recovery is 14 per cent, we would expect to find 98 μg in the extract. When dissolved in 0·5 ml, and 10 μl is applied to the thin-layer plate, as in the Dietz method, then we would expect to find only 1·96 μg ISDN. This is below the amount previously demonstrated to be undetectable in a biological extract containing other fluorescing materials.

On the other hand, the sensitivity of the gas chromatographic method is such that as little as 1×10^{-2} μg may be detected by flame ionization. Some preliminary electron capture experiments have been done and, although these are subject to the problems arising from other contaminating materials, it appears that the sensitivity of the electron capture may be as high as 1×10^{-6}. These values are within the limits of anticipated blood levels and should lend themselves better than previous techniques to the determination of the metabolism of ISDN.

Gas chromatographic separation of ISDN demonstrates that 86 per cent of this drug, intravenously administered, is cleared from rabbit blood within 90 sec.

Thin-layer separation and diphenylamine detection are not satisfactory because of the low levels of ISDN found in rabbit blood.

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The effect of modifying the structure of Rubratoxin B on the acute toxicity to mice

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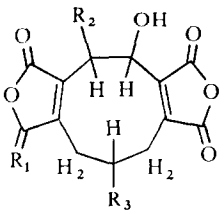
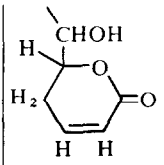
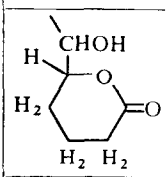
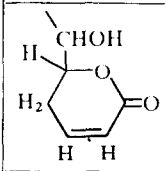
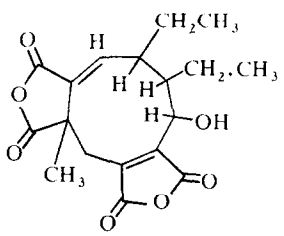
PENICILLIUM RUBRUM Stoll was isolated from mouldy foodstuffs which had proved toxic to cattle and poultry.[1, 2] Grown on liquid medium it provided two toxic metabolites, Rubratoxin A (I) and Rubratoxin B (II) the structures of which have recently been described.[3, 4] A remarkable feature of the toxicity of Rubratoxin B, described by Townsend, Moss and Peck[5] was the association of acute liver damage and death as early as 1 hr after injection. As soon as the presence of an unsaturated delta lactone moiety was identified in the structure it seemed important to relate acute toxicity to changes in chemical structure, and these are now described.

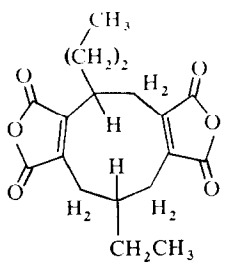
The Rubratoxins are closely related to a group of mould metabolites called collectively the nonadrides.[6] For this reason, two members of this group, Glauconic acid (VI) and Byssochlamic acid (VII), were included in these tests. Byssochlamic acid could be examined only as its tetra sodium salt because of its insolubility. With the exception of the zinc/acetic acid reduction product from Rubratoxin B, all have in common a nine membered ring with two anhydride groups, or one anhydride and one gamma lactol, as in Rubratoxin A. Treatment of Rubratoxin B with zinc and



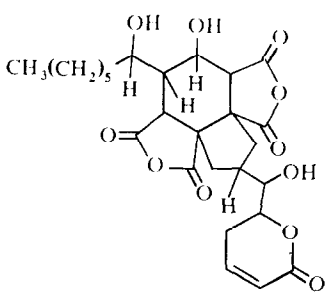
R_1	R_2	R_3
(I) H,OH,	$-CHOH\cdot(CH_2)_5CH_3$,	
(II) =O,	,,	,,
(III) =O,	,,	
(IV) =O,	$CO\cdot(CH_2)_5CH_3$,	
(V) =O,	$-CHOH\cdot(CH_2)_5CH_3$,	$-CHO$



VI



VII



(VIII)

acetic acid gives a compound in which the two double bonds in the ring have disappeared with the addition of only two hydrogen atoms. The most likely explanation, and there is an analogy in the chemistry of Byssochlamic acid,[7] is that the nine membered ring has cyclised further to form a bicyclic system such as, for example, (VIII).

