Biochemical Pharmacology, Vol. 40, No. 10, pp. 2277–2283, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
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METABOLISM OF THE 4-IODO DERIVATIVE OF TAMOXIFEN BY ISOLATED RAT HEPATOCYTES

DEMONSTRATION THAT THE IODINE ATOM REDUCES METABOLIC CONVERSION AND IDENTIFICATION OF FOUR METABOLITES

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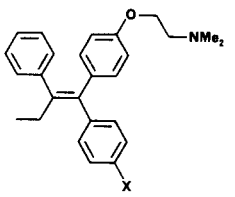
(*Received* 20 *April* 1990; *accepted* 17 *July* 1990)

Abstract—The 4-iodo derivative of tamoxifen, which has been reported to possess improved oestrogen receptor affinity and effectiveness as an inhibitor of breast tumour cell growth *in vitro*, was metabolized by hepatocytes isolated from rats pretreated with phenobarbital four times more slowly than tamoxifen and there was very little formation of glucuronide conjugates. Four principal metabolites were isolated. Examination of mass spectra revealed desmethyl-4-iodotamoxifen, 4-iodotamoxifen *N*-oxide, and α-hydroxydesmethyl-4-iodotamoxifen {4-[4-[2-(methylamino)ethoxy]phenyl]-4-(4-iodophenyl)-3-phenyl-but-3-(*Z*)-en-2-ol}. Their identification was confirmed by comparison with synthesized samples. The structure of the fourth metabolite, 4′-hydroxy-4-iodotamoxifen was revealed by ^{1}H NMR spectroscopy. The iodophenyl moiety is thus retained in all the metabolites. The iodine atom not only blocks metabolism in its vicinity but also reduced the rate of side-chain demethylation and N-oxidation by three-fold. It can be predicted from this study that the presence of the iodine atom should give the compound a greater duration of action *in vivo*.

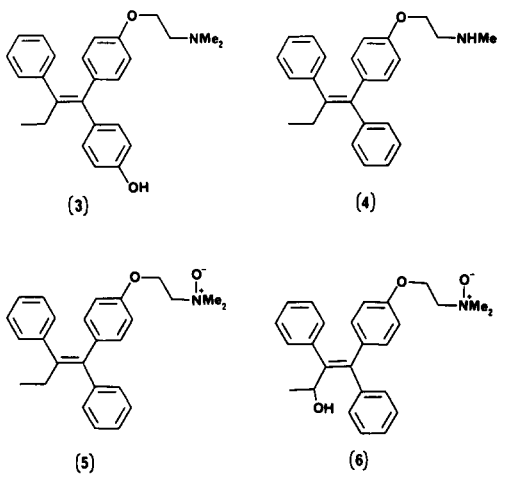


(1) X=I
(2) X=H

Scheme 1.

The 4-iodo derivative (**1**) of tamoxifen is a synthetic antioestrogen that has been found to possess a greater affinity for cytosolic oestrogen receptors than tamoxifen (**2**) (Scheme 1) and a greater ability to inhibit the growth of the human breast cancer cell line. MCF-7 *in vitro* [1]. Its development was brought about through consideration that the tamoxifen metabolite 4-hydroxytamoxifen (**3**) (Scheme 2) is highly potent *in vitro*, having 100 times the affinity of tamoxifen for the oestrogen receptor [2, 3] but has only comparable effectiveness to tamoxifen *in vivo* [4, 5] attributable to rapid deactivating conjugation of the hydroxyl group to form a glucuronide [6]. Consequently, a 4-substituent had been sought that would increase affinity for the receptor without succumbing to metabolic conjugation.

We have previously studied the metabolism of tamoxifen by rat hepatocytes [7]. Inasmuch as several of the metabolites reported in patients' plasma [8] were formed, the rat hepatocytes provide a faithful model for metabolism *in vivo*, at least for tamoxifen. Moreover, rat hepatocytes are capable of forming conjugated metabolites including the glucuronide of 4-hydroxytamoxifen [6]. Here we report a comparison between the metabolism of tamoxifen and its 4-iodo derivative by rat hepatocytes in order to determine whether the 4-iodo substituent could promote any undesirable metabolic deactivation.



Scheme 2. Phase I metabolites of tamoxifen formed by rat hepatocytes.

