Oxidation of the Bisdihydrofuran Moieties of Aflatoxin B_1 and Sterigmatocystin; Conformation of Tetrahydrofurobenzofurans

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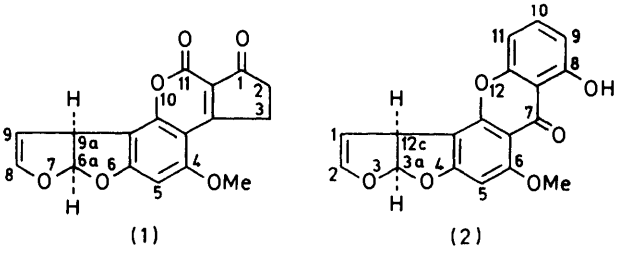
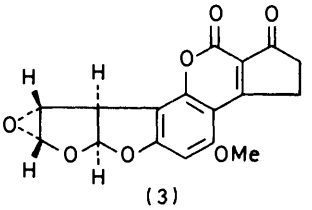
Attempts to prepare and isolate the products of epoxidation of the vinyl ether moieties of the two metabolites aflatoxin B_1 (1) and sterigmatocystin (2) gave only *trans*-glycol derivatives. The rates of consumption of peroxy-acid in the presence of compounds (1) and (2) were measured. Aflatoxin B_1 dichloride and dibromide were prepared and their susceptibilities to nucleophilic attack were examined. The conformations of various di-substituted tetrahydrofurobenzofuran derivatives obtained were studied by ^1H n.m.r. spectroscopy.

AFLATOXIN B_1 (1) and sterigmatocystin (2) are toxic and carcinogenic for a wide species range.[1] Studies on the biochemical mechanism of their action have indicated that their bioactivity is associated with the vinyl ether moiety.[2] Most carcinogens are either electrophilic molecules or are biologically activated by cytochrome P_{450}-dependent mixed function oxygenase. These electrophiles are capable of binding *in vivo* with the informational macromolecules thus inducing cancer.[3] Aflatoxin B_1 lacks reactivity towards nucleophiles and shows only weak *in vitro* association with DNA; this results in inhibition of the template activity of DNA for RNA synthesis.[4] Covalent binding of activated aflatoxin B_1 to the macromolecules appears more likely. Aflatoxin B_1 is metabolised by liver enzymes in different species into several derivatives which are less toxic than aflatoxin B_1.[5] Recently, aflatoxin B_1 oxide (3) has been postulated as the ultimate precarcinogen.[6,7] The epoxide is extremely labile under non-biological conditions (see later) and can be detoxified *in vivo* by conversion into the *trans*-diol (4) by epoxide hydrase. The epoxide intermediate will, therefore, require protection in a hydrophobic environment, if produced on the endoplasmic reticulum, during its migration to the site of action with genetic material (RNA or DNA) in the nucleus; alternatively the epoxidation may occur in the nucleus. Exogenous nucleophiles, *e.g.* sodium thiosulphate, glutathione, methionine, or cysteine, do not compete with water for attack on the aflatoxin B_1 oxide.[8] These findings have been substantiated by similar results with the less reactive epoxide, 1,2-epoxytetradecane.[9]

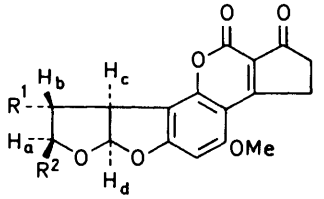
The role of aflatoxin B_1 oxide must be assessed carefully. Peters *et al.*[10] reported that 1,2-epoxy-3,3,3-trichloropropane, an epoxide hydrase inhibitor, does not affect the activation of aflatoxin B_1 by rat liver microsomes. Similarly, pretreatment of Wistar rats with phenobarbitone reduces the aflatoxin B_1 carcinogenicity.[11] However, Raj *et al.*[12] have recently observed the formation of the aflatoxin-B_1–glutathione conjugate.



