Phytochemistry, Vol 24, No 5, pp 933–935, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00
Pergamon Press Ltd

CHEMICAL CONVERSION OF AFLATOXIN B1 TO M1

PAUL CHRISTOU, KATHLEEN D MILLER and ALAN PAAU

Cetus Madison Corporation, 8520 University Green, Middleton, WI 53562, U S A

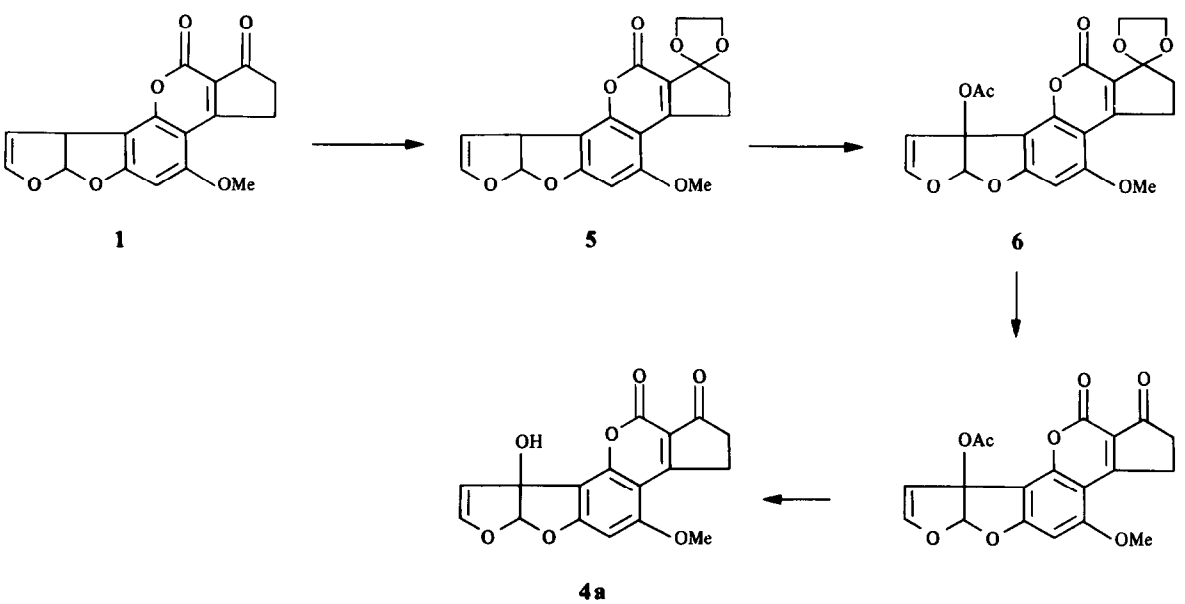
(*Received* 16 *August* 1984)

Key Word Index—*Aspergillus* species, fungus, aflatoxin synthesis

Abstract—Aflatoxin M1, a potent carcinogenic metabolite of aflatoxin B1, was synthesized in four steps, from aflatoxin B1 This reaction is of value because it yields larger quantities of this otherwise difficult to obtain compound for chemical studies and toxicological evaluation

