The Halogenation and Attempted Epoxidation of 3a,8a-Dihydrofuro-[2,3-*b*]benzofuran and Aflatoxin B_1

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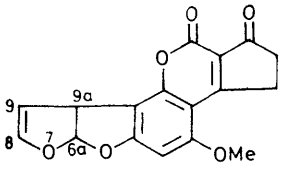
Bromine adds across the enol ether moiety of 3a,8a-dihydrofuro[2,3-*b*]benzofuran and aflatoxin B_1 to produce exclusively *trans*-dibromides, whereas addition of chlorine results in a mixture of *trans*- and *cis*-dichlorides. The resultant dibromides undergo nucleophilic substitution at C-2 or C-8, respectively, with retention of configuration, but the analogous substitution on the dichlorides is not so stereospecific. Peroxoacid oxidation of 3a,8a-dihydrofuro[2,3-*b*]benzofuran results in the addition of the peroxoacid across the terminal furan double bond *via* an epoxide or a resonance-stabilised carbonium ion intermediate to give *cis*- and *trans*-2-(3-chlorobenzoyloxy)-3-hydroxy-2,3,3a,8a-tetrahydrofuro[2,3-*b*]benzofuran and *trans*-8-(3-chlorobenzoyloxy)-9-hydroxy-8,9-dihydroaflatoxin B_1. The *cis*-2-(3-chlorobenzoyloxy)-3-hydroxy-2,3,3a,8a-tetrahydrofuro[2,3-*b*]benzofuran ester rearranges in the reaction conditions to give *trans*-3-(3-chlorobenzoyloxy)-2-hydroxy-2,3,3a,8a-tetrahydrofuro-[2,3-*b*]benzofuran. When ethanol is added to the epoxidising system it acts as a competing nucleophile to give *cis*- and *trans*-hydroxy-acetals.

AFLATOXIN B_1 (1) is a potent liver carcinogen in the rat [1,2] and is suspected of being the causative agent of the high human primary liver cancer incidence in certain areas of the world.[3] It is known that activation of the 8,9-double bond (sometimes numbered 2,3) is necessary for biological activity [4-9] and recently an 8-(7-guanyl)-9-hydroxy-8,9-dihydroaflatoxin B_1 (3; $R^1 = R^3 = H$, R^2 = 7-guanyl, $R^4 = OH$) was found to be the major product isolated on mild acid hydrolysis of DNA-bound aflatoxin B_1 formed *in vitro*,[10] *in vivo*,[11,12] and by chemical (peroxoacid) activation.[13] This adduct is thought to arise by nucleophilic attack of the N-7 positions of guanine in DNA or RNA at C-8 of *exo*-8,9-dihydroaflatoxin B_1 oxide. Since the epoxide is not available Swenson *et al.*[14] prepared an 8,9-dichloro-8,9-dihydroaflatoxin B_1 which has an electrophilic C-8 position and suggested it as a model for the 8,9-oxide.

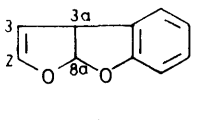
We report here our studies on the halogenation of dihydrofurobenzofurans, of nucleophilic substitution on the product dihalides, and of our attempts to make epoxides of dihydrofurobenzofurans. We have mainly used the simpler, 3a,8a-dihydrofuro[2,3-*b*]benzofuran (2), which we believe is a good model for aflatoxin B_1[15] for studying the reactions of the furan double bond.

RESULTS AND DISCUSSION

Syntheses of Dihalides derived from Aflatoxin B_1 (1) and the Dihydrofurobenzofuran (2) and their Reactions with Nucleophiles.—Addition of bromine across the furan

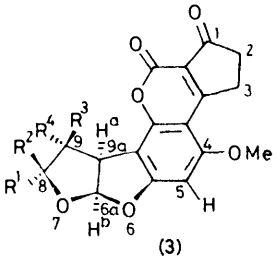


(1)

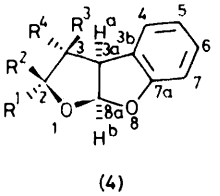


(2)

double bond of (1) or (2) gives the *trans*-dibromides (3a) and (4a) respectively resulting from initial attack by bromine on the less hindered side of the alkene bond. The configuration about C-8 and C-9 or C-2 and C-3 in the products from (1) and (2), respectively, was assigned by ^1H n.m.r. spectroscopy.[10,16] The dibromides (Tables 1 and 2) show no measurable coupling between protons H^1 and H^3, and only slight coupling, causing broadening



(3)



(4)

(3) a; $R^1 = R^3 = H$, $R^2 = R^4 = Br$
b; $R^1 = R^3 = H$, $R^2 = OEt$, $R^4 = Br$
c; $R^1 = R^3 = H$, $R^2 = R^4 = Cl$
d; $R^1 = R^4 = Cl$, $R^2 = R^3 = H$
e; $R^1 = R^3 = H$, $R^2 = 3\text{-}ClC_6H_4CO_2$, $R^4 = OH$
f; $R^1 = R^3 = H$, $R^2 = OEt$, $R^4 = OH$

(4) a; $R^1 = R^3 = H$, $R^2 = R^4 = Br$
b; $R^1 = R^3 = H$, $R^2 = OEt$, $R^4 = Br$
c; $R^1 = R^3 = H$, $R^2 = MeCO_2$, $R^4 = Br$
d; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = Br$
e; $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = Br$
f; $R^1 = R^3 = H$, $R^2 = R^4 = Cl$
g; $R^1 = R^4 = Cl$, $R^2 = R^3 = H$
h; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = Cl$
i; $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = Cl$
j; $R^1 = R^3 = H$, $R^2 = OEt$, $R^4 = Cl$
k; $R^1 = OEt$, $R^2 = R^3 = H$, $R^4 = Cl$
l; $R^1 = R^3 = H$, $R^2 = 3\text{-}ClC_6H_4CO_2$, $R^4 = OH$
m; $R^1 = 3\text{-}ClC_6H_4CO_2$, $R^2 = R^3 = H$, $R^4 = OH$
n; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = 3\text{-}ClC_6H_4CO_2$
o; $R^1 = R^3 = H$, $R^2 = OEt$, $R^4 = OH$
p; $R^1 = OEt$, $R^2 = R^3 = H$, $R^4 = OH$
q; $R^1 = R^3 = H$, $R^2 = 3\text{-}ClC_6H_4CO_2$, $R^4 = MeCO_2$

of the signals, between H^3 and H^a. It can be seen from models, and Newman projection formulae (Table 2, Figure) that in the preferred conformations of the *trans*-dibromides the C–H bonds to H^1, H^3, and H^a are almost mutually perpendicular so that, from the Karplus equation, negligible coupling between these protons would be expected. In the *cis*-configurations ($R^2 = R^3 = H$ or $R^1 = R^4 = H$) the dihedral angles between the *vicinal* C–H bonds are approximately 30°, for which

TABLE 1

^{1}H N.m.r. spectra of 8,9-disubstituted-8,9-dihydro-derivatives of aflatoxin B_1 (δ from internal tetramethylsilane)