(1) (2)



(3)



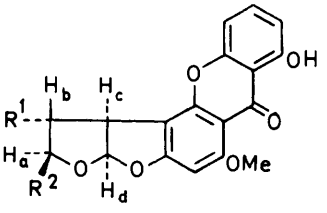
(4) $R^1 = R^2 = OH$
(5) $R^1 = OH, R^2 = OEt$
(6) $R^1 = OH, R^2 = O_2C{\cdot}C_6H_4Cl-m$
(7) $R^1 = R^2 = Cl$
(8) $R^1 = R^2 = Br$
(9) $R^1 = R^2 = H$

From the above evidence it is apparent that the epoxidation of aflatoxin B_1 may represent not only an

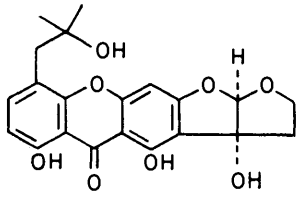
[1] G. N. Wogan and P. M. Newberne, *Cancer Res.*, 1967, **27**, 2370.
[2] J. C. Engelbrecht and B. Altenkirk, *J. Nat. Cancer Inst.*, 1972, **48**, 1647.
[3] J. A. Miller, *Cancer Res.*, 1970, **30**, 559.
[4] G. S. Edwards and G. N. Wogan, *Biochim. Biophys. Acta*, 1970, **224**, 597.
[5] T. C. Campbell and J. R. Hayes, *Toxicol. Appl. Pharmacol.*, 1976, **35**, 119.
[6] R. C. Garner, E. C. Miller, and J. A. Miller, *Cancer Res.*, 1972, **32**, 2058.
[7] R. C. Garner, *F.E.B.S. Letters*, 1973, **36**, 261.
[8] R. C. Garner and C. M. Wright, *Brit. J. Cancer*, 1973, **28**, 544.
[9] R. P. Hanzlik, M. Edelman, W. J. Michaely, and G. Scott, *J. Amer. Chem. Soc.*, 1976, **98**, 1952.
[10] J. W. Peters, R. M. Cooke, F. O. O'Neal, and S. D. Aust, *Fed. Proc.*, 1975, **34**, 784.
[11] R. C. Garner, *Biochem. Pharmacol.*, 1975, **24**, 1553.
[12] H. J. Raj, K. Santhaman, R. P. Gupta, and T. A. Venkitasubramanian, *Chem. Biol. Interactions*, 1975, **11**, 301.

activation but also a detoxification mechanism. With this controversy in mind an effort was made to prepare aflatoxin B_1 oxide (3), in order to study its chemical and biological properties.

Treatment of aflatoxin B_1 with an excess of hydrogen peroxide and with either sodium hydroxide or potassium carbonate as base, at controlled pH (7.5—8.5), resulted only in the recovery of starting material. Similarly, use of hydrogen peroxide with either 4-chlorophenyl isocyanate [13] or benzonitrile [14] failed to yield any isolable epoxidation products. As the epoxidation of olefins with organic hydroperoxides in the presence of transition metal catalysts has been reported to occur in high yields,[15] aflatoxin B_1 was treated with t-butyl hydroperoxide, with hexacarbonylmolybdenum or acetylacetonatovanadium as catalyst. No epoxidation products were detected either at room temperature or at elevated temperatures in dichloromethane–toluene.



(10) R^1=OH, R^2=$O_2C{\cdot}C_6H_4Cl-m$
(11) R^1=OH, R^2=OEt
(12) R^1=R^2=H

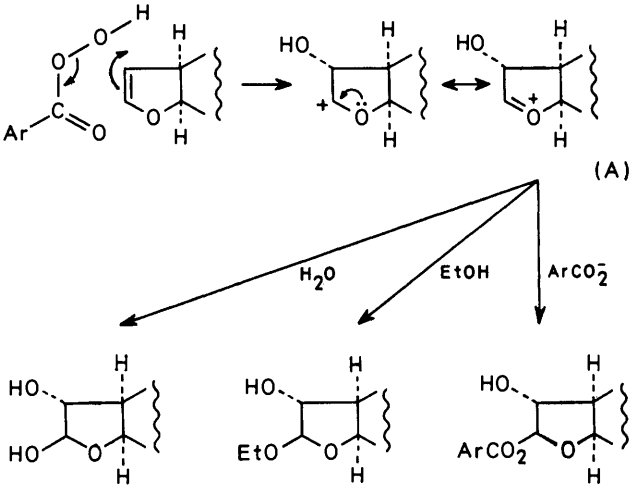


(13)

Attention was subsequently directed towards the epoxidation of aflatoxin B_1 and sterigmatocystin with *m*-chloroperbenzoic acid in chloroform. Under these conditions both aflatoxin B_2 (9) and dihydrosterigmatocystin (12) are stable.

