

# Identification of 4-Hydroxy-*N*-desmethyltamoxifen as a Metabolite of Tamoxifen in Human Bile<sup>1</sup>

Ernst A. Lien,<sup>2</sup> Einar Solheim, Stener Kvinnsland, and Per M. Ueland

Clinical Pharmacology Unit, Department of Pharmacology and Toxicology [E. A. L., P. M. U.], and Department of Pharmacology and Toxicology [E. S.], University of Bergen, N-5021, Bergen, Norway; and Department of Oncology [S. K.], University of Trondheim, 7000 Trondheim, Norway

## ABSTRACT

The occurrence of tamoxifen metabolites in bile was investigated in a 57-year-old female patient receiving chronic treatment with tamoxifen. In bile treated with  $\beta$ -glucuronidase, two major peaks were detected using a chromatographic system developed for the quantitation of tamoxifen metabolites in human serum. One sharp peak coeluted with 4-hydroxytamoxifen whereas a second broad peak eluted slightly ahead of tamoxifen and was separated from all major serum metabolites. This latter peak was identified as the *cis* (about 30%) and *trans* (about 70%) isomers of 4-hydroxy-*N*-desmethyltamoxifen. The identification was based on (a) coelution with authentic standard on reversed-phase chromatography and formation of fluorescent material after photoactivation, (b) a molecular ion ( $M + 1$ )<sup>+</sup> of 374 *m/z* as determined with liquid chromatography-mass spectrometry, and (c) a fragmentogram identical to that of the authentic standard, as obtained by gas chromatography-mass spectrometry.

## INTRODUCTION

Tamoxifen [*trans*-1-(4- $\beta$ -dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene], is a nonsteroidal antiestrogen, which has been widely used during the last 15 years for the palliative treatment of breast cancer (1).

Metabolism is a major determinant of drug action. Biotransformation may lead to inactivation and excretion, and some metabolites may have pharmacological effects. This is certainly the case for tamoxifen, which may be regarded as a prodrug, since it is converted into more active metabolites (2).

Several metabolites of tamoxifen have been identified in human plasma. These include *N*-desmethyltamoxifen (metabolite X), 4-hydroxytamoxifen (metabolite B), *N*-desdimethyltamoxifen (metabolite Z), and the primary alcohol named metabolite Y<sup>3</sup> (3). Among the serum metabolites, 4-hydroxytamoxifen has received particular attention since it has higher *in vitro* affinity towards the estrogen receptor than the parent drug (4). The effect *in vivo* after peroral administration is probably limited by high metabolite clearance resulting in low serum concentration (2). However, 4-hydroxytamoxifen remains unmetabolized *in situ* in human breast for several days after local percutaneous administration to human breast (5).

The routes of excretion of [<sup>14</sup>C]tamoxifen have been investigated in the female rat, mouse, monkey, and dog after i.p. injection or oral administration (6). In all species the major route of excretion was via the faeces. In rats and dogs, a major fraction (22–53%) of radioactivity derived from tamoxifen was excreted into the bile, and a significant amount (16–69%) of biliary radioactivity was reabsorbed and underwent enterohepatic circulation, until it was eliminated into the faeces (6). Investigation of the distribution of [<sup>14</sup>C]tamoxifen in the female

mouse showed that the radioactivity was concentrated and retained in bile and to a lesser degree in liver and pancreas (7).

The excretion of radioactivity following a single oral dose of [<sup>14</sup>C]tamoxifen has been evaluated by Fromson *et al.* in two female patients. Excretion of metabolites into faeces seems to be a major route of elimination also in humans (8).

Most metabolites isolated from faecal extracts of rat and mouse and bile from rat and dog were present as glucuronides and other conjugates. Three metabolites (metabolite A, B, and C) were identified by comparison with authentic standards, whereas the structural assignment of three metabolites (named C, D, and F) was tentative (6). Fromson *et al.* (8) isolated two metabolites (B and F) from  $\beta$ -glucuronidase treated extract of human faeces. Recently, metabolite F has been identified as the phenolic primary alcohol (9).

The data cited above suggest that the major route of tamoxifen metabolite clearance occurs via biliary excretion. Evaluation of this route of elimination in humans by investigating metabolites in faeces may be obscured by intestinal metabolism and reabsorption of metabolites. However, bile drainage of a patient receiving chronic treatment with tamoxifen offers a clinical condition where biliary excretion of some tamoxifen metabolites could be directly assessed. We obtained such bile and analyzed it with a liquid chromatographic system which we have recently developed for measurement of tamoxifen and its metabolites in human serum (10). This method has the advantage of total analytical recovery of both demethylated and hydroxylated metabolites and the parent compound, and analyzes and quantitates these diverse compounds in a single system. We identified a major peak in human bile as 4-hydroxy-*N*-desmethyltamoxifen.

