MUTAGENICITY OF 3a,8a-DIHYDROFURO[2,3-b]BENZOFURAN, A MODEL OF AFLATOXIN B_1, FOR SALMONELLA TYPHIMURIUM TA100

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SUMMARY

Racemic 3a,8a-dihydrofuro[2,3-b]benzofuran has been chemically synthesized as a model of the vinyl ether structure of aflatoxin B1 (AFB_1) and tested for mutagenicity. In the presence of 9000g rat liver supernatant fraction the compound induced his^+ revertant colonies in S. typhimurium TA 100 but with only one five-thousandth the activity of AFB_1. No mutagenicity was found when strain TA98 was used. Omission of the rat liver preparation abolished mutagenic activity. The reduced compound, tetrahydrofurobenzofuran, was inactive as a mutagen either in the presence or absence of the rat liver supernatant.

The 3a,8a-dihydrofuro[2,3-b]benzofuran structure (I) occurs in a number of toxic, mutagenic and carcinogenic natural products, notably the aflatoxins and sterigmatocystins [1-3]. Biological activity is dependent on, although not exclusively determined by, the integrity of the vinyl ether group [4,5]. There is strong evidence that this function in AFB_1 is metabolically activated to a highly reactive epoxide, AFB_1-8,9-oxide (previously called the 2,3-oxide but renumbered according to IUPAC recommendations) [6-8]. The extensive reaction of this epoxide with nucleic acids, in particular with guanine, may be responsible for the mutagenic and carcinogenic properties of this potent mycotoxin [9]. Although it has not been possible, as yet, to synthesize AFB_1-8,9-oxide, a synthetic model, AFB_1-8,9-dichloride, which can readily generate an electrophilic carbon at position 8, exhibits many of the properties associated with liver activated AFB_1 [10].

In connection with our studies on the synthesis and properties of the

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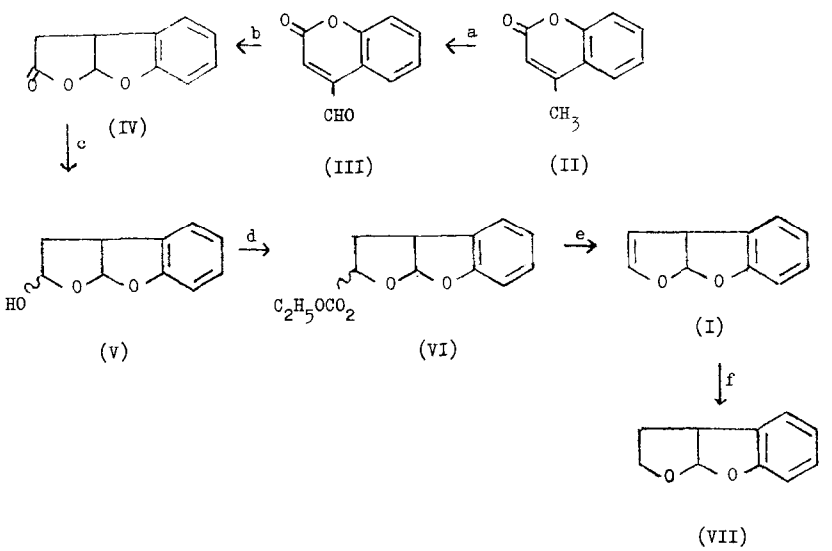
FIGURE 1 SYNTHETIC ROUTE FOR THE PREPARATION OF 3a,8a-DIHYDROFURO[2,3-b]BENZOFURAN



a. SeO_2 in xylene: b. Zn in acetic acid: c. di-isopropylaluminiumhydride in toluene: d. ethylchloroformate in pyridine: e. heat at 150-160°: f. Pd/H_2.

presumptive ultimate carcinogen AFB_1-8,9-oxide we have synthesized (I) and found it to induce his^+ revertants in S. typhimurium TA100 after rat liver activation.

MATERIALS AND METHODS

Chemical Syntheses

3a,8a-Dihydrofuro[2,3-b]benzofuran (I) was synthesized from 4-methylcoumarin [11] (II) essentially by the method developed by Büchi et al. in their syntheses of aflatoxins (Figure 1) [12,13]. The pyrolytic elimination (step e) was slightly modified in that the ethyl carbonate ester (VI), synthesized by the method of O'Connor and Nace [14], was used in place of the acetate to avoid high temperatures and consequent re-arrangement and decomposition. The spectroscopic and analytical data of the intermediates were satisfactory and in agreement with those quoted by Büchi et al. and with that of the hemi-acetal (V) synthesized by an alternative route [15]. (I) is a crystalline solid, m.p. 66-68°. ν_{max} ($CHCl_3$): 3010w, 2920m, 1625m, 1600m cm^{-1}. λ_{max} (ethanol): 210(log E = 4.00), 278(3.56), 284.5(3.49)nm. m/e, M^+=160(67%), 131(100), 103(23), 78(31), 51(20). Proton NMR ($CDCl_3$) δ : 7.1(4H,m), 6.63(1H,d, J=7Hz), 6.37(1H,t, J=2.5Hz), 5.17(1H,t, J=2.5Hz), 4.48(1H,d of t, J=7Hz,2.5Hz). ^{13}C NMR ($CDCl_3$) (noise decoupled spectrum):