Treatment of aflatoxin B_1 with *m*-chloroperbenzoic acid in reagent grade chloroform (stabilized with 1% ethanol) yielded the diol monoethyl ether (5) as the only isolable product. However, in ethanol-free chloroform, the *trans*-glycol ester (6) was the exclusive product. An investigation by silica t.l.c. of the products of a reaction carried out at −78 °C showed no evidence of any epoxide intermediate.

Treatment of sterigmatocystin in ethanol-free chloroform with *m*-chloroperbenzoic acid gave only the *trans*-glycol ester (10). In the presence of ethanol the ester remained the major product, only small quantities of the monoethyl ether (11) being formed. Sterigmatocystin was subsequently treated with *m*-chloroperbenzoic acid in deuteriochloroform in an n.m.r. tube at 32 °C, the reaction being monitored by recording the n.m.r. spectrum every 10 min for 2 h. The only apparent signals were those of sterigmatocystin and the *trans*-glycol ester (10). This implies that an epoxide intermediate, if formed, must be extremely short-lived. Alternatively a resonance-stabilised intermediate such as (A) can be invoked to explain the glycol formation (see Scheme 1).*



SCHEME 1 Postulate for the formation of glycol derivatives

A qualitative comparison by t.l.c. of the reactions of aflatoxin B_1 and sterigmatocystin with *m*-chloroperbenzoic acid indicated that the latter (2) reacted with greater facility. The rates of the reactions were studied by following the disappearance of the perbenzoic acid. The results indicate second-order kinetics with k 1.34×10^{-2} and 2.8×10^{-2} l mol^{-1} s^{-1} for aflatoxin B_1 and sterigmatocystin, respectively. It is possible that the free phenolic hydroxy-group in sterigmatocystin is capable of producing an intramolecular catalytic effect in the epoxidation, as qualitative experiments on sterigmatocystin methyl ether suggest that the reaction rate is decreased by methylation. Steric effects seem to be unimportant, as both compounds are largely planar molecules, except for the dihydrofuran ring which protrudes from the plane.

^{1}H N.m.r. data (see later) indicate dihedral angles of *ca.* 90° between H_a and H_b, and H_b and H_c in the glycol derivatives (5), (6), and (10) (see Table 1). As aflatoxin B_1 and sterigmatocystin have the absolute *S*-configuration at the benzylic carbon atom,[16] the *R*-configuration at C_b is indicated for these glycol products. This was substantiated by application of the Horeau method [17] to compound (10). The above-mentioned stereochemistry

* Proposed by a referee.

[13] N. Matsumura, *Tetrahedron Letters*, 1970, 2029.
[14] G. B. Payne, *Tetrahedron*, 1962, **18**, 763.
[15] M. N. Sheng and J. G. Zajacek, *J. Org. Chem.*, 1970, **35**, 1839.
[16] S. Brechbuhler, G. Büchi, and G. Milne, *J. Org. Chem.*, 1967, **32**, 2641.
[17] A. Horeau, *Tetrahedron Letters*, 1961, 506.

corresponds to attack by the peroxy-acid on the less hindered face of the molecule (α face as drawn) with subsequent *trans*-attack by the nucleophile (*e.g.* $ClC_6H_4{\cdot}CO_2^-$) on the more positive carbon atom C_a. It is uncertain whether the epoxide in fact exists during the reaction.

Swenson *et al.*[18] prepared aflatoxin B_1 dichloride (7) as a model for the proposed ultimate precarcinogen of aflatoxin B_1 (3). In our hands treatment of aflatoxin B_1 with 1 mol. equiv. of chlorine gave at least four products, as established directly by n.m.r. and t.l.c. The main product was isolated and found to be the *trans*-dichloride, as reported by Swenson *et al.*[18] Treatment of aflatoxin B_1 with an equimolar amount of bromine in deuteriochloroform gave only the *trans*-dibromide (8), which could be prepared and isolated under anhydrous conditions. It showed no molecular ion in the mass spectrum but strong peaks at *m/e* 390 and 392, corresponding to the loss of hydrobromic acid from the molecular ion.