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Table 1. Chromatographic mobilities and principal mass spectral fragments for the iodotamoxifen and metabolites isolated

Compound	R_f*	Mass spectral fragments†
Iodotamoxifen (**1**)	0.63	497 (M^+, 6%), 72 [$Me_2N^+(CH_2)_2$, 18] 48 ($Me_2N^+ = CH_2$, 100)
Desmethyl metabolite (**7**)	0.56	483 (M^+, 27%), 426 (43), 58 (79), 44 (100)
N-Oxide (**8**)	0.29	497 (M^+-O, 3%), 452 (M^+-Me_2NOH, 14), 72 (16), 58 (100)
α-Hydroxydesmethyl metabolite (**9**)	0.43	499 (M^+, 9%), 442 (3), 366 (4), 309 (24), 58 (8), 44 (100)
4′-Hydroxy metabolite (**10**)	0.47	513 (M^+, 10%), 72 (37), 58 (100)

* On silica F-254 plates with a solvent system of $CHCl_3$:MeOH:25% aq. NH_3 75:25:0.5. For tamoxifen and metabolites values for comparison are tamoxifen $R_f = 0.63$, tamoxifen *N*-oxide $R_f = 0.25$, 4-hydroxytamoxifen $R_f = 0.55$.

† For mass spectral fragments of tamoxifen metabolites see Refs 7, 11 and 12.

MATERIALS AND METHODS

Materials

4-Iodotamoxifen (**1**) was prepared by the published procedure [1] and a sample of its *N*-oxide was generated by treating a solution in tetrahydrofuran with excess hydrogen peroxide (100 vol.) for 16 hr. Desmethyl-4-iodotamoxifen and the α-hydroxydesmethyl metabolite were synthesized as described below.

In vitro *metabolism*

Isolation of metabolites. Male adult Wistar rats fed *ad lib.* and given phenobarbital in their drinking water (0.5 g/L) for 10–14 days were used to prepare hepatocytes by a two-step perfusion method as described previously [9]. The freshly isolated cells (5 mL of 6–8 × 10^6 cells/mL and 90% viable) were incubated under an atmosphere of oxygen at 37° with 4-iodotamoxifen (100 μg/mL) in 25-mL conical flasks in a phosphate buffered saline medium at pH 7.5 containing Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (20 mmol/mL) and glucose (5 mmol/L). After 60 min, the flasks were cooled in ice, the hepatocytes disintegrated by ultrasonication and the protein precipitated by 3 vol. of acetone. Acetone was removed from the supernatant under vacuum and after adjustment to pH 9.5, phase I nonpolar metabolites and non-utilized 4-iodotamoxifen were extracted with ethyl acetate. The concentrated extract was subjected to normal phase chromatography on silica gel 60F-254 (Merck) plates in chloroform:methanol:25% aqueous ammonia (75:25:0.5), and isolated metabolites subjected to electron impact (E.I.) mass spectrometry (Table 1).

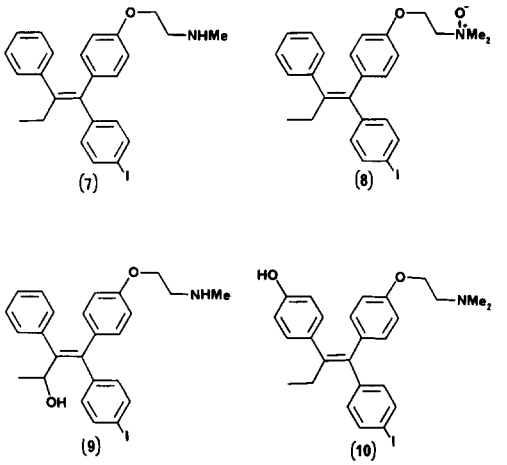
The aqueous fraction remaining after ethyl acetate extraction which contained conjugates was passed through a Sep-Pak cartridge, concentrated and subjected to reverse phase TLC plate chromatography [on Whatman K18CF plates eluting with methanol:water:40% tetrabutylammonium hydroxide (80:20:1)] but the bands observed at R_f values of 0.46 and 0.80 were very weak (probably less than 1% of tamoxifen consumed) and clearly insufficient to enable any conjugated components to be identified.

Comparative rates of metabolism of tamoxifen and 4-iodotamoxifen. The above experiment was repeated for tamoxifen (100 μg/mL, 0.2 mM) and 4-iodotamoxifen (134 μg/mL, 0.2 mM), each in four individual flasks. One flask for each drug was processed immediately by the method described above (ultrasonication and extraction) and acted as control. The remainder were processed after 15, 30 and 60 min and amounts of unmetabolized drug determined by HPLC.