157.6, 145.1, 128.4, 127.6, 124.1, 121.4, 111.4, 110.1, 103.4, 50.3. Found: C, 75.07; H, 5.29. Calculated for $C_{10}H_8O_2$: C, 74.97; H, 5.04.

The tetrahydrofurobenzofuran (VII) was synthesized by reduction of (I) with hydrogen using a 5% palladium/charcoal catalyst; m.p. 37-39°. ν_{max} ($CHCl_3$): 3020w, 2960m, 2880m, 1615w, 1600m cm^{-1}. Proton NMR ($CDCl_3$) δ : 7.1 (4H,m) 6.34(1H,d,J=6Hz), 4.05(2H,m), 3.65(1H,m), 2.15(2H,m).

Bacterial Mutagenicity Assays

25% post-mitochondrial supernatant fractions were prepared from phenobarbitone pre-treated rats as previously described [16]. Overnight nutrient broth cultures of S. typhimurium TA98 and TA100 (kindly provided by Dr. B.N. Ames, University of California, Berkeley) were used to assay for mutagenicity. Test compounds were dissolved in anhydrous dimethylsulphoxide and assayed as reported previously [16]. Control assays contained all ingredients except the test compounds. Results are the mean of duplicate assays from a single experiment. Each test was performed twice to check the reproducibility of the results obtained.

RESULTS AND DISCUSSION

Naturally occurring aflatoxins are optically active whereas (I) and other furobenzofurans of synthetic origin are racemates. Synthetic AFB_1 and AFM_1 have only half the biological activity of the naturally produced compounds indicating that only one enantiomer is biologically active [17].

Table 1 shows the number of histidine revertants of S. typhimurium TA98 and TA100 obtained after exposure to dihydrofurobenzofuran (I) and AFB_1 in the presence and absence of a rat liver preparation. Both compounds have mutagenic activity, but only after liver metabolism. In agreement with previous reports, AFB_1, at very low concentrations, induces both base-substitution and frame-shift mutations. In contrast (I) induces base-substitution mutations only. Taking into account the differences in molecular weight and the fact that (I) is a racemic mixture, dihydrofurobenzofuran has approximately one five-thousandth of the activity of AFB_1.

Reduction of the vinyl ether function (compound VII) abolishes mutagenic activity (data not shown) a finding analogous to the much weaker mutagenic and carcinogenic activity of AFB_2 compared with AFB_1 [5]. Thus the vinyl ether function is essential for mutagenicity, providing strong support that the model compound (I), is activated in a similar manner to AFB_1, probably through the formation of an epoxide.

TABLE 1 MUTAGENICITY OF FUROBENZOFURANS TOWARDS *S. TYPHIMURIUM* TA98 AND TA100

Compound	Concentration (μg/plate)	Liver*	His$^+$ revertants/plate	
			TA98	TA100
Aflatoxin B_1	0.05	+	269	593
		-	12	103
	0.10	+	1174	980
		-	17	108
	0.50	+	1408	1564
		-	17	129
	1.00	+	1328	1372
		-	14	126
Dihydrofurobenzo-furan	20	+	23	118
		-	21	132
	50	+	14	165
		-	15	111
	100	+	28	273
		-	17	130
	200	+	17	367
		-	16	143
Dimethylsulphoxide (Control)		+	23	129
		-	15	123

Results are the mean of duplicate assays. Numbers underlined indicate mutation considered significantly above background.

* Liver post mitochondrial fraction) present +
) absent -

Although there does not at present appear to be a straightforward relationship between carcinogenic and mutagenic potency for diverse chemical classes it is our view that the biological potency of compounds with similar chemical structure undergoing the same metabolic activation step can be

compared. The following factors might account therefore for the great difference in mutagenic potency between (I) and AFB_1 viz: a) A much smaller proportion of (I) is converted to an epoxide either because alternative pathways of metabolism such as ring hydroxylation predominate or there is a large difference in lipid partition coefficients between the two compounds affecting the amount of substrate reaching the site of activation. b) Intercalation is very important for mutagenic activity; (I) causes only base-substitution mutations whereas AFB_1 causes both base-substitution and frame-shift mutations indicating the importance not only of the vinyl ether function but also the fused lactone-cyclopentenone rings.

Work is currently in progress to determine the relative importance of the above factors in furobenzofurans and simpler vinyl ethers.

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