Aflatoxin B_1 dichloride is more carcinogenic than aflatoxin B_1.[18] Aflatoxin B_1 dibromide was considered to be more susceptible to nucleophilic attack at C-8 than the dichloride and thus a better model for the aflatoxin B_1 oxide (3). The rates of hydrolysis of aflatoxin B_1 dichloride (7) and dibromide (8) in aqueous solution at pH 7.4, taken as an indication of the ease of attack at the electronegative C-8, were compared by following the bathochromic shift from 360 to 390 nm due to the formation of the phenolate anion [18] (Scheme 2). The dibromide reaction had $t_{\frac{1}{2}}$ 30 s; *cf.* 185 s for the dichloride at 20 °C. The dibromide will, therefore, react more readily with nucleophilic groups in RNA provided that hydrolysis to the bromohydrin does not occur too rapidly. This aspect is being investigated.

The ^{1}H n.m.r. spectra of compounds (5), (6), (8), and (10) show no discernible coupling between H_a and H_b and between H_b and H_c (see Table 1). This is in agreement with spectra previously recorded for a disubstituted bisdihydrofuran moiety as in multijugin.[19] Small vicinal proton–proton coupling constants are predicted by the Karplus equation [20] if the dihedral angle between the coupling protons is close to 90°. The lack of vicinal coupling can be explained if the tetrahydrofuran ring is in a single envelope (or C_s) conformation, having the CH_aR_2 carbon atom puckered towards the rest of the molecule, with the stereochemistry of the substituents as shown [(5), (6), (8), (10)]. In this conformation the dihedral angles between H_a and H_b and between H_b and H_c are close to 90°. A similar conformation has been determined for aflatoxin B_2 (9) from an *X*-ray study.[21] The above results are in agreement with the observation that dihalogenocyclopentanes [22] and dihalogenotetrahydrofurans exist in solution in a dynamic equilibrium between the two conformers with the substituents either diaxial or diequatorial, the diaxial form being predominant even in highly polar solvents.[22,23]

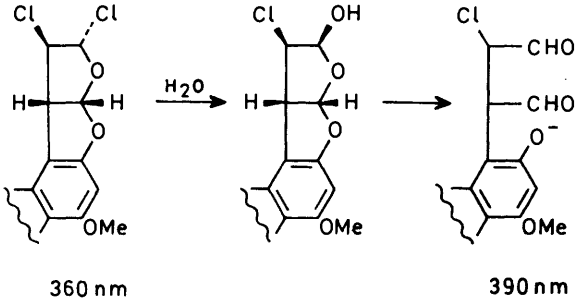
TABLE 1

^{1}H N.m.r. data

	δ					*J*/Hz		
Compound	H_a	H_b	H_c	H_d	OCH_3	a, b	b, c	c, d
(5)	5.21 (s)	4.56 (s)	3.99 (d)	6.66 (d)	3.94	0	0	6
(6)	6.57 (s)	4.90 (s) †	4.20 (d)	6.74 (d)	3.93	0	0	6
(7)	5.92 (s)	4.73 (dd)	4.47 (dd)	6.59 (d)	3.96	3.4	2.4	6
(8)	6.55 (s)	5.22 (s)	4.64 (d)	6.76 (d)	3.98	0	0	6
(10)	6.54 (s)	4.83 (s)	4.24 (d)	6.79 (d)	3.95	0	0	6

† After D_2O exchange.

Aflatoxin B_1 dichloride (7) does show vicinal proton–proton coupling ($J_{a,b}$ 3.4, $J_{b,c}$ 2.4 Hz).[18] This suggests that the dichloride exists in a conformation different from that of aflatoxin B_1 dibromide (8) and the glycol



SCHEME 2 Formation of the phenolate anion on hydrolysis of aflatoxin B_1 dichloride

derivatives (5) and (6). To investigate this anomaly the vicinal coupling constants in the tetrahydrofuran moiety of dihydrosterigmatocystin (12) were determined. In the 100 MHz ^{1}H n.m.r. spectrum of (12) the H_d signal appears as a doublet (*J* 5 Hz) at δ 6.4, and those of H_a, H_b, H_c, R^1 (= H), and R^2 (= H) appear as multiplets at δ 4.2 (2 H, H_a and H_c); δ 3.7 (1 H, R^2) and δ 2.3 (2 H, H_b and R^1). Decoupling of H_d changes the multiplet at δ 4.2 whereas irradiation at the frequency of the multiplet at δ 2.3 results in the formation of an AB system (J_{AB} −8.8 Hz; δ_A 4.2, δ_B 3.9) and a doublet