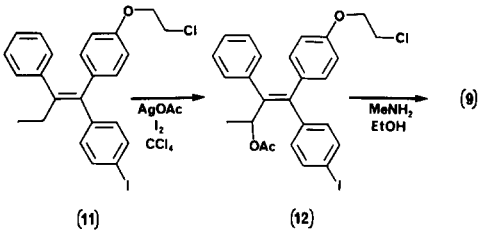
Quantification of metabolites by HPLC. Ethyl acetate extracts of hepatocytes were evaporated under vacuum and redissolved in methanol (500 μL). Aliquots (5 μL) of these solutions were then injected on the HPLC system. The column used was an Apex ODS column (5 μm; 15 cm × 4.6 mm i.d.) (Jones Chromatography, Hengoed, Mid Glamorgan, U.K.) with a mobile phase of methanol–water–diethylamine (85:15:0.1; flow rate 1.5 mL/min). Tamoxifen, iodotamoxifen and metabolites were quantified by UV detection at 254 nm. The retention times for tamoxifen and iodotamoxifen in this system were 330 and 572 sec, respectively. Metabolite peaks were assigned according to their co-elution with synthetic standards both with the mobile phase above and with a mobile phase of methanol–water–diethylamine (80:20:0.1) which gave retention times approximately twice as long. The areas under the metabolite peaks using this latter mobile phase were used to calculate the amount of each metabolite formed using the specific absorption at 254 nm of the appropriate synthetic standard. Unassigned metabolite peaks were quantitated by assuming a specific absorption at 254 nm equal to that of their parent compounds.

Instrumentation

E.I. mass spectra (70 eV) were recorded on a VG7070 H spectrometer with a direct insertion probe coupled to a VG2235 data system. Proton NMR spectra were recorded on a Bruker AC250 spectrometer in 0.5 mL of the appropriate solvent. For the metabolite (**10**), acquisition of data was made over 14.5 hr (22526 transients). HPLC was carried out on a Waters HPLC system comprising a U6K



Scheme 3. Phase I metabolites of 4-iodotamoxifen formed by rat hepatocytes.



Scheme 4. Synthesis of α-hydroxydesmethyl-4-iodotamoxifen.

injector, two model 510 pumps, a model 680 automated gradient controller and a model 440 absorbance detector (Millipore UK Limited, Watford, Herts, U.K.). A Trilab 2000 data system (Trivector) was used to analyse the data.

Synthesis

Desmethyl-4-iodotamoxifen (**7**). (Scheme 3). A solution of E-1-[4-(2-chloroethoxy)phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (**11**, 3.0 g, 6.14 mmol) [1] in 25% methylamine in ethanol (100 mL) was heated in a sealed bomb at 100° for 3 hr, the mixture then cooled and partitioned between aqueous potassium carbonate (1 M; 100 mL) and ether (100 mL). The ether solution was concentrated and the residue applied to a column of silica gel (Merck 15111, 60 g). Elution with 1:20 triethylamine-ether gave E-1-[4-[2-(methylamino)ethoxy]phenyl]-1-(4-iodophenyl)-2-phenylbut-1-ene (**7**, 2.13 g, 72%) as an oil which crystallized from light petroleum (b.p. 80–100°). The crystals had m.p. 89–91°; Anal. Calc. for $C_{25}H_{26}INO$: C, 62.1; H, 5.4; N, 2.9; I 26.25. Found C, 62.0; H, 5.4; N, 2.9; I 26.3%.

Synthesis of α-hydroxydesmethyl-4-iodotamoxifen (**9**). We have reported the synthesis of α-hydroxytamoxifen in which introduction of the hydroxyethyl group was made by coupling of a vinyllithium with acetaldehyde [10]. This method would be incompatible with the presence of an aryl iodide and therefore a new method of synthesis, outlined in Scheme 4, has been used for α-hydroxydesmethyl-4-iodotamoxifen. The chloroethyl-substituted iodotriphenylbutene (**11**), which is an immediate precursor of 4-iodotamoxifen, underwent allylic acetoxylation upon treatment with iodine and silver acetate in tetrachloromethane (Prevost conditions). The product (**12**) was obtained as a mixture of *Z(trans)* and *E(cis)* isomers owing to free bond rotation in a carbenium-ion reaction intermediate, and chromatography was required to isolate the required *Z*-isomer. Treatment with methylamine in ethanol then both replaced chloro- by methylamino- and cleaved the acetoxy substituent.

