The metabolism of dibenz[b,f]-1,4-oxazepine (CR): In vivo hydroxylation of 10,11-dihydrodibenz[b,f]-1,4-oxazepin-11-(1OH)-one and the NIH shift

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Summary. Studies of the in vivo metabolism of 10,11-dihydrodibenz[b,f]-1,4-oxazepin-11-(1OH)-one (**2**) specifically deuteriated at C-7 implicate an arene oxide intermediate during the conversion to 7-hydroxy-**2** (**4**) as evidenced by the observation of the NIH shift.

Dibenz[b,f]-1,4-oxazepine (**1**, CR) is a potent sensory irritant. The metabolism of CR in rats[1] (and other species) after i.v. or intragastric administration results in the renal excretion of essentially the whole dose as monohydroxylated derivatives (**3**–**5**)[2] of dibenz[b,f]-1,4-oxazepin-11-(1OH)-one (**2**, lactam), either unconjugated (9%) or in conjugation with sulphate (78%) by the pathway shown in scheme 1. The 7-hydroxylactam **4** represents > 50% of the administered dose.

The hydroxylation of aromatic substrates by the hepatic monooxygenases usually proceeds through arene oxide intermediates[3] whose potential arylating ability is closely linked to the possession of cytotoxic properties. Evidence for such intermediates in vivo is indirect and based on the formation of mercapturic acid derivatives or dihydrodiols as metabolites or the observation of the NIH shift using suitably labelled substrates. The subject has been reviewed[3–6].

Detailed studies of CR[1] and lactam **2**[7] metabolism have shown that **2** is a primary metabolite and the precursor of the urinary hydroxylated metabolites. To investigate the possibility of arene oxide intermediates formed during hydroxylation, the in vivo metabolism of **2**, specifically deuteriated at C-7, the major site of oxidation, was examined.

Experimental. 11-[^{14}C]-**2**[8] (3.1 mCi/mM) was mixed with 2-7-d_1[9] (containing 85% deuterium at C-7) to give **2** of specific activity 0.16 mCi/mM with 79% deuterium at C-7 which was administered i.v. in propan-1,2-diol solution to male Porton-strain rats at a dose of 15 mg/kg. Urinary metabolites were isolated (after preliminary clean-up over Amberlite XAD-2) by a combination of hydrolysis with aryl sulphatase and repeated column chromatography over silica gel. These procedures will be reported in detail elsewhere[1].

2 experiments were performed. In one, the urine was treated with aryl sulphatase prior to the separation of conjugated and non-conjugated metabolites. In the other, non-conjugated **3** and **4** were separated from their conjugates prior to hydrolysis of the latter. In each case, metabolites were purified to chemical homogeneity prior to analysis for deuterium content by mass spectrometry using a V.G. Micromass 7070F instrument at 70 eV with a source temperature of 200 °C. NMR-spectra were determined on a Jeol JNM-PS-100 spectrometer.

Results. The deuterium content of metabolites **2**, **3** and **4** is shown in the table. In the 1st experiment but not in the 2nd, a trace of unchanged **2** was isolated. There was good agreement between experiments.

The position of the deuterium in **4** was assigned by ^{1}H NMR spectroscopy[10]. In DMSO-d_6 solution, a 1st order interpretation of the resonances for H-9 (δ 6.98, d, J_{98} 8.3 Hz), H-6 (δ 6.72, d, J_{68} 2.5 Hz) and H-8 (δ 6.60, q, J_{68} 2.5 and J_{98} 8.3 Hz) was possible. By comparison of the spectra of synthetic **4** (non-deuteriated) and metabolite **4** (14% deuterium) and taking an average of many repeated integrations, the deuterium was tentatively assigned to C-8. The 9-hydroxylactam (**5**), formed in both experiments was not isolated.

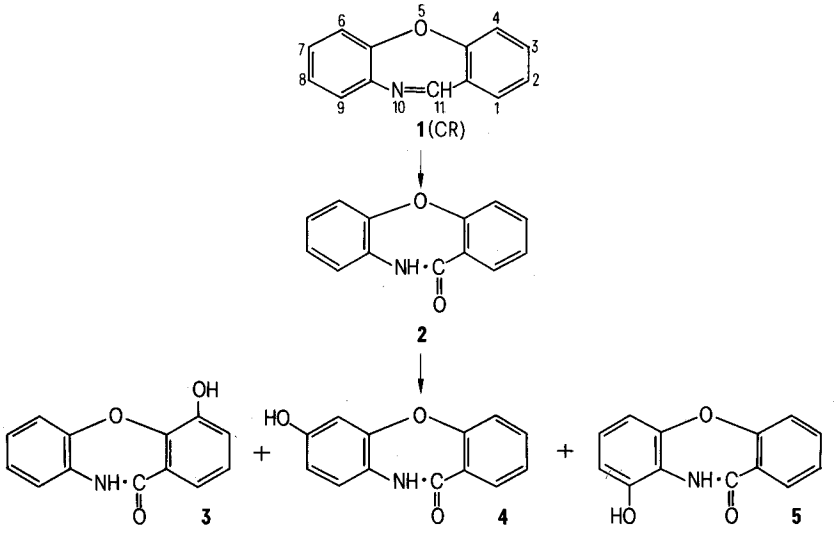
Discussion. The migration of deuterium or tritium (and also methyl or halogen) from the site of enzymic hydroxylation

Deuterium content of urinary metabolites isolated from the in vivo metabolism of 10,11-dihydrodibenz[b,f]-1,4-oxazepin-11-(10H)-one-7-d_1 (**2**)