	R^1	R^2	R^3	R^4	H^a	H^b	OMe	Aromatic-H
(3a)	H 6.56 (s)	Br	H 5.24 (s)	Br	4.64 (d, *J* 5.8 Hz)	6.78 (d, *J* 5.8 Hz)	3.98 (s)	6.42 (s)
(3b)	H 5.59 (s)	OEt 0.80 (t) 3.6—4.2 (m)	H 4.56 (s)	Br	4.43 (d, *J* 5.9 Hz)	6.73 (d, *J* 5.9 Hz)	4.00 (s)	6.40 (s)
(3c)	H 6.24 (s)	Cl	H 5.02 (s)	Cl	4.70 (d, *J* 6.0 Hz)	6.74 (d, *J* 6.0 Hz)	4.00 (s)	6.45 (s)
(3d)	Cl	H 5.96 (d, *J* 3.2 Hz)	H 4.75 (dd, *J* 3.2 and 2.5 Hz)	Cl	4.49 (dd, *J* 6.1 and 2.5 Hz)	6.61 (d, *J* 6.1 Hz)	3.98 (s)	6.45 (s)
(3e)	H 6.66 (s)	3-ClC$_6$H$_4$CO$_2$ 7.30—7.80 (m)	H 4.96 (s)	OH	4.29 (d, *J* 5.9 Hz)	6.85 (d, *J* 5.9 Hz)	3.98 (s)	6.45 (s)
(3f)	H 5.20 (s)	OEt 0.93 (t) 3.40—3.80 (m)	H 4.55 (s)	OH	4.00 (d, *J* 6.0 Hz)	6.65 (d, *J* 6.0 Hz)	3.95 (s)	6.30 (s)

s = Singlet, d = doublet, t = triplet, m = multiplet.

TABLE 2

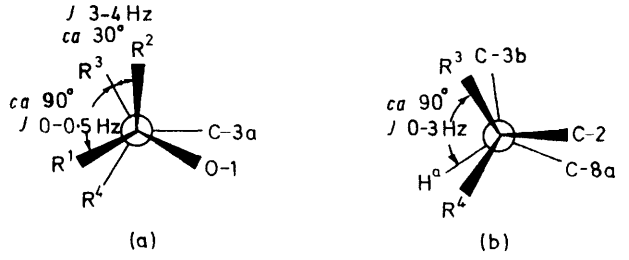
^{1}H N.m.r. spectra of 2,3-disubstituted-2,3,3a,8a-tetrahydrofuro[2,3-*b*]benzofurans (δ from internal tetramethylsilane)

	R^1	R^2	R^3	R^4	H^a	H^b	Aromatic-H
(4a)	H 6.64 (s)	Br	H 5.03 (s)	Br	4.47 (d, *J* 5.8 Hz)	6.72 (d, *J* 5.8 Hz)	6.8—7.5 (m)
(4b)	H 5.40 (s)	OEt 0.80 (t), 3.1—3.8 (m)	H 4.36 (s)	Br	4.28 (d, *J* 6.0 Hz)	6.54 (d, *J* 6.0 Hz)	6.7—7.2 (m)
(4c)	H 6.45 (s)	OAc 1.55 (s)	H 4.50 (s)	Br	4.42 (d, *J* 5.9 Hz)	6.62 (d, *J* 5.9 Hz)	6.8—7.5 (m)
(4d)	H 5.79 [a] (d, *J* 4.5 Hz)	OH 2.45 [b] (d, *J* 4.5 Hz)	H 4.39 (s)	Br	4.25 (d, *J* 5.6 Hz)	6.56 (d, *J* 5.6 Hz)	6.7—7.4 (m)
(4e)	OH 3.4 [b] (d, *J* 12.0 Hz)	H 5.05 [c] (dd, *J* 12.0 and 3.5 Hz)	H 4.57 (d, *J* 3.5 Hz)	Br	4.28 (d, *J* 5.6 Hz)	6.32 (d, *J* 5.6 Hz)	6.7—7.2 (m)
(4f)	H 6.13 (s)	Cl	H 4.75 (s)	Cl	4.33 (d, *J* 5.8 Hz)	6.54 (d, *J* 5.8 Hz)	6.8—7.4 (m)
(4g)	Cl	H 6.02 (d, *J* 3.6 Hz)	H 4.34 (dd, *J* 5.9 and 3.6 Hz)	Cl	4.16 (dd, *J* 6.1 and 5.9 Hz)	6.42 (d, *J* 6.1 Hz)	6.8—7.4 (m)
(4h)	H 5.51 (s)	OH	H 4.32 (s)	Cl	4.18 (d, *J* 6.2 Hz)	6.57 (d, *J* 6.2 Hz)	6.7—7.2 (m)
(4i)	OH	H 5.23 (br s)	H 4.38 (d, *J* 2.9 Hz)	Cl	4.18 (d, *J* 6.0 Hz)	6.35 (d, *J* 6.0 Hz)	6.7—7.2 (m)
(4j)	H 5.23 (s)	OEt 0.79 (t), 3.2—3.8 (m)	H 4.32 (s)	Cl	4.11 (d, *J* 6.5 Hz)	6.47 (d, *J* 6.5 Hz)	6.7—7.3 (m)
(4k)	OEt 1.30 (t), 3.56—4.10 (m)	H 5.11 (d, *J* 3.2 Hz)	H 4.31 (br dd, *J ca.* 3 and 1.5 Hz)	Cl	4.20 (br dd, *J ca.* 6 and 1.5 Hz)	6.44 (d, *J* 5.5 Hz)	6.8—7.4 (m)
(4l)	H 6.44 (s)	3-ClC$_6$H$_4$CO$_2$ 6.8—7.4 (m)	H 4.62 (s)	OH 2.95 (br s) [a]	4.06 (d, *J* 5.6 Hz)	6.54 (d, *J* 5.6 Hz)	6.75—7.4 (m)
(4m)	3-ClC$_6$H$_4$CO$_2$ 6.7—7.9 (m)	H 6.35 (d, *J* 3.9 Hz)	H 4.62 (m) [d]	OH 2.42 [b] (d, *J* 4.3 Hz)	4.11 (br d, *J ca.* 6 Hz)	6.52 (d, *J* 5.8 Hz)	6.7—7.2 (m)
(4n)	H 5.70 [a] (d, *J* 4.2 Hz)	OH 2.54 [b] (d, *J* 4.2 Hz)	H 5.48 (s)	3-ClC$_6$H$_4$CO$_2$ 6.7—7.9 (m)	4.11 (br d, *J ca.* 6 Hz)	6.56 (d, *J* 5.8 Hz)	6.8—7.6 (m)
(4o)	H 5.11 (s)	OEt 1.02 (t), 3.50—4.08 (m)	H 4.38 (s)	OH 2.80 (br s) [b]	3.88 (d, *J* 6.1 Hz)	6.50 (d, *J* 6.1 Hz)	6.7—7.2 (m)
(4p)	OEt 0.79 (t), 3.04—3.72 (m)	H 5.16 (d, *J* 3.3 Hz)	H 4.36 (dd, *J* 3.3 and 1.5 Hz)	OH 1.90 (br s) [b]	4.02 (br d, *J ca.* 6 Hz)	6.52 (d, *J* 6.2 Hz)	6.7—7.2 (m)
(4q)	H 6.50 (s)	3-ClC$_6$H$_4$CO$_2$ 6.8—7.4 (m)	H 5.39 (s)	OAc 2.18 (s)	4.07 (d, *J* 5.6 Hz)	6.51 (d, *J* 5.6 Hz)	6.8—7.3 (m)

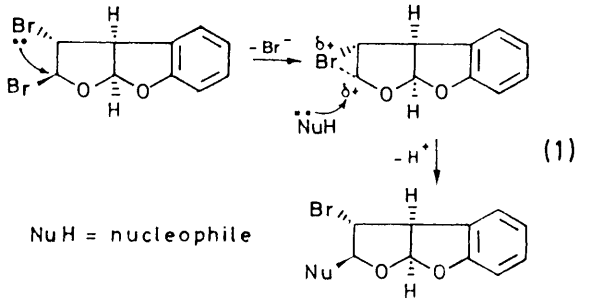
s = Singlet, d = doublet, t = triplet, m = multiplet, br = broad.