[18] D. H. Swenson, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1975, **35**, 3811.
[19] R. Vleggaar, T. M. Smallberger, and A. J. van den Berg, *Tetrahedron*, 1975, **31**, 2571.
[20] C. Romers, C. Altona, H. R. Buys, and E. Havinga, *Topics Stereochem.*, 1969, **4**, 39.
[21] T. C. van Soest and A. F. Peerdeman, *Acta Cryst.*, 1970, **B26**, 1956.
[22] C. Altona, H. R. Buys, and E. Havinga, *Rec. Trav. chim.*, 1966, **85**, 983.
[23] H. R. Buys, C. Altona, and E. Havinga, *Tetrahedron*, 1968, **24**, 3019.

(J 5 Hz; δ 4.2). The complete assignments are given in Table 2. At 32 °C the resonances are broad ($\Delta\nu_{\frac{1}{2}}$ 2 Hz) but these are sharpened ($\Delta\nu_{\frac{1}{2}}$ 0.5 Hz) when the temperature is raised to 95 °C. The vicinal coupling constants of (12) at the two temperatures are probably the same, as the pattern of the multiplets does not change with temperature.

The spectrum of (12) at 95 °C was analysed by using the program LAME.[24] The results are given in Table 2, together with the coupling constants obtained from a first-order analysis of the 100 MHz ^{1}H n.m.r. spectrum of dihydroaustocystin D (13);[25] the dihedral angles between the coupling protons as determined from the reported X-ray data of aflatoxin B_2 (9); and the coupling constants calculated for these dihedral angles by using the Karplus equation derived by Altona and Sundaralingam[26] for the sugar moieties in nucleosides and nucleotides.

TABLE 2

^{1}H N.m.r. data for dihydrosterigmatocystin (12) and dihydroaustocystin D (13)

Parameter *	Dihydrosterigmatocystin (12) †	Dihydroaustocystin D (13)	Dihedral angle between protons (°) ‡	Calculated ^3J values from ref. 27
$^3J(H_a,H_b)$	0.67	1.0	−107	0
$^3J(H_a,R^1)$	8.14	7.6	30	7.82
$^3J(R^2,H_b)$	5.61	4.9	32	6.52
$^3J(R^2,R^1)$	11.53	12.2	169	11.12
$^3J(H_b,H_c)$	1.13		90	0
$^3J(R^1,H_c)$	8.49		−23	7.82
$^3J(H_c,H_d)$	5.59		13	8.80
$^2J(H_a,R^2)$	−8.81	8.8		
$^2J(H_b,R^1)$	−12.5	12.5		

Parameter	Dihydrosterigmatocystin (12) †	Parameter	
δ_a	4.130	δ_{OMe}	3.958
δ_{R^2}	3.700	δ_5	6.326
δ_b	2.300	δ_9	6.720
δ_{R^1}	2.286	δ_{10}	7.437
δ_c	4.168	δ_{11}	6.764
δ_n	6.447	$^3J(9,10)$	8.3
		$^4J(9,11)$	1.1

* J in Hz; δ in p.p.m. from internal Me_4Si. † At 95 °C. ‡ From the X-ray study of aflatoxin B_2 (9).[21]

From these results it was concluded that the tetrahydrofurobenzofuran moieties of dihydrosterigmatocystin (12) and dihydroaustocystin D (13) must exist in solution in a conformation close to that determined for aflatoxin B_2 (9) in the solid state,[21] and for the disubstituted derivatives discussed above, excluding aflatoxin B_1 dichloride (7). The potential energy barrier between the diaxial and diequatorial conformations in dihalogenotetrahydrofuran was calculated to be 2.9 kcal mol^{-1},[23] with a fairly broad energy minimum.[22,23] The temperature dependence of the ^{1}H n.m.r. spectrum of (12) can therefore be attributed to pseudolibration.[27] In aflatoxin B_1 dichloride (7) the chlorine atoms are diaxial and the tetrahydrofuran ring is flexible as in (12). It appears that the puckering of the ring is reduced in (7), resulting in a vicinal coupling constant of 3.4 Hz between H_a and H_b.

The chemical shift data of the two protons corresponding to H_c and R^2 (= H) were previously assigned incorrectly for the dihydroautocystins.[25] These should be reassigned to conform to Table 2.

