eosinophils/section vs 1.07 ± 0.15 in controls); it did not suppress that provoked by a further 24 hr treatment with estradiol (3.8 ± 0.6 eosinophils/section, with 5 μg estradiol alone; 13.8 ± 3.4 in *o-p'*-DDT pretreated estradiol treated rats).

In summary, for all parameters investigated, *o-p'*-DDT quantitatively and qualitatively behaves like estradiol, i.e. as a long acting, purely agonistic estrogen, thus distinct from estrogenic compounds that elicit short (like estriol) or overprolonged (like triphenylethylene derivatives) nuclear retention of the estrogen receptor.

Therefore any adverse effect that pharmacological doses of *o-p'*-DDT might have on rat uterine physiology would be attributable to undue (but otherwise normal) estrogenic stimulation rather than to an estrogen-antagonist action.

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The inhibitory effect of *p*-trifluoromethyl substitution on the hepatic microsomal metabolism of benzyl phenyl sulphide

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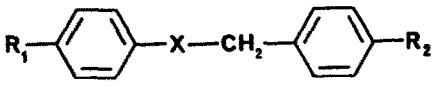
Many drugs contain sulphur and readily undergo metabolic *S*-oxidation and *S*-dealkylation which may result in inactivation and render the molecule more polar, facilitating excretion. This unwanted metabolism may perhaps be modified by the introduction of appropriately placed fluorine substituents since the inclusion of fluorine into a molecule can inhibit metabolism up to several carbon atoms removed from the fluorine atom [1–4]. In this study we have used a rat hepatic microsomal system to investigate the inhibitory effect of substituting *p*-trifluoromethyl groups into the model compound benzyl phenyl sulphide (**Ia**, see Fig. 1). The extents of overall metabolism and amounts of sulphoxides and sulphones formed have been determined. The results obtained demonstrate that while *C*-oxidation is markedly inhibited by fluorine substitution there is only a minor effect on *S*-oxidation.



	R_1	R_2		X
I	H	H	a	S
II	H	CF_3	b	SO
III	CF_3	H	c	SO_2
IV	CF_3	CF_3		
V	OH	CF_3		

Fig. 1.

Methods

Metabolism. Hepatic microsomes were prepared from sodium phenobarbitone pretreated male Wistar albino rats as previously described [5]. Incubations were carried out in stoppered 25 ml Ehrlenmeyer flasks at 37° for 60 min in a shaking water bath, the contents purged with O_2 at 30-min intervals. Microsomes (1.0 ml, 15–20 mg/ml protein; 1.7 nmol cyt P_{450}/mg protein) and substrate (0.5 mg) were incubated with a cofactor system [5]. For controls, substrate was added with internal standard at the end of the incubation. Sulphones (0.5 mg) were used as internal standards and were added as follows: **IIIc** for **Ia**, **IVc** for **IIa** and **Ic** for **IIIa** and **IVa**. Metabolism was terminated by placing flasks on ice and addition of extraction solvent. Extractions were carried out with ethyl acetate (3 × 7 ml) over NaCl (1 g) and the combined organic phases concentrated to dryness. The residues were dissolved in HPLC acetonitrile (500 μl) and aliquots (4 μl) analysed by HPLC using a system previously described [6] with a μBondpak C_{18} column (30 cm × 0.39 mm i.d.). The column was eluted with acetonitrile: water (65:35) at a flow rate of 1.0 ml/min and detection was by u.v. absorbance at 254 nm. Peak areas were calculated using a Trivector Trilab 2000 analysis programme and concentrations determined from standard curves generated with the synthesised products.

Mass spectrometry was carried out on a VG 7070H mass spectrometer, ionising voltage 70 eV, trap current 100 μA.

Synthesis

Benzyl phenyl sulphide (**Ia**) is commercially available and was used without further purification. The trifluoromethyl substituted benzyl phenyl sulphides (**IIa**, **IIIa**, **IVa**, and **Va**) were prepared by condensation of benzyl bromide or 4-(trifluoromethyl) benzyl bromide [7] with thiophenol, 4-hydroxythiophenol, or 4-(trifluoromethyl)thiophenol [8] using sodium ethoxide in ethanol as reported for the preparation of **IIa** [7].