[a] Resonance collapses to a singlet on D_2O shake. [b] Resonance vanishes on D_2O shake. [c] Resonances collapses to a doublet on D_2O shake. [d] Resonance collapses to a broad doublet on D_2O shake.

spin–spin interactions of the order of 3—4 Hz would be expected. The two *cis* arrangements can be distinguished by the coupling of H^a with H^3 (0—3 Hz) or with H^4 (*ca.* 6 Hz) respectively.



Nucleophilic attack on the dibromide (4a) by ethanol, or by acetate in acetic acid, results in substitution at C-2 with retention of configuration [indicated by the n.m.r. spectra of the products (4b, c), Table 2] arising through neighbouring-group participation of bromine on C-3 [equation (1)] during substitution.[17] Similarly, the stereochemistry of 8,9-dibromo-8,9-dihydroaflatoxin B_1 (3a) was retained during attack of ethanol at C-8 [Table 1, (3b)]. Unexpectedly, propane-1-thiol or its anion in

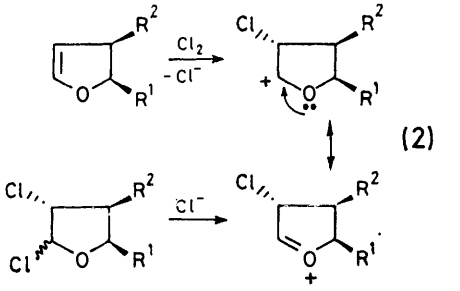


thiol suspension were poor nucleophiles towards the dibromides, and the formation of traces of thiol substitution products was confirmed only by mass spectroscopy. (It is noteworthy that the mutagenicity of aflatoxin B_1 is unaffected by the presence of several sulphur nucleophiles.[18])

Hydrolysis of the dibromide (4a) is also apparently stereospecific, since the bromohemiacetal which was isolated after a single crystallisation contained 88% of the *trans* isomer (4d). The remaining 12% of the product was the anomer (4e) derived from (4d) by inversion at C-2, comparable with the more familiar anomerisation of sugars. The proportion of the *cis* isomer in a deuteriochloroform solution of the hemiacetals increased to 40% on standing. [The stereochemistries of the bromohemiacetals (4d) and (4e) have been assigned by examination of the couplings between the protons on C-2, C-3, C-3a, and the hydroxy group, Table 2.]

The tertiary amines, triethylamine, and pyridine, and the hindered secondary amine di-t-butylamine,[19] did not react with the dibromide (4a). Diethylamine hydrobromide was formed by the reaction of diethylamine with (4a). However, we could not determine whether the hydrobromide arose from nucleophilic attack at C-2 with the consequent formation of hydrogen bromide, or from base-catalysed elimination of hydrogen bromide, since the product consisted of a mixture of inseparable compounds.

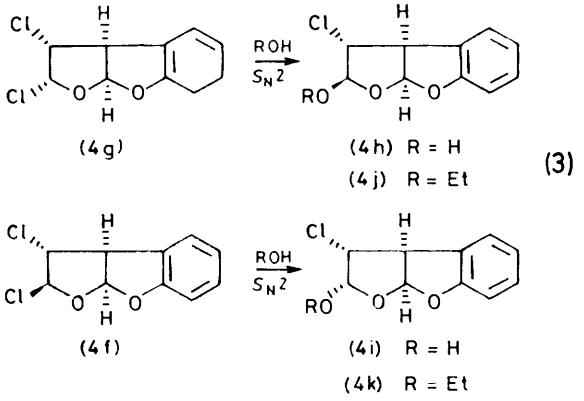
Chlorination of (2) with chlorine in dichloromethane solution gave the two dichlorides (4f) and (4g), which we were unable to separate. However, chlorination of (2) in the presence of 1 equiv. of pyridine, or by the use of *N*-chloropyridinium chloride, gave mixtures in which either dichloride (4f) was present alone or was the largely predominant product. This isomer is assigned the same *trans* configuration as the dibromide (4a) on the basis of its n.m.r. spectrum, in which the resonances of H^1 and H^3 are singlets. Dana and Roos [20] found that addition



of chlorine to a carbon tetrachloride solution of 2,3-disubstituted-2,3-dihydrofurans proceeds *via* a carbonium ion stabilised by the furan oxygen, and that the ratio of *trans* to *cis* products is under steric control [equation (2)]. When $R^1 = R^2 = H$ this ratio was 50 : 50 indicating symmetrical attack on the carbonium ion intermediate, whereas when R^1 = Ph, R^2 = Me the ratio was 22 : 78. Comparison of our results [in which the ratio (4f) : (4g) formed in carbon tetrachloride, dichloromethane, and nitrobenzene was 20 : 80, 50 : 50, and 66 : 34, respectively] with those of Dana and Roos show that (4g) is the *cis*-dichloride. The abnormally large coupling of 5.9 Hz between H^a and H^3 must be due to steric and dipole interactions of the *cis,vic* chlorine atoms of (4g), which distort the molecule from the conformations shown in the Figure. The configuration of (4g) is confirmed by the results of nucleophilic substitution on the dichlorides (4f) and (4g) in which ethanolysis of the *cis*-dichloride (4g) results in an acetal with a *trans* stereochemistry, see below.

Hydrolysis of the dichlorides (4f) and (4g) gives the chloro-hemiacetals (4h) and (4i) which, like the bromohemiacetals (4d) and (4e), anomerise at C-2 in deuteriochloroform solution. Ethanolysis of the *trans*-dichloride (4f) produces approximately equal amounts of the *trans*- and *cis*-chloroacetals (4j) and (4k) respectively, whereas ethanolysis of a mixture of dichlorides [(4f) : (4g) 20 : 80] under similar conditions, results in the same *trans*- and *cis*-chloro-acetals in a 90 : 10 ratio. The dichlorides (4f) and (4g) do not solvolyse entirely by an S_N1 mechanism, for the *trans* : *cis* ratio of the products from an S_N1 solvolysis should be independent of the configuration at C-2. Since a chloronium ion is apparently energetically

unfavourable in these systems,[20] an S_N2 mechanism must also be involved [equation (3)].



(3)

Although Swenson *et al.*[14] obtained a single dichloride from the chlorination of aflatoxin B_1 in dichloromethane solution, other workers [16] have found a complex mixture to be formed. In our study the chlorination of aflatoxin B_1 with chlorine in the absence of pyridine gave mixtures which were not readily purified, whereas the inclusion of 1 equiv. of pyridine during chlorination, or the use of *N*-chloropyridinium chloride as the chlorinating agent, gave mixtures which contained the *trans* and *cis* dichlorides (3c) and (3d) respectively. Our n.m.r. data for the *cis*-dichloride (3d) agree with those of the dichloride synthesised by Swenson *et al.*[14] and Gorst-Allman *et al.*[16] but our conclusions, based on the n.m.r. spectral data presented in Tables 1 and 2, conflict with their assignment of the configuration. Gorst-Allman *et al.*[16] have suggested that the 8,9-substituents of 8,9-disubstituted-8,9-dihydroaflatoxin derivatives exist in two interconvertible orientations, *i.e.* axial-axial and equatorial-equatorial. They assigned a *trans* configuration to the 8,9-dichloro-8,9-dihydroaflatoxin B_1 they prepared, even though the n.m.r. spectrum was significantly different from those of other *trans*-8,9-disubstituted derivatives of aflatoxin B_1. They interpreted the difference as arising from the preferred diaxial conformation of the dichloro-compound, which would give couplings between protons on C-8 and C-9 in the n.m.r. spectrum of *ca.* 3.4 Hz. However, our n.m.r. spectral data, which show the existence of two dichloro-isomers of aflatoxin B_1, and of 2,3,3a,8a-tetrahydrofurobenzofuran, one with the typical n.m.r. spectrum of the *trans* isomers and the other with the couplings observed by Gorst-Allman *et al.* and Swenson *et al.*, do not support this view. It seems likely that the dichloride of Swenson *et al.* and Gorst-Allman *et al.* has a *cis* configuration about the 8,9-bond.