EXPERIMENTAL

U.v. absorptions were measured for solutions in methanol (Unicam SP 800 spectrometer). I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer for solutions in chloroform. Mass spectra were taken on an A.E.I. MS9 double-focusing spectrometer. N.m.r. spectra were recorded on a Varian HA-100 spectrometer and a Varian XL-100-15FT spectrometer for solutions in $CDCl_3$ with Me_4Si as internal reference. T.l.c. was carried out on Merck precoated silica plates (thicknesses 0.25 and 2 mm for analytical and preparative separations, respectively). For column chromatography Merck silica, particle size 0.063—0.200, was used.

Epoxidation of Aflatoxin B_1.—(*a*) A mixture of *m*-chloroperbenzoic acid (70 mg) and aflatoxin B_1 (75 mg) in dry, ethanol-free chloroform (15 ml) was stirred at 20 °C for 72 h. The chloroform was removed in a stream of nitrogen and the residue was separated by column chromatography. Elution with chloroform–methanol (99 : 1 v/v) gave the trans-8,9-*diol monoester* (6) (30 mg) as a brown amorphous powder; $\lambda_{max.}$ 356, 329, 263, and 215 nm (log ε 4.26, 4.24, 3.98, and 4.40); $\nu_{max.}$ 2 927, 1 763, 1 632, 1 602, 1 487, and 1 444 cm^{-1}; *m/e* 484.0537 (M^+, $C_{24}H_{17}ClO_9$ requires 484.0560).

(*b*) A mixture of *m*-chloroperbenzoic acid (40 mg) and aflatoxin B_1 (30 mg) in ethanol–chloroform (1 : 99 v/v) (10 ml) was stirred at 20 °C for 72 h. The solvent was removed in a stream of nitrogen and the residue was separated by column chromatography as above to yield the trans-*diol monoethyl ether* (5) (6 mg) as a white amorphous powder; $\lambda_{max.}$ 360, 265, 239, and 220 nm (log ε 4.23, 3.94, 3.97, and 4.16); $\nu_{max.}$ 2 930, 1 759, 1 634, 1 602, 1 487, and 1 446 cm^{-1}; *m/e* 374.1008 (M^+, $C_{19}H_{18}O_8$ requires 374.1001).

Epoxidation of Sterigmatocystin.—(*a*) A mixture of *m*-chloroperbenzoic acid (40 mg) and sterigmatocystin (50 mg) in dry, ethanol-free chloroform (5 ml) was stirred at 20 °C for 3 h. Work-up as above gave the trans-1,2-*diol monoester* (10) (49 mg) as a yellow glass; $\lambda_{max.}$ 342, 265, 242, and 231 nm (log ε 3.97, 3.95, 4.28, and 4.41); $\nu_{max.}$ 1 649, 1 621, 1 591, 1 462, 1 270, and 1 130 cm^{-1}; *m/e* 496.0549 (M^+, $C_{25}H_{17}ClO_9$ requires 496.0561).

(*b*) A mixture of *m*-chloroperbenzoic acid (40 mg) and sterigmatocystin (50 mg) in ethanol–chloroform (1 : 99 v/v) (10 ml) was stirred at 20 °C for 3 h. Removal of the solvent in a stream of nitrogen left an inseparable mixture of the ester (10) and the ethyl ether (11).

Reaction of Aflatoxin B_1 with [Bis-(m-chlorobenzoyldioxy)-iodo]benzene.—A mixture of aflatoxin B_1 (5 mg) and [bis-(*m*-chlorobenzoyldioxy)iodo]benzene (20 mg) in dry, ethanol-free chloroform (3 ml) was stirred at 20 °C for 6 h. The product was separated by t.l.c. in chloroform–methanol

[24] C. W. Haigh, *Ann. Reports N.M.R. Spectroscopy*, 1971, **4**, 311.

[25] P. S. Steyn and R. Vleggaar, *J.C.S. Perkin I*, 1974, 2250.

[26] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1973, **95**, 2333.

[27] C. Altona, H. R. Buys, and E. Havinga, *Rec. Trav. chim.*, 1966, **85**, 739.

(97 : 3 v/v) to yield the ester (6) (2 mg), identical with that obtained previously.