## MATERIALS AND METHODS

**Chemicals.** Tamoxifen, 4-hydroxytamoxifen and *N*-desmethyltamoxifen, were obtained from Pharmachemie B. V., Haarlem, Holland. Metabolite Y, *N*-desdimethyltamoxifen and 4-hydroxy-*N*-desmethyltamoxifen were gifts from Imperial Chemical Industries PLC, Pharmaceuticals Division, Macclesfield, Cheshire, UK. The latter reference compound was obtained as a mixture of about 85% *cis* isomer and 15% *trans* isomer. Attempts to enrich the *trans* isomer by boiling in ethanol, were not successful. Glusulase (a preparation of the intestinal juice of the snail, *Helix Pomatia*, containing  $\beta$ -glucuronidase and sulfatase) was from E. I. DuPont de Nemours & Co. Inc., Wilmington, DE. Normotest, testing for blood clotting factors II, VII, and X, is a product of Nycomed AS, Oslo, Norway. Other reagents were purchased from commercial sources given previously (10).

**Patient.** The patient was a 57-year-old female who had breast cancer with pleural effusion and axillary and liver metastases. She received chronic treatment with tamoxifen (30 mg daily) since 12/18/86. Due to total occlusion of the common bile duct with a serum bilirubin of 303  $\mu$ M (normal < 18  $\mu$ M) a T tube biliary drainage was established on 2/6/87. From this date, her liver function parameters improved (Table 1).

On 3/3/87 she received 10 mg Adriamycin, and on 3/5/87 the endocrine treatment was changed from tamoxifen to megestrolate, 160 mg daily. She was transferred to a local hospital on 3/12/87, and expired on 3/26/87.

Received 9/10/87; revised 11/19/87; accepted 1/20/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> This work was supported by grants from the Norwegian Cancer Society, the Norwegian Society for Fighting Cancer, and The Norwegian Medical Depot.

<sup>2</sup> To whom requests for reprints should be addressed.

<sup>3</sup> The abbreviations and trivial name used are: metabolite Y, *trans*-1-(4- $\beta$ -hydroxy-ethoxyphenyl)-1,2-diphenylbut-1-ene; LC/MS, liquid chromatography/mass spectrometry; GC/MS, gas chromatography/mass spectrometry.

Table 1 Patient data

Date	Serum parameters						
	Bilirubin ( $\mu\text{M}$ )	AP <sup>a</sup> (IU/liter)	ASAT <sup>b</sup> (IU/liter)	ALAT <sup>c</sup> (IU/liter)	$\gamma$ -GT <sup>d</sup> (IU/liter)	Albumin (g/liter)	NT(%) <sup>e</sup>
2/2/87	303	999	117	193	469	23	0.77
2/27/87	75	287		103	186	32	0.65
3/9/87	43	184	51	104	121	29	0.65

<sup>a</sup> Alkaline phosphatase.

<sup>b</sup> Aspartate aminotransferase.

<sup>c</sup> Alanine aminotransferase.

<sup>d</sup>  $\gamma$ -Glutamyl transpeptidase.

<sup>e</sup> Normotest (normal 75–125).

Bile was collected for 5 days (3/1/87–3/5/87) while she received chronic treatment with tamoxifen, and for 20 days (3/5/87–3/25/87) during drug washout. The laboratory findings during this period are summarized in Table 1.

**Sample Collection and Processing.** The bile was collected in polyethylene bags which were replaced twice daily. The collected bile was stored in glass bottles at  $-20^{\circ}\text{C}$ .

The bile was thawed, and treated with glucusase, as described by Bakke *et al.* (11). The pH was then adjusted to 7 by adding 1 M NaOH. The neutralized samples were mixed with equal volume of acetonitrile, and the precipitate removed by centrifugation. The supernates were transferred to sample vials, capped, and analyzed.

**High-Performance Liquid Chromatography.** We used a liquid chromatography system which was developed for the determination of tamoxifen and metabolites in serum (10). The assay was modified to improve the separation and isolation of the early eluting, hydrophilic metabolites. The method and the modification are briefly described below.