Attempted Epoxidations of Aflatoxin B_1 (1) *and* 3a,8a-*Dihydrofuro*[2,3-b]*benzofuran* (2).—It would be expected that epoxidation of the enol ether moiety of the terminal furan ring of a 3a,8a-dihydrofuro[2,3-*b*]benzofuran would result in a highly reactive oxide. For example, although it has been possible to epoxidise the six-membered cyclic enol ether, 2,3-dihydro-4*H*-pyran,[21] no-one has isolated an oxide of a five-membered cyclic enol ether. Enol ether oxides are reactive towards nucleophilic reagents [22,23] and are readily cleaved by the acids formed during peroxoacid oxidation of the enol ether.[22]

Aflatoxin B_1 has been shown to react with 3-chloroperoxybenzoic acid in dichloromethane to form the *trans*-hydroxy-ester (3e) [16] and, in the presence of ethanol and 3-chloroperoxybenzoic acid, reacts to give the *trans*-hydroxy-acetal (3f).[16] (Sterigmatocystin is known to behave similarly.[16]) We have confirmed these observations for aflatoxin B_1 and have investigated the peroxoacid oxidation of (2) in greater detail.

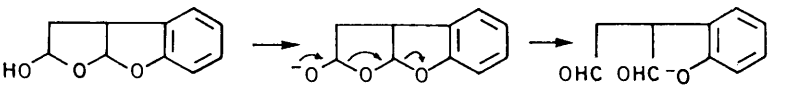
Three isomeric hydroxy-3-chlorobenzoates can be isolated from the reaction of 3-chloroperoxybenzoic acid with (2). In addition other less polar compounds account for some 2—20% of the products, but as the chromatographic profile of these was unchanged when the reaction was performed in the presence of ethanol we conclude that they do not include the 2,3-oxide. The proportions of the hydroxy-esters formed in the oxidation are solvent-dependent (Table 3). On the basis of their n.m.r. spectra, the hydroxy-esters have been assigned the structures (4l), (4m), and (4n), in which the 2,3-substituents are *trans* in (4l) and (4n), and *cis* in (4m). The *trans*-hydroxy-esters (4l) and (4n) are stable to rearrangement of the 3-chlorobenzoyloxy-moiety under the conditions of oxidation, whereas the *cis*-hydroxy-ester (4m) rearranges in the presence of 3-chloroperoxybenzoic acid to the *trans* isomer (4n). It would seem, therefore, that (4n) arises by rearrangement of the initially formed *cis*-hydroxy-ester (4m).

TABLE 3

Reactions of 3a,8a-dihydrofuro[2,3-*b*]benzofuran (2) with 3-chloroperoxobenzoic acid (PBA)

Solvent	Reactants (10^{-5} mol) PBA	(2)	Time/h	Products (%) (4l)	(4m)	(4n)	(4o)	(4p)
CH_2Cl_2	6.6	6.3	16	90	0	10		
Et_2O	2.8	1.9	30	85	0	15		
THF	5.9	7.5	16	0	100	0		
THF	18	1.2	50	0	30	70		
THF	20	1.1	80	0	0	100		
EtOH–$CHCl_3$ (2 : 98)	9.0	0.7	30	55	0	15	28	2
EtOH	60	1.3	50	0	0	80	80	20

On addition of base the u.v. spectra of all three esters show a bathochromic shift of 13 nm, which is associated with ionisation of hydroxy at C-2 and isomerism to a phenolate anion [24] [equation (4)]. This observation, however, cannot be used to distinguish (4n), which has a hydroxy on C-2, from (4l) and (4m), which have a 3-chlorobenzoyloxy-group on C-2, since at the pH neces-

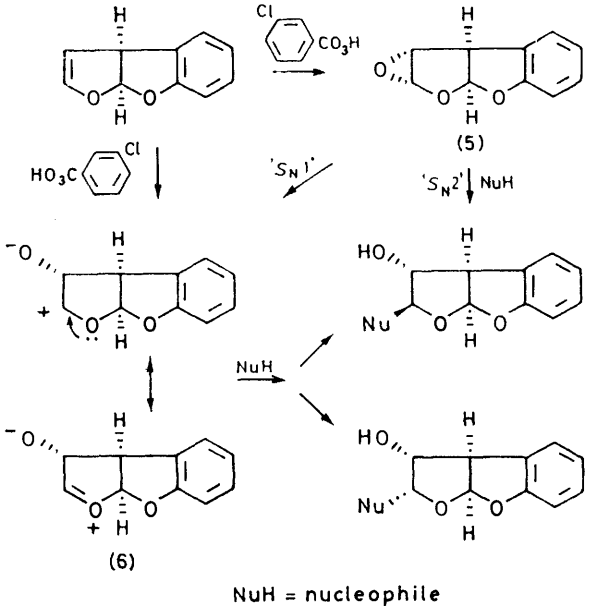


(4)

sary to ionise this hydroxy (*ca.* pH 9) hydrolysis of the hydroxy-ester to a diol also occurs. [Acetylation of the hydroxy group of the hydroxy-ester (4l) results in a deshielding of the *gem*-proton of the diester product (4q), so that the resonance of H^3 shifts downfield to a value close to that observed for the hydroxy-ester (4n).]

Two hydroxy-acetals were isolated from the peroxyacid oxidation of (2) in the presence of ethanol,[16,23] namely (4o) and (4p), corresponding to the hydroxy-esters (4l) and (4m); no hydroxy-acetal corresponding to hydroxy-ester (4n) was detected (Table 3). The hydroxy-acetals arise from nucleophilic attack by ethanol on an intermediate in the oxidation, rather than from further reaction of the hydroxy-esters (4l) and (4n), since these are stable to nucleophilic displacement of the 3-chlorobenzoyloxy moiety by ethanol under the conditions of epoxidation. Hydroxy-ester (4m) forms a 3-chlorobenzoyloxy-ethyl acetal under these conditions. [The rearrangement of *cis*-hydroxy-ester (4m) to *trans*-hydroxy-ester (4n) and the reaction with ethanol probably proceed *via* a cyclic ester. This type of acyl migration is commonly found in the reactions of carbohydrate esters.[25]]

3-Chloroperoxybenzoic acid attacks 3a,8a-dihydrofuro[2,3-*b*]benzofuran (and aflatoxin B_1) preferentially from the less hindered side to give either the *exo*-2,3-oxide (5) or a resonance-stabilised carbonium ion (6). Gorst-Allman *et al.*[16] concluded that the latter was the more likely intermediate. The compounds (4l, m, o, and p) arise when either of these intermediates is trapped by 3-chlorobenzoic acid formed during 3-chloroperoxybenzoic acid oxidation, or by ethanol [equation (5)]. If



the reactive intermediate is the epoxide, the ring-opening cannot be by a concerted S_N2 type process, which would give only the *trans* products (4l) and (4o). The alternative S_N1-type attack, however, *via* the resonance-stabilised carbonium ion (6), would lead to both *cis* (4m) and (4p) and *trans* (4l) and (4o) products. Interestingly, *cis* addition products are favoured in the more polar solvent tetrahydrofuran, which is more capable of stabilising such a carbonium ion intermediate.