Z - 1 - [4 - (2 - Chloroethoxy)phenyl] - 1 - (4 - iodophenyl) - 2 - phenyl - 3 - acetoxy - 1 - butene (**12**). A solution of *E*-1-[4-(2-chloroethoxy)phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (**11**, 1.01 g, 2.05 mmol) [1] and iodine (1.04 g, 4.10 mmol) in tetrachloromethane (20 mL) and containing silver (I) acetate (1.36 g, 8.20 mmol) was stirred at 20°. After 16 hr, the mixture was diluted with dichloromethane (50 mL) and filtered through Celite. The pale yellow filtrate was concentrated and the residue applied to a column of silica gel (Merck 15111; 40 g). Elution with 2:3 dichloromethane–light petroleum (b.p. 60–80°) gave (i) *the title compound* (295 mg, 26%) as a foam which crystallized from light petroleum (b.p. 80–100°), m.p. 124–126°; NMR ($CDCl_3$) 1.27 (d, J = 6.6 Hz, 3H, CH_3CH), 3.71 (t, J = 5.9 Hz, 2H, OCH_2CH_2Cl), 4.07 (t, J = 5.9 Hz, 2H, OCH_2CH_2Cl), 5.71 (q, J = 6.6 Hz, 1H, CH_3CH), 6.53 (d, J = 8.8 Hz, 2H, ArH *ortho* to OCH_2), 6.77 (d, 2H, ArH *meta* to OCH_2), 7.03 (d, J = 8.3 Hz, 2H, ArH *meta* to I), 7.11–7.23 (m, 5H, Ph), 7.71 (d, 2H, ArH *ortho* to I); Anal. Calc. for $C_{26}H_{24}ClIO_3$: C, 57.1; H, 4.4; Cl, 6.5; I, 23.2. Found: C, 56.9, H, 4.4; Cl, 6.8; I, 23.3%.

Further elution of the column gave mixtures of Z- and E-isomers (337 mg) and then the unwanted E-isomer (235 mg). NMR ($CDCl_3$) δH *inter alia* 1.26 (d, J = 6.6 Hz, 3H, CH_3CH), 3.82 (t, J = 5.9 Hz, 2H, OCH_2CH_2Cl), 4.25 (t, J = 5.9 Hz, 2H, OCH_2CH_2Cl). The total yield of mixed isomers was 77%.

4-[4-[2-(Methylamino)ethoxy]phenyl]-4-(4-iodophenyl)-3-phenylbut-3-(Z)-en-2-ol (**9**). A solution of (**12**) (109 mg) in 25% methylamine in ethanol (50 mL) was heated in a sealed bomb at 100° (60 p.s.i.) for 6 hr. The solution was then concentrated and the residue partitioned between ether (10 mL) and 1 M sodium hydroxide (40 mL). The ether solution was concentrated and the residue applied to a column of silica gel (Merck 15111; 8 g). Elution with 1:1:50 methanol–triethylamine–ether gave *the title compound* (72.7 mg, 73%) as an oil which crystallized from light petroleum (b.p. 80–100°), m.p. 140–142°; NMR ($CDCl_3$) δ_H 1.17 (d, J = 6.5 Hz, 3H, CH_3CH), 2.43 (s, 3H, NHMe), 2.85 (t, J = 5.1 Hz, 2H, OCH_2CH_2N), 3.89 (t, 2H, OCH_2CH_2N), 4.78 (q, J = 6.5 Hz, 1H, CH_3CH), 6.52 (d, J = 8.8 Hz, 2H, ArH *ortho* to OCH_2), 6.76 (d, 2H, ArH *meta* to OCH_2), 6.99 (d, J = 8.3 Hz, 2H, ArH *meta* to I), 7.10–7.22 (m, 5H, Ph), 7.68 (d, 2H, ArH *ortho* to I); Anal. Calc. for $C_{25}H_{26}INO_2$ C, 60.45; H, 5.5; N,

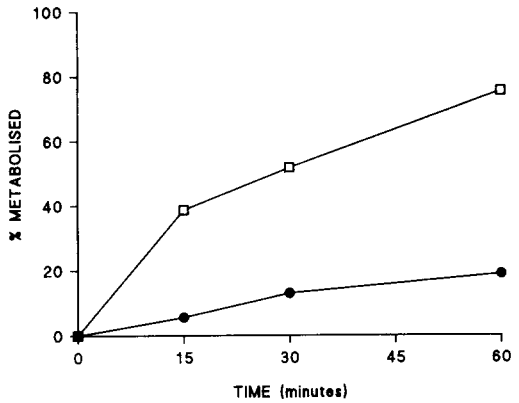


Fig. 1. Comparison of the rate of metabolism of tamoxifen (□) vs its 4-iodo derivative (●) by isolated rat hepatocytes.