Large samples of 250  $\mu\text{l}$  were injected into a small precolumn with an internal diameter of 0.21 cm, packed with 5  $\mu\text{m}$  octadecylsilane material. The length of this column was increased from 2 cm, as in the original method (10), to 3 cm. The samples were on-column concentrated by equilibrating the precolumn with 50% acetonitrile in water, containing 3 mM acetic acid and 2 mM diethylamine. The analytes were then directed into an analytical octadecylsilane-Hypersil column (0.21 x 10 cm) by changing the mobile phase followed by column switching. The composition of the mobile phase was 91% acetonitrile containing 1 mM acetic acid and 0.67 mM diethylamine, and the flow rate was 0.3 ml/min. Tamoxifen and its metabolites were eluted in the following order: metabolite Y, metabolite B, tamoxifen, metabolite Z, and metabolite X. These compounds were postcolumn converted to fluorophors by UV illumination while passing through a quartz tube, and then monitored by a fluorescence detector.

The instruments and the construction of the postcolumn converter have been described (10).

**Liquid Chromatography/Mass Spectrometry.** The analytical column was connected to a LC/MS, thermospray system (model 201; Vestec, Houston, TX). Before entering the thermospray, the effluent from the column was mixed with 0.1 M ammonium acetate reagent, delivered at a rate of 0.7 ml/min via a zero dead volume T connector.

**Gas Chromatography/Mass Spectrometry.** Samples of bile were chromatographed on the liquid chromatographic system described above, and the material to be identified was isolated by collecting effluent from the column, using the programmable fraction collector model, Foxy, from ISCO. The desired fraction from 30 samples were pooled, and lyophilized. The lyophilized sample was silylated with  $\text{N}_1\text{O}$ -bis(trimethylsilyl) acetamid (1 ml), prior to GC/MS analysis.

Samples of 5  $\mu\text{l}$  of the material in ethyl acetate were subjected to GC/MS, using a Hewlett-Packard 5970 MSD (GC/MS) mass spectrometry system. The column was an SP-255 wall-coated, open-tubular glass capillary column (0.30 mm i.d. x 6 m) and the temperature was programmed from 150 to  $280^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$ . On column injection technique was used, and helium (2 ml/min) was used as a carrier gas.

## RESULTS

**Tamoxifen and its Metabolites in Serum.** Table 2 shows the amount of tamoxifen and its major metabolites in serum at

different time points during the investigation. The concentrations of tamoxifen, metabolite Y, *N*-desmethyltamoxifen, and *N*-didesmethyltamoxifen (Table 2) are in the range reported for patients receiving chronic tamoxifen treatment (3, 12). The concentrations of drug and these metabolites in serum are progressively decreased following tamoxifen withdrawal.

**Liquid Chromatographic Analysis of Bile.** Fig. 1 shows fluorescence traces of UV illuminated effluent from the reversed-phase column.

Chromatogram of bile from a patient not treated with tamoxifen is shown in trace A. No peaks cochromatographed with standards (*traces B* and *C*).

Analysis of bile from a patient receiving chronic treatment with tamoxifen is shown in *traces D* and *E*. Only trace amounts of fluorescent material, tentatively identified as tamoxifen, *N*-desdimethyltamoxifen, and *N*-desmethyltamoxifen, was found in bile not treated with glucusase (*trace D*). Two major peaks appeared following glucusase treatment. One cochromatographed with 4-hydroxytamoxifen and another (retention time of 9 min) eluted slightly ahead of tamoxifen and was separated from all major serum metabolites (shaded area, *traces E* and *F*). The latter peak was broad and skewed, suggesting inhomogeneity. Our further efforts were directed towards the identification of this peak.

*Trace C* shows a chromatogram from bile spiked with 4-hydroxy-*N*-desmethyltamoxifen. The standard contained a mixture of *trans* (15%) and *cis* isomer (85%), which showed retention times of 8.95 and 9.42 min, respectively. The minor portion (*trans* isomer) coeluted with the main part of the unknown peak (*trace E*), whereas the *cis* isomer eluted in the down-slope region of the unknown peak. These findings are consistent with the possibility that the unknown material contains a mixture of *trans*- and *cis*-4-hydroxy-*N*-desmethyltamoxifen, and the former isomer is prevailing.

Chromatogram of bile 18 days after the last tamoxifen dose is shown in *trace F*. The material in bile, tentatively identified as 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen, is markedly decreased during drug washout (Fig. 1, *trace F*).

**Identification of Tamoxifen Metabolite in Bile by Mass Spectrometry.** Extracted bile and reference compounds were chromatographed on the reversed-phase system described above, and the effluent directed into the mass spectrometry thermospray system.

The spectrum of authentic 4-hydroxy-*N*-desmethyltamoxifen showed only one major peak corresponding to the  $(\text{M}+1)^+$  ion of 374  $m/z$  (data not shown).