Attempts were made to remove acidic materials from the oxidising system, since acids act as catalysts for the oxiran ring-opening of dihydrofuran oxides by protonation of oxiran or furan (O-1) oxygen. The inclusion of solid disodium hydrogenphosphate during 3-chloroperoxybenzoic acid or trifluoroperoxyacetic acid[26] oxidations of (2) had no effect on the product distribution. In the latter case it was hoped that the increased acidity of trifluoroperoxyacetic and trifluoroacetic acids, compared with the corresponding aromatic acids, would be offset by their more ready removal from solution by base and by their lower nucleophilicity, compared with the corresponding aromatic acids, but this was not observed since hydroxy-trifluoroacetates were formed (these are being further investigated).

Attempts to use the transition-metal-catalysed decomposition of t-butyl hydroperoxide[27] or a peroxomolybdenum–hexamethylphosphoramide complex[28] to epoxidise (2) failed. Compound (2) was recovered in the former reaction, but use of the latter reagent resulted in a complex mixture of products which could not be characterised.

The elimination of hydrogen halide from a *trans*-1,2-halogenohydrin is a common route to epoxides which, by selection of a suitable base, can be accomplished under non-nucleophilic conditions. In this way some of the diol-epoxides of benzo[*a*]pyrene have been synthesised using potassium t-butoxide or an anionic Amberlite resin as base.[29,30]

We attempted the elimination of hydrogen bromide from the bromo-hemiacetal (4d) although we were aware that only the *endo*-oxide could result from this reaction, whereas peroxyacid oxidation of (2) involves oxidation of the *exo* side of the alkene bond, and that the *endo*- and *exo*-oxides would not be expected to have the same stability to rearrangement or to nucleophilic attack. [Elimination of hydrogen bromide under such mild conditions is favourable only if the bromide and hydroxy-substituents can attain an *anti*-periplanar arrangement. The epoxide which could be formed from the bromo-hemiacetals (4d) or (4e) therefore depends on the fixed configuration at C-3, since that at C-2 is readily inverted.]

Elimination of hydrogen bromide from the bromo-hemiacetal (4d) by means of di-t-butylamine took place readily in dichloromethane, chloroform, acetone, or ethanol solution. We were unable to isolate any of the polar products from the complex product mixture, but a single non-polar product (7) was formed, in low yield, independently of solvent. In ethanol, no products resulting from nucleophilic attack on an epoxide intermediate were detected, suggesting that such an intermediate is not involved or is too rapidly rearranged to be trapped by ethanol. Compound (7) resembled a formyl-

coumarin in its n.m.r. and u.v. spectra and although we were unable to characterise it fully it was not 4-formylcoumarin, a product which might be expected from rearrangement of the furobenzofuran 2,3-oxides. 3,3,3a,8a-Tetrahydrofuro[2,3-*b*]benzofuran-2-one or 2,2,-3a,8a-tetrahydrofuro[2,3-*b*]benzofuran-3-one, which are also products expected from rearrangement of the furobenzofuran 2,3-oxides, were not found in the reaction mixtures. N.m.r. and u.v. spectroscopy showed that the polar products no longer retained the furobenzofuran moiety.

Although we have been unable to isolate the oxides of aflatoxin B_1 or (2), the *in vivo* formation of a guanine adduct, which has been isolated from DNA-bound aflatoxin B_1, is compatable with the 8,9-*exo*-oxide or a resonance-stabilised cation such as (6) being the active metabolite of aflatoxin B_1. We are at present investigating the reactivity and mutagenicity of several hydroxy-esters, derived from aflatoxin B_1 or (2), which may provide models for their respective oxides.

EXPERIMENTAL

Experiments were conducted under nitrogen (British Oxygen, white-spot grade) using dried solvents. Products were routinely isolated by preparative h.p.l.c. using a Dupont 830 chromatograph in conjunction with an 837 u.v. spectrophotometer, fitted with a Whatman 'Magnum 9' column (250 × 9.4 mm) packed with Partisil-10 and using dichloromethane or ethanol–dichloromethane (1 : 99) as solvent. Reverse-phase h.p.l.c. was performed using a Partisil-10 ODS column (250 × 3 mm) and an elution gradient of methanol–water (1 : 10) to 100% methanol. The molar proportions of products were calculated from h.p.l.c. peak areas and coefficients of absorptivity, whereas yields represent the weights of purified materials. U.v. spectra were recorded on a Pye–Unicam SP 1800 spectrophotometer and i.r. spectra were recorded on a Perkin-Elmer 257 spectrophotometer. Mass spectra were obtained with an AEI MS 30 spectrometer. Each compound for which an accurate mass measurement is recorded was homogeneous by h.p.l.c.; however, there was insufficient material available for m.p. determinations. ^{1}H N.m.r. spectra were measured with a JEOL HA 100 spectrometer and the n.m.r. spectral data are given in Tables 1 and 2.

trans-2,3-*Dibromo*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]*benzofuran* (4a).—A 10% solution of bromine in carbon tetrachloride was added, with stirring, to a solution of (2) [15] (8.0 mg, 0.05 mmol) in dichloromethane (1 cm^3) cooled to 0 °C, until the colour of bromine was no longer discharged. Solvent removal gave the *dibromide* as a crystalline solid in almost quantitative yield. On standing at 20 °C (in the absence of a nucleophilic solvent) the *dibromide* decomposed within a few hours; $\lambda_{max.}$ (CH_2Cl_2) 239, 285, and 291 nm (log ε 3.58, 3.51, and 3.47); *m/e* 319.8857 (M^+, 0.5%. $C_{10}H_8{}^{79}Br^{81}BrO_2$ requires *M*, 319.8871).

trans-8,9-*Dibromo*-8,9-*dihydroaflatoxin* B_1 (3a).—This was prepared by the method above, and had analytical data in agreement with those in the literature.[16]

trans-3-*Bromo*-2-*ethoxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]-*benzofuran* (4b). The dibromide (4a) [prepared from (2) (6.0 mg, 0.037 mmol)] was dissolved in ethanol (1 cm^3) and allowed to stand at 20 °C for 30 h. The trans-*bromoacetal* (4b) was isolated by h.p.l.c. (9.5 mg, 90%); $\lambda_{max.}$ (CH_2Cl_2) 233, 282, and 290 nm (log ε 3.35, 3.49, and 3.44); *m/e* 284.0046 (M^+, 0.7%. $C_{12}H_{13}{}^{79}BrO_3$ requires *M*, 284.0048). No other substitution products were detected in the reaction mixture.

trans-2-*Acetoxy*-3-*bromo*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]-*benzofuran* (4c).—The dibromide (4a) [prepared from (2) (8.0 mg, 0.05 mmol)] was dissolved in a mixture of anhydrous sodium acetate (0.1 g) and glacial acetic acid (1.5 cm^3) and stirred at 30 °C. After 30 h, solvent removal gave a residue which was extracted with dichloromethane, and the *bromoester* (4c) was isolated by h.p.l.c. (10.5 mg, 70%); $\lambda_{max.}$ (CH_2Cl_2) 233, 281, and 287 nm (log ε 4.19, 3.52, and 3.46); $\nu_{max.}$ (CH_2Cl_2) 1 760 cm^{-1} (C=O); *m/e* 297.9852 (M^+, 1%. $C_{12}H_{11}{}^{79}BrO_4$ requires *M*, 297.9841).

trans-9-*Bromo*-8-*ethoxy*-8,9-*dihydroaflatoxin* B_1 (3b).—The dibromide (3a) [prepared from aflatoxin B_1 (6.0 mg, 0.019 mmol)] was dissolved in ethanol (1.5 cm^3) and allowed to stand at 50 °C for 3 h, and at 20 °C for 30 h. A deuteriochloroform solution of the residue after solvent removal was used for spectroscopic analysis; $\lambda_{max.}$ (CH_2Cl_2) 230, 260, and 346 nm (log ε 4.29, 4.10, and 4.21); *m/e* 436.0157 (M^+, 3.5%. $C_{19}H_{17}{}^{79}BrO_7$ requires *M*, 436.0158).