2.9; I, 25.1. Found: C, 60.1; H, 5.25; N, 2.8; I, 25.4%.

RESULTS

Comparative rates of metabolism of tamoxifen and its 4-iodo derivative

Figure 1 displays amounts of compound consumed after parallel incubations with rat hepatocytes after 15, 30 and 60 min. Since a suitable radiolabelled form of the iodotamoxifen was not available, the amounts of unmetabolized drug was in each case detected by HPLC analysis with detection by UV absorbancy at 254 nm. Percentage not metabolized was then determined by the ratio of peak areas between extracts from the incubations and the controls.

It is clearly seen from Fig. 1 that the tamoxifen was consumed at about four times the rate of its iodo derivative. For both compounds, amount consumed as a proportion of that remaining was approximately linear with time. The amount of tamoxifen remaining after 1 hr (25%) was practically identical to that obtained in our previous study on tamoxifen (24%) [7] and therefore the activity of the hepatocytes used in this study was essentially the same as those used previously.

Identification of metabolites

Table 1 gives chromatographic mobilities and mass spectral data of the four principal metabolites of 4-iodotamoxifen. Two of these are immediately identifiable from their mass spectra, which parallel those of known tamoxifen metabolites (i.e. **4** and **5**). Thus, the desmethyl metabolite (**7**) shows a molecular ion at 14 mass units less than 4-iodotamoxifen and major fragments at m/z 58 and 44 which characterize a demethylated side chain. The *N*-oxide (**8**), though not giving a molecular ion, showed a characteristic fragment due to Cope elimination of *N*,*N*-dimethylhydroxylamine at m/z 452 [11]. These iodotamoxifen metabolites could be confirmed by comparison with synthetic materials.

For metabolite (**9**) there was no tamoxifen metabolite giving a directly corresponding mass spectrum, but the presence of major fragments at m/z 58 and 44 showed a desmethyl side chain and the molecular ion at m/z 499 indicated the presence of a hydroxy group. The fragments at m/z 366 and 309 are due to cleavage at the central double bond. Such fragmentation has been observed previously in the case of α-hydroxytamoxifen-*N*-oxide (**6**) [12], and consequently the metabolite was suspected to be α-hydroxydesmethyl-4-iodotamoxifen. Confirmation of this identification was made by comparison of the mass spectrum with that of a synthetic sample (Fig. 2).

The fourth metabolite gave a mass spectrum with a molecular ion at m/z 513 consistent with aromatic hydroxylation but because of the absence of fragments above m/z 72 the position of the hydroxyl function could not be deduced. Further information was gained through NMR spectroscopy. Figure 3 illustrates the spectrum obtained using d_4-methanol as solvent. There are two components in 4:1 ratio and the relatively high frequency position of the signals for the minor component indicate that this is a *cis* isomer formed through isomerization. The major component showed in the region for aromatic protons (δ 6.5–7.7), a pattern of six doublets. The only substitution arrangement that can give this pattern is for all three aromatic rings to be *para*-substituted and therefore the metabolite can be confidently identified as 4′-hydroxy-4-iodotamoxifen (**10**). An earlier spectrum of the metabolite, recorded in deuteriochloroform had originally proved too complex to interpret but could now be assigned to a 1:1 mixture of *trans* and *cis* isomers of 4′-hydroxy-4-iodotamoxifen where, in addition the side chains had been protonated by contaminating hydrogen chloride in the solvent. 4′-Hydroxytamoxifen, reported by Reunitz *et al.* as a rat liver microsomal metabolite of tamoxifen [13] is known to be susceptible to isomerization [14] and we have previously seen that isomerization of 4′-hydroxytamoxifen in deuteriochloroform is rapid.