Oxidation to the sulphoxides (**Ib**, **IIb**, **IIIb**, **IVb**) was by treatment of a solution of the sulphide (0.5 g) in acetic acid (10 ml) with hydrogen peroxide (20 vol.; 1 ml) at room temperature for 24 hr. The mixtures were poured into water (20 ml), neutralised with saturated aqueous sodium hydrogen carbonate, and extracted with ether (3 × 30 ml). Concentration of the extracts gave the crude sulphoxides.

Oxidation of the sulphide (**IIa**) to the sulphone (**IIc**) was with hydrogen peroxide (100 vol.; 5 equiv.) in acetic acid at reflux for 2 hr. The mixture was cooled to room temperature and the crude sulphone (**IIc**) which crystallised was collected by filtration and washed with ethanol.

Oxidation of the sulphides (**IIIa** and **IVa**) to the sulphones was with potassium permanganate (2.5 equiv.) in acetic acid at 20° for 30 min. The mixtures were partitioned between equal volumes of saturated aqueous sodium hydrogen carbonate and 3:1 ether–dichloromethane and the organic layer concentrated to give the crude sulphones (**IIIc** and **IVc**).

Products **IIb**, **IIIa**, **IIIb**, **IIIc**, **IVb**, **IVc** and **Va** were purified by column chromatography on silica, eluting with dichloromethane for **IIb** and **IVb**, with chloroform for **IIIa**, **IIIb** and **Va**, and with 6:4 dichloromethane–light petroleum (b.p. 40–60°) for **IIIc** and **IVc**. Solvents for recrystallisation where used and yields and physical data of products are given in Table 3.

Results and discussion

Following hepatic microsomal metabolism the extracted metabolites were initially identified by their HPLC retention times in comparison with synthesised compounds and peaks from HPLC were collected and the structures confirmed by electron impact (EI) mass spectrometry (Table 1). The extent of microsomal metabolism of the various benzyl phenyl sulphides studied was determined from the difference in ratios of peak area of substrate to internal standard between test and control incubates and the amounts of sulphoxide and sulphone formed were calculated from calibration curves obtained for the synthesised compounds (**IIb** and **c** to **IVb** and **c**); the results being displayed in Table 2.

Metabolism of benzyl phenyl sulphide itself (**Ia**) resulted in almost total disappearance of the substrate (0.5 mg) within 60 min at 37°. The quantity of sulphoxide and sulphone detected did not account for the total substrate consumed (see Table 2), most likely to be because of extensive *C*-oxidation giving rise to *S*-dealkylated products not detectable using the HPLC system herein described. In particular, benzoic acid was not detected, despite its being fully extractable and detectable in our HPLC system.

As predicted from previous investigations [1–4] the inclusion of one or two *p*-trifluoromethyl groups into **Ia** does reduce the total metabolism with di-substitution (**IVa**) resulting in total inhibition of carbon metabolism, the substrate consumption being accounted for by sulphoxidation; the over-estimation in metabolites formed is due to the high sensitivity of the HPLC data system together with low concentrations of compounds present. Substitution of the trifluoromethyl group into only one of the rings (**IIa** or **IIIa**) resulted in a greater amount of metabolism than for **IVa**, not all accounted for by sulphoxidation; however, there was less substrate consumption than determined for the unsubstituted compound (**Ia**). For compound **IIIa** less sulphoxidation was detected than with **IIa** indicating a greater inhibitory effect of the trifluoromethyl group through a phenyl ring as compared with the benzyl group, as expected. However, more unaccountable metabolism was detected for **IIIa** than with **IIa**, possibly due to debenzylation via oxidation at the relatively susceptible benzyl carbon [9], again benzoic acid was not detected as a metabolism product.

In an *in vivo* metabolism study with 2-benzylthio-5-trifluoromethylbenzoic acid a small amount of the sulphoxide together with much larger quantities of the debenzylation products 2-mercapto-5-trifluoromethylbenzoic acid and the disulphide 2,2′-dithiobis-[5-trifluoromethylbenzoic acid have been identified in rat and man [10]. This compound closely resembles **IIIa** and substantiates our suggestion that unaccounted substrate may be due to metabolic *S*-debenzylation. No other products were observed on HPLC; compound **Va** was not detected as a metabolite of **IIa**.