Iodimetric Determination of the Rate of Consumption of m-*Chloroperbenzoic Acid.*—(a) *Aflatoxin* B_1. Aflatoxin B_1 (46.9 mg) was dissolved in chloroform (50 ml) and the solution was stirred at 25 °C. *m*-Chloroperbenzoic acid (79.8%; 69.5 mg) was added and samples (5 ml) were withdrawn after 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min. To each was added saturated potassium iodide solution (2 ml), and after 5 min at 25 °C the sample was titrated against sodium thiosulphate solution (1.008×10^{-2}N) with starch as indicator. The rate constant for the consumption of *m*-chloroperbenzoic acid by aflatoxin B_1 could then be determined.[28]

(b) *Sterigmatocystin.* The same procedure was adopted, using sterigmatocystin (49.8 mg) and *m*-chloroperbenzoic acid (70.3 mg). The determinations were repeated in triplicate.

Absolute Configuration of Sterigmatocystin Glycol Monobenzoate (10).—A mixture of α-phenylbutyric acid anhydride (15 mg) and sterigmatocystin glycol monobenzoate (10) (11 mg) in anhydrous pyridine (2 ml) was stirred at 25 °C for 48 h. Water (0.5 ml) was added and the stirring was continued for 4 h. Chloroform (15 ml) was added and the solution was extracted with saturated aqueous sodium hydrogen carbonate (3 × 5 ml), washed (6N-hydrochloric acid), dried (Na_2SO_4), and evaporated. The residual ester (11 mg) had M^+ 642 ($C_{35}H_{27}ClO_{10}$).

The combined sodium hydrogen carbonate extracts were acidified (6N-hydrochloric acid) and extracted with chloroform to yield α-phenylbutyric acid (8 mg), $[\alpha]_D^{22}$ +6.2°.

Chlorination of Aflatoxin B_1.—To a solution of aflatoxin B_1 (32 mg) in dry, ethanol-free chloroform (1 ml) was added a saturated solution of chlorine in dry chloroform, 0.1 ml at a time, until t.l.c. in acetone–dichloromethane (10 : 95 v/v) showed no unchanged aflatoxin B_1. After 1 h the solvent was removed in a stream of nitrogen and the residue was separated on t.l.c. to give *aflatoxin* B_1 *dichloride* (7) (15 mg) as a white amorphous powder; $\lambda_{max.}$ 353, 262, and 221 nm (log ε 4.23, 4.01, and 4.29); $\nu_{max.}$ 1 763, 1 629, 1 601, 1 481, and 1 440 cm^{-1}; *m/e* 381.9998 (M^+, $C_{17}H_{12}Cl_2O_6$ requires 382.0011).

Bromination of Aflatoxin B_1.—To a solution of aflatoxin B_1 (32 mg) in dry, ethanol-free chloroform (2 ml) was added a solution containing 1 mol. equiv. of bromine in dry chloroform (0.1 ml). After 10 min at 20 °C the solvent was removed in a stream of nitrogen to yield *aflatoxin* B_1 *dibromide* (8) (46 mg) as a brown amorphous powder; $\lambda_{max.}$ 356, 264, and 222 nm (log ε 4.10, 3.88, and 4.12); $\nu_{max.}$ 1 767, 1 631, 1 604, 1 485, and 1 443 cm^{-1}; *m/e* 389.9724 (M^+ − HBr, $C_{17}H_{11}BrO_6$ requires 389.9739).

Hydrolysis of Aflatoxin B_1 *Dihalides.*—(a) *Dibromide.* To a solution of aflatoxin B_1 dibromide (*ca.* 30 μg) in dry acetone (0.15 ml) was added phosphate buffer solution (2.85 ml) (8.695mM-KH_2PO_4, 30.43mM-Na_2HPO_4; pH 7.4) in a cuvette at 21 °C. The absorbance at 392 nm was measured every 10 s for 2 min. Values of $t_{\frac{1}{2}}$ for hydrolysis could then be determined as described by Swenson *et al.*[18] For the dibromide $t_{\frac{1}{2}}$ 30 s.

(b) *Dichloride.* The procedure followed was as above, the readings being taken over 6 min. For the dichloride $t_{\frac{1}{2}}$ 185 s.

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[28] I. M. Kolthoff, T. S. Lee, and M. A. Mairs, *J. Polymer Sci.*, 1947, **2**, 199.