The full NMR spectral data for the metabolite (**10**) are (a) in $CDCl_3$ (99.96 atom % D) *inter alia* 2.40 (q, J = 7 Hz, 2H + 2H, $CH_3C\underline{H}_2$), 2.88 (br. s, 6H, $-N^+H\underline{Me}_2$ of *trans* isomer), 2.94 (br. s, 6H, $-N^+H\underline{Me}_2$ of *cis*), 3.38 (br, 2H, $OCH_2C\underline{H}_2N$ of *trans*), 3.48 (br, 2H, $OCH_2C\underline{H}_2N$ of *cis*), 4.40 (br, 2H, $OC\underline{H}_2CH_2N$ of *trans*), 4.54 (br, 2H, $OC\underline{H}_2CH_2N$ of *cis*), 6.5–7.4 (aromatic signals, complex), 7.66 (d, J = 9 Hz, 2H, Ar<u>H</u> *ortho* to I of *trans*). (b) in d_4-MeOH (99.6 atom % D) 0.88 (t, J = 7.4 Hz 3H), 2.27 (s, 6H), 2.37 (q, J = 7.4 Hz, 2H), 2.67 (t, J = 5.5 Hz, 2H), 3.95 (t, J = 5.5 Hz, 2H), 6.55 (d, 2H), 6.58 (d, 2H), 6.73 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 8.3 Hz, 2H)—see Fig. 3 for assignments; the minor component gives *inter alia* 2.33 (s, NMe_2), 2.75 (t, J = 6 Hz, $OCH_2C\underline{H}_2N$), 4.10 (t, J = 6 Hz, $OC\underline{H}_2CH_2N$).

Quantification of metabolites formed

In our previous study with tamoxifen [7], amounts of metabolites were readily determined since radiolabelled drug was commercially available. This was not the case for the iodotamoxifen (**1**) and consequently HPLC was employed for quantification using

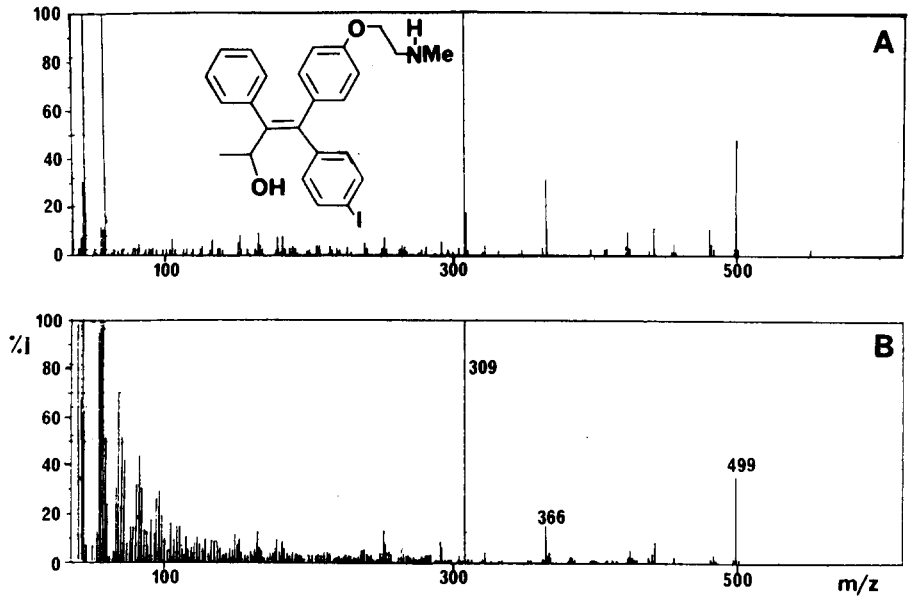


Fig. 2. Electron impact mass spectra of α-hydroxydesmethyl-4-iodotamoxifen; (A) synthesized compound, (B) isolated metabolite.