Our results are in general agreement with previous metabolism studies of sulphur-containing compounds in that the sulphur moiety is readily oxidised to the sulphoxide and sulphone. The enzyme characteristics of *S*-oxidation have been less well studied than for *N*- and *C*-oxidation but a number of systems are indicated including cytochrome P_{450} [11–15], Ziegler's flavoprotein [16] and a soluble guinea-pig protein [17]. Further oxidation may result in *S*-dealkylation and for a number of compounds this reaction has been shown to require microsomes, an NADPH-regenerating system and the soluble fraction [18, 19]. However, for the substrates used in this study metabolic *S*-dealkylation appears to proceed in the absence of the soluble fraction.

Table 1. HPLC retention times and principal mass spectrum fragments of benzyl phenyl sulphide (**Ia**), its *p*-trifluoromethyl analogues (**IIa–IVa**) and their isolated metabolites

	RT in HPLC (sec)	Mass spectral fragments (m/z)
Ia	730	200(M^+,23%), 91(100), 65(14), 51(4)
Ib	280	216(M^+,1%), 91(100), 65(10), 51(5)
Ic	320	232(M^+,2%), 91(100), 65(9), 51(5)
IIa	1000	268(M^+,20%), 159(100), 109(34), 65(13), 51(10)
IIb	350	284(M^+,4%), 159(100), 109(13), 77(9), 51(10)
IIc	420	300(M^+,3%), 281(2), 159(100), 109(11), 77(11), 51(12)
IIIa	1050	268(M^+,15%), 159(13), 91(100), 65(12)
IIIb	365	284(M^+,0.2%), 91(100), 65(12), 51(2)
IIIc	450	300(M^+,0.4%), 281(1), 91(100), 65(12), 51(3)
IVa	1425	336(M^+,22%), 159(100), 109(5)
IVb	470	352(M^+,1%), 159(100), 109(12), 91(13)
IVc	590	368(M^+,0.3%), 349(3), 159(100), 109(11)

Table 2. Microsomal metabolism of benzyl phenyl sulphide (**Ia**) and its *p*-trifluoromethyl analogues (**IIa–IVa**) quantified by HPLC (see text for methods)

Initial amount of substrate		Total metabolism*		Metabolites recovered			
				Sulphoxide		Sulphone	
	(μmole)	(μmole)	(% of dose)	(μmole)	(% of dose)	(μmole)	(% of dose)
Ia	2.50	2.47	99	0.38	15	0.63	25
IIa	1.87	1.28	68	0.25	13	0.62	33
IIIa	1.87	1.18	63	0.19	10	0.36	19
IVa	1.49	0.38	25	0.14	9	0.31	21

* Determined from amount of recovered substrate in test incubate compared with that in control incubate.

Table 3. Physical data for synthetic compounds

	Yield (%)	M.p. (°C)	Analytical data			
			Found %		Calculated %	
			C	H	C	H
Ib	81	124–125*				
IIa	78	66–67 (MeOH)†	62.4	3.8	62.7	4.1
IIb	62	203–205 ($CHCl_3$–petroleum 1:1)	58.8	3.9	59.1	3.9
IIc	92	239–241 ($CHCl_3$)‡				
IIIa	63	73–75 (MeOH)	62.4	4.0	62.7	4.1
IIIb	83	155–157	58.9	3.8	59.1	3.9
IIIc	61	168–170 (EtOH)	56.0	3.75	56.0	3.7
IVa	71	60–61 (EtOH–H_2O 1:1)	53.9	2.7	53.6	3.0
IVb	61	140–141 (EtOH)	51.0	2.9	51.1	2.9
IVc	83	184–185 (EtOH)	48.8	2.9	48.9	2.7
Va	71	98–100	58.9	3.9	59.1	3.9

* Lit. 125.5 [20]; †lit. 72–73.5 [7]; ‡lit. 234–235 [7].
Compounds **Ia** and **Ic** are commercially available (Lancaster Synthesis, Morecambe, U.K.).

In conclusion, although trifluoromethyl substitution into benzyl phenyl sulphide results in a reduction in the extent of microsomal metabolism this is primarily due to an inhibitory effect on carbon metabolism, oxidation at sulphur being only slightly reduced.

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