3-*Bromo*-2-*hydroxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]*benzofuran* (4d, e).—Water (20 cm^3) was added to a solution of the dibromide (4a) [prepared from (2) (18.0 mg. 0.11 mmol)] in acetone (10 cm^3). The solution was warmed to 40 °C for 7 min, cooled, and extracted with dichloromethane (20 cm^3). Removal of solvent from the organic extract gave a residue which was crystallised (dichloromethane–pentane) and gave a single peak by h.p.l.c. analysis (7.0 mg, 25%); $\lambda_{max.}$ (CH_2Cl_2) 233, 281, and 287 nm (log ε 3.48, 3.55, and 3.50); *m/e* 255.9734 (M^+, 10.5%. $C_{10}H_9{}^{79}BrO_3$ requires *M*, 255.9735). N.m.r. spectra were recorded for a freshly prepared solution and for a solution which had been allowed to stand at 20 °C for 60 h. The ratio (4d) : (4e) was calculated from the relative integrated intensities of the low-field resonances of the bridgehead proton (H^b).

Reaction of (3a) *and* (4a) *with Propane*-1-*thiol and Propane*-1-*thiolate Anion*.—The dibromides (3a) or (4a) (1.0 mg) were dissolved in freshly distilled propane-1-thiol at 30 °C and the reactions were monitored by h.p.l.c. Since some unreacted dibromides remained after several days, the mixtures were warmed to *ca.* 50 °C for 10 h when most of the dibromides were consumed. The reactions were repeated with 1.05 equiv. of propane-1-thiolate in propane-1-thiol at 50 °C. In all the experiments no products could be isolated although traces of substitution products were shown to be present by mass spectrometry of the reaction mixtures. The mass spectra contained the ions at *m/e* 316 [reaction of (4a)] and 468 [reaction of (3a)].

Reaction of (4a) *with Amines*.—A 10% solution of di-t-butylamine [19] (0.1 cm^3) in dichloromethane was added to a solution of the dibromide (4a) in dichloromethane (0.5 cm^3) [prepared from (2) (5.0 mg, 0.031 mmol)]. After several days at 20 °C, h.p.l.c. analysis showed that the dibromide was still present. Repeating the experiment with a five-fold excess of triethylamine or with a 10% excess of pyridine in place of di-t-butylamine gave the same result. The reaction was repeated with 4 equiv. of diethylamine and crystals separated from the mixture after 2 h at 30 °C. The crystals were found to be diethylamine hydrobromide. Reverse-phase h.p.l.c. of the filtrate showed the presence of a complex series of compounds which could not be isolated.

2,3-*Dichloro*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]*benzofurans* (4f) *and* (4g).—Chlorine (1.05 equiv.) in carbon tetrachloride (*ca.* 0.1 cm^3) was added to a stirred, ice-cooled solution of (2) (8.2 mg, 0.05 mmol) in carbon tetrachloride (1 cm^3) and the mixture was warmed to 20 °C. After 5 min the solvent was removed and the residue of dichlorides was purified by h.p.l.c. (7.0 mg, 60%); $\lambda_{max.}$ (CH_2Cl_2) 230, 277, and 284 nm (log ε 3.30, 3.49, and 3.43); *m/e* 229.9880 (M^+, 3.6%. $C_{10}H_8{}^{35}Cl_2O_2$ requires *M*, 229.9902). The chlorination was repeated in dichloromethane or nitrobenzene solution and the relative proportions of the dichlorides (4f) and (4g) were determined from the integrated intensities of the resonances of the low-field bridgehead protons in the n.m.r. spectra. The chlorination was also repeated with (2) (10.0 mg, 0.06 mmol) with chlorine in carbon tetrachloride in the presence of pyridine (1.1 equiv.) or with *N*-chloropyridinium chloride (1.1 equiv.). Purification by h.p.l.c. gave a mixture of the chlorides (4f) and (4g) in the ratio 95 : 5 respectively (chlorine + pyridine) (9.5 mg, 65%) and 90 : 10 (*N*-chloropyridinium chloride) (9.0 mg, 62%).

Hydrolysis of the Dichlorides (4f) *and* (4g); 3-*Chloro*-2-*hydroxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]*benzofurans* (4h) *and* (4i).—A solution of (4f) and (4g) was prepared by the chlorination of (2) (8.2 mg, 0.051 mmol) in carbon tetrachloride. The solvent was removed, the residue dissolved in acetone (10 cm^3), and water (20 cm^3) was added to this solution, which was then heated to 40 °C for 20 min, cooled, and extracted with dichloromethane. The crude extract contained unhydrolysed dichloride (20%) and was purified by h.p.l.c. to give (4h) and (4i) in the ratio 80 : 20 (n.m.r. analysis). When a deuteriochloroform solution was left to stand at *ca.* 20 °C for 60 h these proportions changed to 60 : 40 (4.6 mg, 42%); $\lambda_{max.}$ (CH_2Cl_2) 233, 282, and 288 nm (log ε 3.29, 3.51, and 3.44); *m/e* 212.0245 (M^+, 2.4%. $C_{10}H_9{}^{35}ClO_3$ requires *M*, 212.0240).

Ethanolysis of the Dichlorides (4f) *and* (4g); 3-*Chloro*-2-*ethoxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]*benzofurans* (4j) *and* (4k).—(*a*) A mixture of (4g) and (4f) (proportions 80 : 20 by n.m.r. analysis) was made by the chlorination of (2) (13.4 mg, 0.08 mmol) in carbon tetrachloride and allowed to stand for 60 h in ethanol (1 cm^3) at 30 °C. The resulting chloroacetals (4j) and (4k), which were purified by h.p.l.c., were present in the ratio 90 : 10, respectively; the *chloroacetal* (4j) (10.9 mg, 60%) had $\lambda_{max.}$ (CH_2Cl_2) 231, 282, and 288 nm (log ε 3.17, 3.48, and 3.42); *m/e* 240.0552 (M^+, 32%. $C_{12}H_{13}{}^{35}ClO_3$ requires *M*, 240.0553).

(*b*) The experiment was repeated with a mixture of (4f) containing 5% of (4g) (confirmed by n.m.r. analysis), prepared by the chlorination of (2) (10.1 mg, 0.06 mmol) in dichloromethane containing pyridine (6 mm^3, 0.07 mmol). The ratio of the product chloroacetals (4j) : (4k), separated by h.p.l.c., was 40 : 60; the *chloroacetal* (4k) (3.2 mg, 21%) had $\lambda_{max.}$ (CH_2Cl_2) 230, 282, and 287 nm (log ε 3.16, 3.48, and 3.42); *m/e* 240.0548 (M^+, 13%. $C_{12}H_{13}{}^{35}ClO_3$ requires *M*, 240.0553).