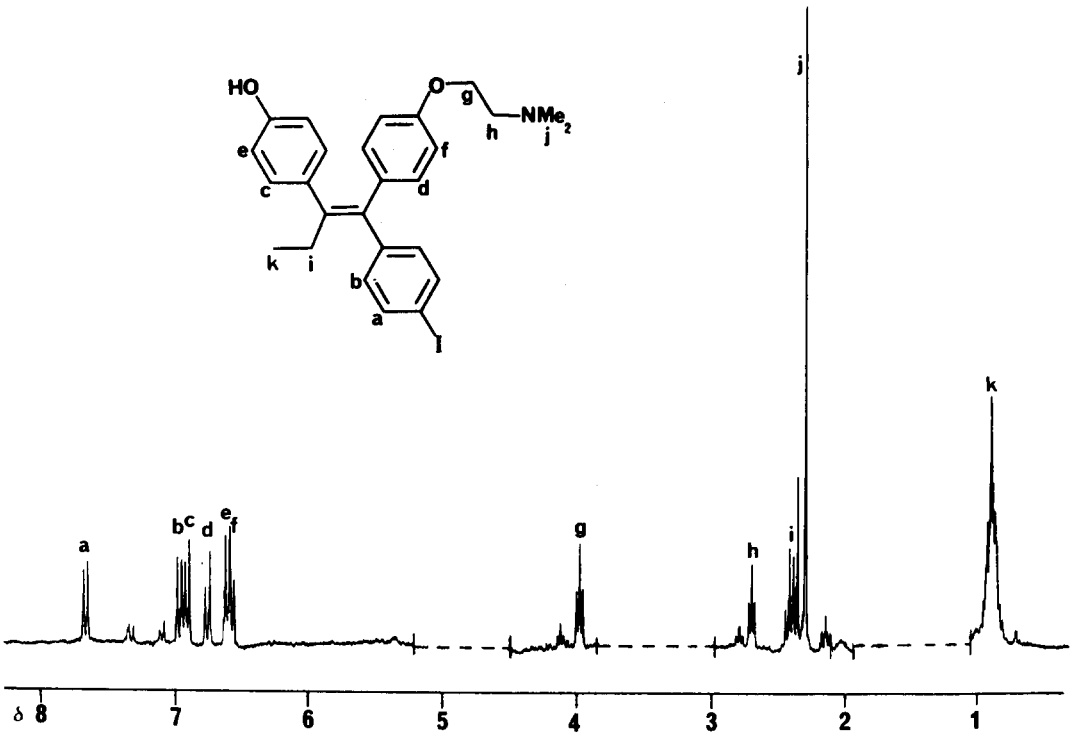


Fig. 3. NMR spectrum (250 MHz, d_4-methanol) of the metabolite identified as 4′-hydroxy-4-iodotamoxifen. Parts of the spectrum containing intense solvent or water signals have been deleted for clarity.

UV absorption detection at 254 nm and synthetic materials as standards both to locate the relevant HPLC peak and to standardize the absorbance. For the 4′-hydroxy metabolite (**10**), which we did not have synthetic material for comparison, assignment of the HPLC peak could not be confidently made but quantification could be obtained from the NMR spectrum by comparing the intensity of signals for

Table 2. Quantification of metabolites by HPLC: retention times (R_t) values and percentage obtained*

Compound	Percentage			
	R_{tr}† in extract (sec)	from control	in extract after 60 min incubation	60 min − control
Recovered iodotamoxifen (**1**)	1360	70	57	−13
Desmethyl metabolite (**7**)	1060	0.2‡	5.5	5.3
N-Oxide (**8**)	456	6.6	12.1	5.5
α-Hydroxydesmethyl metabolite (**9**)	214	0	0.8	0.8

* Four minor attributes (unassigned) were also observed having retention times 271, 370, 607 and 726 sec representing 0.4, 0.2, 0.3 and 0.1%, respectively.

† On an APEX ODS column eluting with methanol–water–diethylamine (85:15:1).

‡ Attributable to background.

the metabolite with those of the carbon-13 satellites of the residual monoprotonated solvent. Thus 0.5 mL, 99.8 atom % D-methanol used as solvent contained 10^{-5} cm^3 (6×10^{-6} g, 0.18 μmol) of $^{13}CD_2HOD$ and each of the two satellite peaks represented 0.09 μmol. The peak for the dimethylamino group (6H) of the metabolite integrated for 30% of the solvent satellite, so amount of the 4′-hydroxy metabolite was $0.027/6 = 0.0045$ μmol = 2.3 μg which from 1800 μg 4-iodotamoxifen used represented 0.13% conversion.

Table 2 gives the percentages of metabolites obtained as a proportion of the initial amount of iodotamoxifen both in the control extract and that after 60 min incubation with the hepatocytes. It is seen that in the control, only 70% of the iodotamoxifen could be recovered. Part of the loss is due to the facile chemical 6.6% *N*-oxide formation. This is about twice the amount of *N*-oxide (3%), formed chemically in control experiments for tamoxifen [7]. After incubation with the hepatocytes for 1 hr, 57% of the iodotamoxifen could be recovered. The extent of metabolism of 18% ($13/70 \times 100\%$) is the figure obtained in the above comparison of metabolism rate between tamoxifen and 4-iodotamoxifen (Fig. 1). The identified metabolites comprised the bulk of those obtained. Only very small amounts of three unidentified metabolites were observed. Together, the Phase I metabolites add up to 12.6% of the original amount of iodotamoxifen used in the experiment. This correlated well with the amount of iodotamoxifen consumed by the metabolism (13% of original amount) and is consistent with very little formation (<0.4%) of non-extractable conjugates such as glucuronides, which is what was observed when the aqueous fraction from the metabolite identification experiment was examined on TLC.