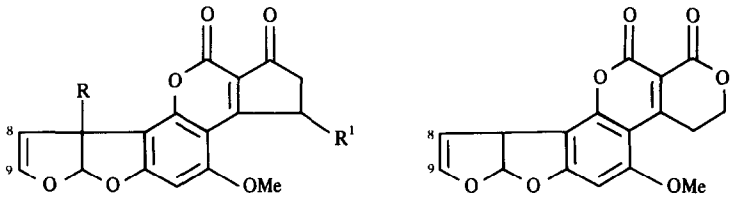
INTRODUCTION

Aflatoxins are a class of secondary metabolites of fungal origin, which are very potent carcinogens and mutagens They are produced by various molds in foods and feeds under specific conditions of temperature and humidity Aflatoxins B and G, produced by *Aspergillus* species, are common contaminants in corn, peanuts, cottonseed and a range of other grains, nuts and cereals Since the recognition of the disastrous effects these compounds have on animals and humans [1–4], much effort has been directed into research for the detection and detoxification of these compounds Research is also active on the toxicology and chemistry of the aflatoxins as well as on the elucidation of the mechanism of their carcinogenicity, mutagenicity and toxicity Aflatoxin B1 (**1**) is the primary toxic metabolite of *Aspergillus flavus* and *A parasiticus*, it can easily be produced in culture in fairly large quantities [5, 6] The same applies to aflatoxins in the G group (**2**, **3**) In the case of the M group of toxins (**4**), however, this is not the case Aflatoxin M1 was first detected in the milk of cows fed aflatoxin B1 contaminated corn and peanut meal [7, 8] Although its acute toxicity has been established [9], the most important questions concerning its carcinogenicity remain unanswered because only minute quantities of this metabolite have been available for study This fact, coupled with the expense of purchasing even minute amounts of aflatoxin M1, and our desire to develop an assay for the detection of aflatoxin M1 in milk and milk products, prompted us to investigate the feasibility of the



1 **5** **6** **4a**

Fig 1 Conversion of aflatoxin B1 to aflatoxin M1



1 R, R^1 = H,
4a R = OH, R^1 = H
4b R = OH, R^1 = H, No $\Delta^{8,9}$
7 R = H, R^1 = OH

2
3 No $\Delta^{8,9}$

Fig 2 Aflatoxin structures **1**, B1, **2**, G1, **3**, G2, **4a**, M1, **4b**, M2, **7**, Q

chemical conversion of aflatoxin B1 to aflatoxin M1 Buchi and Weinreb [10] described an elegant but very long synthesis of aflatoxin M1, starting from 4,6-dihydroxycoumaran-3-one The most common source of the toxin is *Aspergillus flavus*, this strain is one of the best producers of the compound, yielding 28–30 mg/100 ml of medium [11]

RESULTS AND DISCUSSION

Our initial attempts focused on the direct conversion of aflatoxin B1 to aflatoxin M1 using selenium dioxide oxidation because of the specificity of this reagent in allylic oxidations This oxidation, however, afforded a complex mixture of products when carried out in acetic acid–acetic anhydride, ethanol, dioxane or tetrahydrofuran, the usual selenium dioxide oxidation solvents None of these products corresponded to either aflatoxin M1 or aflatoxin M1 acetate, prepared by direct acetylation of a standard We attributed this complexity to the fact that aflatoxin B1 possesses a number of reactive sites which would be prone to oxidation

We decided therefore to include a protection/deprotection sequence in our reaction sequence in order to overcome the problem of multiple reactive sites The cyclopentenone moiety in aflatoxin B1 was protected as the ketal (**5**), using standard conditions (ethylene glycol–*p*-toluenesulphonic acid, with azeotropic removal of water) The ketal was purified by column chromatography and characterized by mass spectroscopy Aflatoxin B1 ketal was subjected to selenium dioxide oxidation under a variety of conditions (solvents, concentration and temperature) We found that when the reaction was performed in acetic acid–acetic anhydride, we obtained a mixture of three compounds, two of which were later characterized as aflatoxins M1, and Q (**7**) acetates, after deketalization, the ratio of these compounds was 3 1 We noticed that the purity of the reagent was extremely important for this conversion Use of selenium dioxide which had not been resublimed prior to the reaction afforded an extremely low yield of the desired product and the complexity of the products increased dramatically Deketalization and deacetylation of the above compounds followed by preparative TLC afforded pure, crystalline aflatoxin M1, the identity of which was confirmed by TLC comparison with a standard using three different solvent systems and a reaction with aflatoxin M1 antibodies (to be reported)

A significant amount of material was lost during the deketalization and deacetylation steps and the overall yield of pure aflatoxin M1 from aflatoxin B1 was only 0 03% Serious problems were encountered in the purification of aflatoxin M1 ketal acetate (**6**) as we were not able to convert all the colloidal red selenium to the black allotrope, for this reason, column chromatography proved ineffective in removing traces of selenium and organoselenium compounds, we managed to overcome this problem, however, by performing three successive preparative TLC purifications Safety considerations and limitations did not allow the use of sophisticated techniques such as HPLC for the purification of our reaction intermediates and products