8,9-*Dichloro*-8,9-*dihydroaflatoxin* B_1 (3c) *and* (3d).—Aflatoxin B_1 (5.0 mg, 0.02 mmol) in dichloromethane (1 cm^3) was chlorinated with chlorine (1.05 equiv.) in dichloromethane as described above. After removal of solvent, n.m.r. spectrometry showed that the residual solid was impure. Purification by preparative t.l.c. [silica gel using chloroform–acetone (9 : 1)] in a nitrogen atmosphere resulted in decomposition of the products. When the chlorination was repeated in the presence of 1 equiv. of pyridine or with *N*-chloropyridinium chloride, as described above, the products were predominantly the dichlorides (3c) and (3d) in the ratio 20 : 80, respectively (n.m.r. analysis); $\lambda_{max.}$ (CH_2Cl_2) 233, 263, and 353 nm (log ε 4.22, 4.11, and 4.23); *m/e* 382.0012 (M^+, 1.3%. $C_{17}H_{12}{}^{35}Cl_2O_6$ requires *M*, 382.0011).

3-*Chloroperoxybenzoic Acid Oxidation of* (2): 2-(3-*Chlorobenzoyloxy*)-3-*hydroxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]-*benzofurans* (4l) *and* (4m), 3-(3-*Chlorobenzoyloxy*)-2-*hydroxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]*benzofuran* (4n), *and* trans-*and* cis-2-*Ethoxy*-3-*hydroxy*-2,3,3a,8a-*tetrahydrofuro*[2,3-b]-*benzofurans* (4o) *and* (4p).—3-Chloroperoxybenzoic acid was added to solutions of (2) in solvent (1 cm^3), and the mixtures were left to stand at 20 °C for the time stated in Table 3. The mixtures were concentrated *in vacuo* and the products (4l—4p) purified by h.p.l.c. The experiments were repeated in the presence of dry disodium hydrogenphosphate (*ca.* 100 mg) with the same results. The h.p.l.c. profile of the less polar products, using dried dichloromethane as h.p.l.c. solvent, was compared with that obtained when these products were prepared in the presence of ethanol. The *hydroxy-ester* (4l) had $\lambda_{max.}$ (CH_2Cl_2) 238, 278, and 284 nm (log ε 3.91, 3.50, and 3.49); $\nu_{max.}$ ($CHCl_3$) 1 740 cm^{-1} (C=O); *m/e* 332.0455 (M^+, 0.4%. $C_{17}H_{13}{}^{35}ClO_5$ requires *M*, 332.0451). The *hydroxy-ester* (4m) had $\lambda_{max.}$ (CH_2Cl_2) 238, 278, and 283 nm (log ε 3.90, 3.52, and 3.51); $\nu_{max.}$ ($CHCl_3$) 1 740 cm^{-1} (C=O); *m/e* 332.0466 (M^+, 0.4%. $C_{17}H_{13}{}^{35}ClO_5$ requires *M*, 332.0451). The *hydroxy-ester* (4n) had $\lambda_{max.}$ (CH_2Cl_2) 239, 278, and 284 nm (log ε 3.90, 2.54, and 3.47); $\nu_{max.}$ ($CHCl_3$) 1 740 cm^{-1} (C=O); *m/e* 332.0485 (M^+, 0.5%. $C_{17}H_{13}{}^{35}ClO_5$ requires *M*, 332.0451). The *hydroxy-acetal* (4o) had $\lambda_{max.}$ (CH_2Cl_2) 233, 281, and 288 nm (log ε 3.15, 3.47, and 3.42); *m/e* 222.0891 (M^+, 0.7%. $C_{12}H_{14}O_4$ requires *M*, 222.0892). The *hydroxy-acetal* (4p) had $\lambda_{max.}$ (CH_2Cl_2) 233, 281, and 288 nm (log ε 3.15, 3.46, and 3.43); *m/e* 222.0919 (M^+, 0.5%. $C_{12}H_{14}O_4$ requires *M*, 222.0892).

3-*Acetoxy*-2-(3-*chlorobenzoyloxy*)-2,3,3a,8a-*tetrahydrofuro*-[2,3-b]*benzofuran* (4q).—A mixture of (4l) (8.0 mg, 0.025 mmol), anhydrous sodium acetate (20 mg, 0.25 mmol), and acetic anhydride (0.1 cm^3) was heated to 60 °C for 2 h, cooled, and the acetic anhydride removed *in vacuo*. The solid residue was extracted with dichloromethane (3 cm^3), and the extract purified by h.p.l.c. to give the *diester* (4q) (8.0 mg, 88%); $\lambda_{max.}$ (CH_2Cl_2) 235, 279, and 285 cm^{-1} (log ε 3.82, 3.48, and 3.47); $\nu_{max.}$ ($CHCl_3$) 1 740 and 1 760 cm^{-1} (C=O); *m/e* 374.0528 (M^+, 0.8%. $C_{19}H_{15}{}^{35}ClO_6$ requires *M*, 374.0557).

3-*Chloroperoxybenzoic Acid Oxidations of Aflatoxin* B_1; 8-(3-*Chlorobenzoyloxy*)-9-*hydroxy*-8,9-*dihydroaflatoxin* B_1 (3e) *and* 8-*Ethoxy*-9-*hydroxy*-8,9-*dihydroaflatoxin* B_1 (3f).—(*a*) 3-Chloroperoxybenzoic acid (5.0 mg, 0.028 mmol) was added to a solution of aflatoxin B_1 (5.0 mg, 0.016 mmol) in dichloromethane (1 cm^3) and the mixture allowed to stand at 20 °C). After 30 h the solvent was removed *in vacuo* and the residue recrystallised from dichloromethane–ether to give the hydroxy-ester (3e) (4.0 mg, 51%) which had analytical data in agreement with those in the literature.[16]

(*b*) The experiment was repeated using aflatoxin B_1 (7.0 mg, 0.022 mmol) in chloroform (3 cm^3) containing 2% ethanol and 3-chloroperoxybenzoic acid (6.0 mg, 0.03 mmol). The ratio of products (3e) : (3f) was determined by reverse-phase h.p.l.c. to be 1 : 3. The analytical data for (3f) agree with those quoted in the literature.[16]

Attempted Reaction of the Hydroxy-esters (3e) *and* (4l—n) *with Ethanol.*—The hydroxy-esters (3e), (4l), or (4n) (*ca.* 0.5 mg) were dissolved in ethanol (0.5 cm^3) containing a

mixture of 3-chloroperoxybenzoic acid (*ca.* 0.1 mg) and 3-chlorobenzoic acid (*ca.* 0.1 mg). These mixtures were left to stand at 20 °C for several days and monitoring by h.p.l.c. showed that the hydroxy-esters remained unchanged. 3-Chloroperoxybenzoic acid (0.5 mg)was added to the hydroxy-ester (4m) [4.0 mg in deuteriochloroform (0.5 cm^3)]. After 16 h at 20 °C the n.m.r. spectrum showed the mixture contained (4m) and (4n) in the ratio 7 : 3, respectively. Ethanol (0.1 cm^3) was added to this mixture, which was left to stand for a further 16 h. The two products, separated by h.p.l.c., were found to be (4n) and the ethyl acetal of (4n) *m/e* 360 (M^+, 3%).

Attempted Reaction of the Dihydrofurobenzofuran (2) *with t-Butyl Hydroperoxide and Bis(pentane-2,4-dionato)dioxomolybdenum*(VI). The dihydrofurobenzofuran (2), (9.0 mg, 0.056 mmol) and t-butyl hydroperoxide (6.0 mg, 0.073 mmol) were dissolved in dichloromethane (0.5 cm^3) and bis(pentane-2,4-dionato)dioxomolybdenum [31] (*ca.* 100 μg) was added. After 4 h at 20 °C, followed by 4 h at reflux no reaction was apparent.