For comparison, the amounts of metabolites obtained in our previous study with tamoxifen were: recovered tamoxifen (24%), 4-hydroxytamoxifen (**3**) (6%), *N*-desmethyltamoxifen (**4**) (15%), tamoxifen *N*-oxide (**5**) (15%), α-hydroxytamoxifen *N*-oxide (**6**) (2%), unidentified Phase I metabolites (10%), glucuronide conjugates (7.2%) [7].

DISCUSSION

The four identified metabolites (**7–10**) of the 4-iodo derivative of tamoxifen formed by isolated rat hepatocytes retained the 4-iodophenyl ring. Therefore the iodine atom inhibits metabolism in its environment. The prevention of 4-hydroxylation, however, can only partly account for the four-fold slower consumption of the iodo derivative compared with tamoxifen since less than half the amount of tamoxifen metabolized under these conditions is hydroxylated [7] The presence of the iodine apparently also reduces the rate of side chain modification, i.e. N-oxidation and demethylation, each of these metabolic processes occurring to an extent of 15% from tamoxifen [7] but only 5% from the iodo derivative.

As a result of the prevention of 4-hydroxylation, it would be expected that oxidative metabolism would be redirected elsewhere. Indeed, the 4′-hydroxylation to give metabolite (**10**) is not a pathway we had observed in the hepatocyte metabolism of tamoxifen, although Ruenitz *et al.* [13] have observed rat liver microsomal 4′-hydroxylation of tamoxifen. 4′-Hydroxytamoxifen is reported to be an antiestrogen with a five-fold greater affinity for the oestrogen receptor than tamoxifen [14], but this is much weaker than that of 4-hydroxytamoxifen and so if 4′-hydroxy-4-iodotamoxifen (**10**) is rapidly conjugated, then this metabolite would not be a major contributor of activity *in vivo*. This is particularly likely since the very small proportion of this metabolite formed (*ca.* 0.1%) is considerably less than the proportion of 4-hydroxytamoxifen (8%) formed from tamoxifen.

Formation of desmethyl-4-iodotamoxifen (**7**) and the *N*-oxide (**8**) are pathways that parallel the metabolism of tamoxifen. Both desmethyltamoxifen and tamoxifen *N*-oxide have an antitumour potency similar to that of tamoxifen [4, 15]. The former is the major circulating metabolite of tamoxifen in patients [16] whereas the latter has only been reported *in vitro* and is possibly an artefact arising from the relatively high oxygen availability during *in vitro* metabolism [7]. The formation of the α-hydroxylated desmethyl metabolite **9** is curious. It presumably forms only from the desmethyl metabolite **7** since no α-hydroxy-4-iodotamoxifen was observed. In contrast, for tamoxifen, α-hydroxylation was observed only after N-oxidation rather than demethylation [12]. This difference, together with the remote effect of the iodine in inhibiting side

chain modification, underlines the difficulty of prediction of the preferred metabolism route or metabolism rate from the structure. Presumably, the iodine atom can alter the orientation of binding to or affinity for the enzymes responsible for oxidative metabolism, as can the precise nature of the side chain. Since α-hydroxylation of tamoxifen has been found to reduce the affinity for the oestrogen receptor by 10-fold [10], α-hydroxylation subsequent to demethylation will represent a deactivation pathway of the 4-iodo derivative of tamoxifen if the results of hepatocyte experiments are paralleled *in vivo*.

The slower rate of metabolism caused by the iodine incorporation is predictive of a long duration of action of 4-iodotamoxifen *in vivo*, a quality which is likely to be of benefit for breast cancer treatment where sustained circulating drug levels are needed [17]. A further possible use of the iodo derivative of tamoxifen described, as well as for other iodotamoxifens having the iodine in a different position [18, 19], is with the iodine atom radiolabelled for site selective radiotherapy or for imaging of target tissues. The retention of the iodine atom in all of the observed metabolites is a promising feature since liberation of free radioactive iodine would require measures to counter thyroid radiotoxicity. On the other hand, long duration of action would not be beneficial for this purpose, since rapid clearance from the plasma of imaging agents is thought to be necessary to achieve good selectivity for uptake in the target tissues [20].

The conclusion of this paper that 4-iodotamoxifen is a "metabolism restricted" analogue of tamoxifen ensures that a pharmacokinetic study on 4-iodotamoxifen will be valuable. Also, introduction of iodine into drug types which suffer rapid deactivating metabolism could be a strategy to overcome any consequent limitation to their therapeutic use.

Acknowledgements—This investigation was supported by grants from the Cancer Research Campaign and Medical Research Council to the Institute of Cancer Research. We thank Mr M. H. Baker for the mass spectra.

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