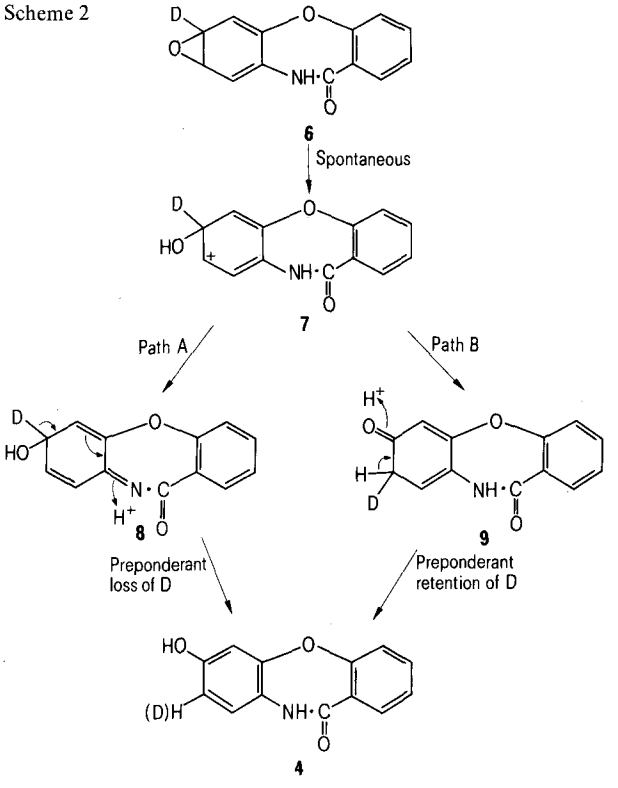
Experiment	Metabolite	Deuterium (%)
1	Lactam **2**	79
	4-Hydroxylactam **3**	80*
	7-Hydroxylactam **4**	12*
2	Lactam **2**	Not observed
	4-Hydroxylactam **3**	81**
		79***
	7-Hydroxylactam **4**	14**
		14***

*From metabolites excreted as both non-conjugates and sulphate conjugates; **excreted as non-conjugate; ***excreted as sulphate conjugate.

Scheme 1



Scheme 2



in an aromatic substrate to an adjacent position is a characteristic of a process involving in whole or in part, an arene oxide intermediate[3]. The results in the table implicate an arene oxide intermediate in the hydroxylation of lactam **2** to 7-hydroxylactam **4**. The quantitative retention of deuterium in both unmetabolised **2** and in the 4-hydroxylactam **3**, where metabolism occurs remote from the site of deuteriation, indicate that low deuterium retention in **4** is a consequence of hydroxylation at C-7 and not of alternative exchange reactions. The absence of metabolites hydroxylated at C-6 or C-8 suggests the formation of only 1 oxide which rearranges selectively to give **4**. The tentative assignment of the deuterium in **4** to C-8 is consistent only with the 7,8-oxide (**6**) (scheme 2).

The low retention (about 13%) of deuterium in **4** is consistent with an oxide in which the substituent para to the deuterium has a readily ionisable proton. It has been suggested[3,11] that after the initial spontaneous ring-opening leading in this instance to **7**, that rearrangement via path A (scheme 2) is favoured where the intervention of intermediates such as **8** result mainly in the loss of deuterium. Rearrangement via path B involving the dienone intermediate **9** result largely in the retention of deuterium. Low retentions of deuterium have been reported with other aromatic amides. Thus, acetanilide-4-d_1 showed 34% retention (in vivo, rat)[12] and the more closely related benzanilide-4-d_1, 21% retention[11].

The absence of metabolites derived from the reaction of nucleophiles with the oxide (**6**), especially mercapturic acid derivatives, suggests low arylating potential. This is consistent with the anticipated facile ring-opening and consequent ready isomerisation to the phenol that is associated with oxides from electron-rich arenes such as **2**. Experiments designed to differentiate between insertion and addition mechanisms for the in vitro conversion of **1** to **2** using a kinetic approach were unsuccessful[15].

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15 Incubation of **1** with the cytosol fraction prepared from rat liver homogenate gave good conversions (> 70%) into the lactam **2**. In an attempt to distinguish between the alternative insertion and addition pathways, i.e. between enol lactam and oxaziridine intermediates for this transformation, a kinetic isotope approach was adopted. Using a 1:1 mixture of **1**-11-d and non-deuteriated **1** (H/D = 1), the H/D ratio was determined in unchanged **1** throughout the course of the incubation (0–45 min). The H/D ratio did not deviate significantly from unity with time. In the absence of a full kinetic analysis of the system and a certain knowledge of the rate-determining step, no conclusions can be drawn from this result. A similar view has been suggested recently[13] and must cast doubt on the validity of a meaningful interpretation based on the absence of a primary kinetic isotope effect[14].

Conversion of β-sitosterol into both fucosterol and isofucosterol in *Tenebrio molitor*[1]

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Summary. It has been demonstrated with cold trap experiments that *Tenebrio molitor* transforms β-sitosterol not only into fucosterol, but also into isofucosterol.

Many species of insects are known to convert phytosterols into cholesterol[2], fucosterol (**I**) having been implicated[3-5] as intermediate in this process, since it has been isolated as a metabolite of β-sitosterol (**II**).

On the other hand, as isofucosterol (**III**) is utilized by *Bombix mori*[5] and *Tenebrio molitor*[6], the question arises whether this compound also is a real β-sitosterol metabolite, or whether its utilization is due to enzymes arising from an inductive process.

We wish now to report experimental evidence that, not only fucosterol (**I**), but also isofucosterol (**III**) is formed when β-sitosterol (**II**) is metabolized by *Tenebrio molitor*.