All experiments were done on groups of ten male mice of the I.C.I. strain, the compounds being injected by the i.p. route. The animals were closely observed for the first 24 hr after injection and were then kept for a further 6 days before being killed and examined post-mortem. Rubratoxin B is only slightly soluble in water but is readily soluble in propylene glycol (P.G.), solution being aided by gentle warming, and most experiments were made with this solvent in volumes of 0·1 ml/20g mouse. The sodium salts of Glauconic and Byssochlamic acids were studied in solution in distilled water. LD_{50} values with their 95 per cent confidence limits were usually calculated by the method of Litchfield and Wilcoxon[8] although some compounds were available in such small quantities that only approximate values could be estimated (see Table 1).

The results in Table 1 demonstrate that an alteration of any of the functional groups in the structure of Rubratoxin B leads to a decrease in toxicity. It is especially interesting to note the presence of an unsaturated delta lactone in the structure of the toxin. The association of toxicity with alpha beta unsaturated lactones has been the subject of several studies.[9, 10] Hydrogenation of the group

TABLE 1. INTRAPERITONEAL TOXICITY OF THE RUBRATOXINS, DERIVATIVES AND RELATED COMPOUNDS TO MALE MICE

Compound	Solvent*	Purity	LD_{50} (mg/kg)
Rubratoxin A (I)	P.G.	pure	6·6 (9·2–4·8)
Rubratoxin B (II)	P.G.	pure	3·0 (4·2–2·1)
Mono sodium Rubratoxin B	P.G.	pure	4·2 (6·7–2·6)
Tri sodium Rubratoxin B	P.G.	pure	8·8 (12·1–6·4)
Tetra sodium Rubratoxin B	P.G.	pure	12·0 (18·0–8·0)
Penta sodium Rubratoxin B	P.G.	crude	ca. 75
Dihydrorubratoxin B (III)	P.G.	pure	12·0 (15·4–9·5)
Ketorubratoxin B (IV)	P.G.	pure	ca. 20
Rubratoxin B triacetate	P.G.	pure	ca. 125
Zn/HAc reduction of Rubratoxin B	P.G.	crude	not toxic at 100
Periodate cleavage product (V)	P.G.	crude	greater than 50
Glauconic acid (VI)	P.G.	pure	88·0 (88·5–8·75)
Tetra sodium glauconic acid	D.W.	pure	not toxic at 400
Tetra sodium byssochlamic acid	D.W.	pure	ca. 200

* P.G. propylene glycol, D.W. distilled water.

to the saturated lactone (III) leads to a significant drop in the toxicity but removal of this group completely, by periodate cleavage of the alpha diol formed on hydrolysis of the lactone, to give the compound (V) causes a large decrease of toxicity. The series of sodium salts from mono- to penta-, represents the sequential opening of the two anhydride and the lactone functions. There is some evidence to indicate that the lactone ring begins to open before four equivalents of alkali are consumed, but the large change of toxicity from the tetra to the penta sodium salt would indicate that in the tetra sodium salt the lactone is still largely intact.

The Rubratoxin B molecule contains three secondary hydroxyl groups. The partially acetylated derivatives have not been isolated as characterisable compounds but complete acetylation leads to a large loss of activity, and the specific oxidation of the hydroxyl group in the *n*-alkyl side chain gives rise to a dihydroxymonoketone (IV) which is less toxic than the trihydroxy parent compound.

It is not possible, at this stage, to comment on the effect of differences in solubility on the relative toxicities of these compounds although this may be a contributory factor. The poly-functional nature of the Rubratoxins gave us an opportunity to compare empirically the effect of altering one or more

of the functional groups on the toxicity of the metabolites. In no instance did any alteration in the structure of Rubratoxin B lead to an increase in toxicity and the results show that all the functional groups of this poly-functional molecule contribute to its toxicity.

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The effect of prolonged pretreatment with 6-substituted benzo [a] pyrene derivatives upon zoxazolamine paralysis times in mice

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IT HAS recently been shown[1] that pretreatment of mice with a single dose of a number of 6-substituted benzo [a] pyrene derivatives caused a shortening of the zoxazolamine paralysis time 24 hr later. In the case of 6-hydroxymethyl-benzo [a] pyrene, a marked prolongation was observed whilst benzo-[a] pyrene-6-carboxaldehyde was without effect. It seemed worth examining the effect of repeated pretreatment with benzo [a] pyrene derivatives upon zoxazolamine paralysis time. Accordingly groups of ten mice were injected with either arachis oil or 6-substituted benzo [a] pyrene derivatives in arachis oil and 72 hr and 96 hr later further doses given. A further 24 hr after the final dose zoxazolamine was administered and the paralysis time determined. The dose levels and experimental procedures have been described previously.[1]