas chromatograph Sterols were identified using R_t of the unknown sterols and comparing these to the R_t of cholesterol on three columns (SE 30, QF1 and PMPE) Fatty acid ester determinations were made by GLC with a Hi-EFF 1BP column and the esters identified by comparison with known fatty acid esters on basis of R_t also by TLC using $AgNO_3$-silica gel to separate the esters on the basis of the number of double bonds per molecule

Extraction of water soluble sterol Mycelia from the $CHCl_3$–MeOH extraction was dried at room temp then covered with DMSO The mycelia and DMSO were heated for 30 min After cooling, the DMSO was decanted off and extracted with hexane The hexane soluble material was fractionated on an alumina column, Woelm grade II and the sterol fraction collected and analyzed for sterols by GLC

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