Reaction of the Dihydrofurobenzofuran (2) *with Oxodiperoxo(hexamethylphosphoramido)molybdenum*(VI).— Oxodiperoxo(hexamethylphosphoramido)molybdenum(VI) [28] (15 mg, 0.04 mmol) was added to a solution of (2), (11.0 mg, 0.069 mmol) in dichloromethane. After 30 h at 20 °C, when no reaction was apparent, the mixture was heated to reflux when it became green and a solid separated. After 2 h all the substrate had completely reacted; however, no products could be isolated.

Reaction of the Dihydrofurobenzofuran (2) *with Trifluoroperoxyacetic acid.*—1 equiv. of trifluoroperoxyacetic acid [26] as a solution in dichloromethane was added to a stirred mixture of (2) (5.0 mg, 0.031 mmol) in dichloromethane (1 cm^3) and dried disodium hydrogenphosphate (80 mg). After 30 h at 20 °C the mixture was filtered and concentrated. H.p.l.c. analysis (silica, dry dichloromethane as solvent) showed non-polar products to be absent. The crude product was used to obtain analytical data; $\nu_{max.}$ ($CHCl_3$) 1 780 cm^{-1} ($CF_3C{=}O$); δ 4.00 (d, *J* 6 Hz), 4.56 (s), 5.35 (s), 5.58 (s), 6.22 (s), 6.49 (d, *J* 6 Hz), 6.55 (d, *J* 6 Hz), and 6.75—6.4 (m); *m/e* 290 (M^+, 75%), 243 (40), 194 (45), 147 (100), and 131 (85).

Elimination of Hydrogen Bromide from (4d) *and* (4e).— Samples of bromo-hemiacetal (4d) and (4d) (*ca.* 1 mg) were dissolved in dichloromethane, chloroform, acetone, or ethanol and to each was added a *ca.* 10% excess of di-t-butylamine in dichloromethane. Within 15 min the elimination was complete. Samples of the mixture were examined by h.p.l.c. (silica) which showed the presence of a single product, and by reverse-phase h.p.l.c. which showed a complex series of polar compounds to be present. The reaction was repeated with the bromo-hemiacetal (5.0 mg, 0.019 mmol) in deuteriochloroform (0.5 cm^3) in an n.m.r. tube and the reaction was monitored by n.m.r. After 15 min only the resonances due to the aromatic protons (δ 6.8—7.4) and di-t-butylamine hydrobromide (δ 1.30 and 4.95) were clearly evident. Concentration of the mixture, followed by extraction (1 : 1 pentane–dichloromethane) left a residue of crystalline di-t-butylamine hydrobromide. The non-polar compound was isolated from the extract by h.p.l.c. (silica) (0.5 mg, *ca.* 10%); $\lambda_{max.}$ (CH_2Cl_2) 214, 260, and 282 nm; $\nu_{max.}$ ($CHCl_3$) 3 570, 2 930, 1 705, 1 710, 1 490, 1 460, 1 220, 1 160, and 1 020 cm^{-1}; δ 5.5 (1 H, m), *ca.* 6.2 (1 H, m), 6.64 (1 H, d, *J* 4.9 Hz), *ca.* 7.2 (4 H, m), and 8.3 (1 H, dd, *J* 8 and 3 Hz); *m/e* 176 (2.9%), 159 (2.0), 147 (3.0), and 131 (3.0). The product mixture resulting from elimination in ethanol was examined by mass spectrometry. The spectrum resembled that quoted above, in particular no ion of *m/e* 222 (that of the hydroxyethyl acetals) could be detectd.

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REFERENCES

[1] M. C. Lancaster, F. P. Jenkins, and J. Mc. L. Philip, *Nature*, 1961, **192**, 1095.
[2] G. N. Wogan, 'Aflatoxin Carcinogenesis,' in 'Methods in Cancer Research,' (ed. Busch), Academic Press, New York, 1973, pp. 309—344.
[3] F. G. Peers, G. A. Gilman, and C. A. Linsell, *Internat. J. Cancer*, 1976, **17**, 167.
[4] G. N. Wogan, G. S. Edwards, and P. M. Newberne, *Cancer Res.*, 1971, **31**, 1936.
[5] J. L. Ayres, D. J. Lee, J. H. Wales, and R. O. Sinnhuber, *J. Nat. Cancer Inst.*, 1971, **46**, 561.
[6] R. C. Garner, E. C. Miller, J. A. Miller, J. V. Garner, and R. S. Hanson, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 774.
[7] R. C. Garner, E. C. Miller, and J. A. Miller, *Cancer Res.*, 1972, **32**, 2058.
[8] D. H. Swenson, J. A. Miller, and E. C. Miller, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 1260.
[9] D. H. Swenson, J. K. Lin, E. C. Miller, and J. A. Miller, *Cancer Res.*, 1977, **37**, 172.
[10] J. M. Essignman, R. G. Croy, A. M. Nadzan, W. F. Busby, jun., V. N. Reinhold, G. Büchi, and G. N. Wogan, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 1870.
[11] J. K. Lin, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1977, **37**, 4430.
[12] R. G. Croy, J. M. Essigman, V. N. Reinhold, and G. N. Wogan, *Proc. Nat. Acad. Sci. U.S.A.*, 1978, **75**, 1745.
[13] C. N. Martin and R. C. Garner, *Nature*, 1977, **267**, 863.
[14] D. H. Swenson, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1975, **35**, 3811.
[15] B. F. Coles, J. R. Lindsay Smith, and R. C. Garner, *Biochem. Biophys. Res. Comm.*, 1977, **76**, 888.
[16] C. P. Gorst-Allman, P. S. Steyn, and P. L. Wessels, *J.C.S. Perkin I*, 1977, 1360.
[17] B. Capon, *Quart. Rev.*, 1964, **18**, 45.
[18] R. C. Garner and C. M. Wright, *Brit. J. Cancer*, 1973, **28**, 544.
[19] T. G. Back and D. H. R. Barton, *J.C.S. Perkin I*, 1977, 924.
[20] G. Dana and C. Roos, *Bull. Soc. chim. France*, 1973, 371.
[21] J. J. Havel and K. H. Chan, *J. Org. Chem.*, 1976, **41**, 513.
[22] C. Stevens and J. Tazuna, *J. Amer. Chem. Soc.*, 1954, **76**, 715.
[23] A. A. Frimer, *Synthesis*, 1977, 578.
[24] G. Büchi, D. M. Foulkes, M. Kurono, G. F. Mitchell, and R. S. Schneider, *J. Amer. Chem. Soc.*, 1967, **89**, 6745.
[25] E. Pascu, *Adv. Carbohydrate Chem.*, 1945, **1**, 77.
[26] W. D. Emmons and A. S. Pagano, *J. Amer. Chem. Soc.*, 1953, **77**, 89.
[27] N. Indicator and W. F. Brill, *J. Org. Chem.*, 1965, **30**, 2074.
[28] H. Mimoun, I. Seree de Roche, and L. Sajus, *Tetrahedron*, 1970, **26**, 37.
[29] R. E. Lehr, M. Schaefer-Ridder, and D. M. Jerina, *Tetrahedron Letters*, 1977, 539.
[30] P. P. Fu and R. G. Harvey, *Tetrahedron Letters*, 1977, 2059.
[31] W. C. Fernelius, K. Terada, and B. E. Bryant, *Inorg. Synth.* 1960, **6**, 147.