EXPERIMENTAL

To a solution of aflatoxin B1 (0 1788 g, 0 573 mmol) in dry C_6H_6 (5 ml) was added ethylene glycol (0 354 g, 5 71 mmol) and *p*-toluenesulphonic acid (0 038 g, 0 2 mmol), the mixture was heated under reflux for 12 hr with azeotropic removal of H_2O, cooled to room temp and diluted with Et_2O (10 ml) The organic phase was washed with 5% bicarbonate (5 ml) and dried (Na_2SO_4), evapn of the solvent under red pres, purification of the residue by CC (silica gel-60, $CHCl_3$–MeOH, 19 1) and crystallization afforded the ketal in 80% yield The structure was confirmed by MS

A soln of SeO_2 in HOAc–Ac_2O (1 1) (0 016 g in 2 ml) was added dropwise to a rapidly stirred soln of the ketal in the same solvent (0 05 g in 5 ml) The soln became increasingly coloured as Se precipitated After the initial reaction, the mixture was heated at reflux for 12 hr, cooled and filtered with the addition of diatomaceous earth in order to remove colloidal Se The mixture was diluted with $CHCl_3$ (10 ml), washed (H_2O), dried and evapd under red pres to yield an oil, which was purified by prep TLC (silica gel-60, isopropanol–Me_2CO–$CHCl_3$, 1 1 8) Three major products were obtained, two of which were later characterized as the acetates of the ketals of aflatoxins M1 and Q, three successive purifications by prep TLC were required in order to obtain pure samples of the three compounds, because of this, the yields of these compounds were small

The ketal of aflatoxin M1 acetate (1 mmol) was dissolved in a mixture of Ac_2O–THF–H_2O (3 2 2, 10 ml) and stirred at 45° under N_2 for 12 hr The mixture was cooled to room temperature, diluted with $CHCl_3$ (10 ml), washed (H_2O) and dried Evapn of the solvent under red pres afforded a viscous oil, which was purified by prep TLC (silica gel-60, isopropanol–Me_2CO–

$CHCl_3$, 1 1 8) The major product was found to be aflatoxin M1 acetate, characterized by co-chromatography with authentic material prepared via a different route Aflatoxin Q was also characterized by comparison with a standard

The acetate was dissolved in dioxane (5 ml), the soln made alkaline (pH 8–9) with a saturated aq soln of $Ba(OH)_2$, and heated at reflux for 2 hr After cooling, the product was taken up in $CHCl_3$, washed with 2 M HCl, 5% $NaHCO_3$ and H_2O The organic phase was dried, evapd under red pres and the residue purified by prep TLC as above to afford pure, crystalline aflatoxin M1, the identity of which was confirmed by co-chromatography with authentic material and reaction with aflatoxin M1 antibodies

Acknowledgements—We thank the U W Department of Biochemistry for MS facilities and Cetus Corp, Emeryville, CA for facilities and equipment

REFERENCES

1 Blount, W P (1961) *Turkeys* **9**, 52, 55, 61
2 Spensley, P C (1963) *Endeavour* **22**, 75
3 Dunham, L J and Bailar, J C (1968) *J Natl Cancer Inst* **41**, 155
4 World Health Organization (1968) IARC Working Conf Studies Role Aflatoxin Human Disease, Lyon, France
5 Shotwell, O L, Hesseltine, C W, Stubblefield, R D and Sorenson, W G (1966) *Appl Microbiol* **14**, 425
6 Stubblefield, R D, Shannon, G M and Shotwell, O L (1970) *J Am Oil Chem Soc* **47**, 38
7 Allcroft, R and Carnaghan, R B A (1963) *Vet Rec* **75**, 259
8 DeIongh, H, Vles, R O and van Pelt, J G (1964) *Nature (London)* **202**, 466
9 Purchase, I F H (1967) *Food Cosmet Toxicol* **5**, 339
10 Buchi, G and Weinreb, S M (1971) **93**, 746
11 Reddy, T V, Viswanathan, L and Venkitasubramanian (1971) *Appl Microbiol* **22**, 393