Fig. 2 shows a single ion monitoring trace of bile from nontreated patients (*trace A*), bile spiked with 4-hydroxy-*N*-desmethyltamoxifen (*trace B*) and glucusase treated bile from patient receiving chronic treatment with tamoxifen (*trace C*). The instrument was set to monitor 374  $m/z$  (4-hydroxy-*N*-desmethyltamoxifen). Material containing the same  $(\text{M}+1)^+$

Table 2. Concentrations of tamoxifen and metabolites in serum

Last tamoxifen tablet given in the morning of 3/5/87. The patient expired on 3/26/87.

Date	Tam <sup>a</sup> (ng/ml)	Met Y <sup>b</sup> (ng/ml)	4-OH-T <sup>c</sup> (ng/ml)	Desdim-T <sup>d</sup> (ng/ml)	Desm-T <sup>e</sup> (ng/ml)
Before tamoxifen withdrawal					
2/27/87	128.3	12.7	3.6	34.4	189.6
3/4/87	153.8	15.2	4.9	32.6	186.6
After tamoxifen withdrawal					
3/9/87	90.1	14.7	4.7	33.3	169.2
3/12/87	61.9	10.1	3.3	30.5	130.5
3/23/87	28.0	4.0	1.8	17.9	67.3

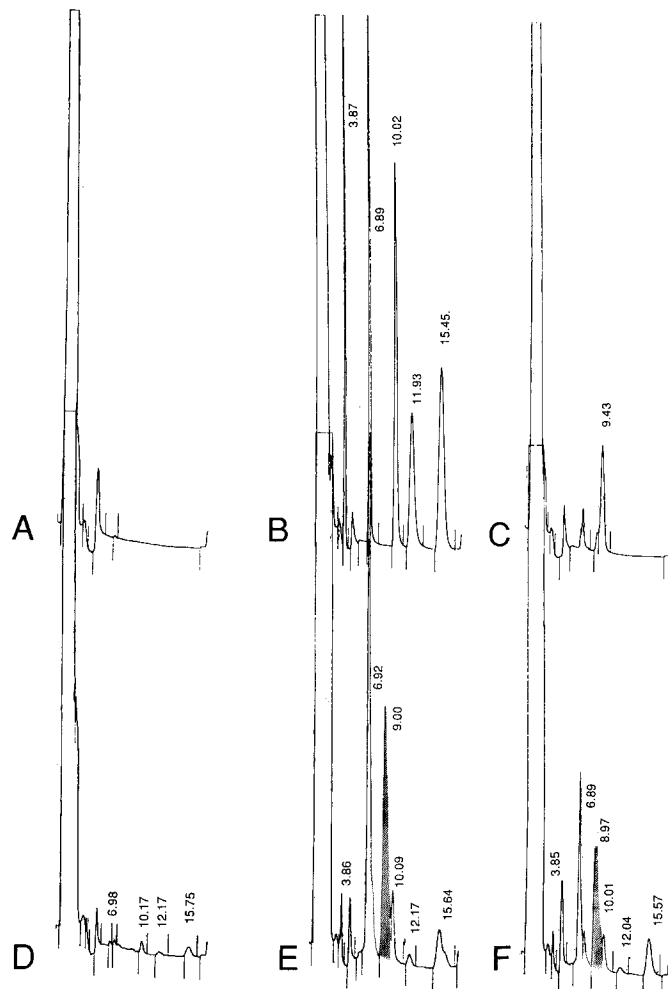
<sup>a</sup> Tamoxifen.<sup>b</sup> Metabolite Y.<sup>c</sup> 4-Hydroxytamoxifen.<sup>d</sup> *N*-Desdimethyltamoxifen.<sup>e</sup> *N*-Desmethyltamoxifen.

Fig. 1. Analysis of bile by reversed-phase liquid chromatography postcolumn photoactivation and fluorescence detection. *A*, control bile from patient not taking tamoxifen; *B*, blank bile spiked with metabolite Y (3.87 min), 4-hydroxytamoxifen (6.89 min), tamoxifen (10.02 min), *N*-desdimethyltamoxifen (11.93 min), and *N*-desmethyltamoxifen (15.45 min); *C*, blank bile spiked with 4-hydroxy-*N*-desmethyltamoxifen (the standard contained 85% *cis* form and 15% *trans* form); *D*, bile from the patient with biliary drainage, receiving chronic tamoxifen treatment; *E*, the same sample of bile as shown in trace *D*, but after deconjugation with glucusase; *F*, glucusase treated bile from patient 18 days after the last tamoxifen dose. Shaded areas, peaks tentatively identified as 4-hydroxy-*N*-desmethyltamoxifen.

ion and eluting at the same retention times as this reference compound, was present in bile of patient receiving tamoxifen. The standard is composed of 85% *cis* isomer and 15% *trans* isomer (trace *B*) whereas in bile from patient the compound coeluting with the *trans* isomer is the prevailing species (trace *C*). When the bile shown in trace *C* was spiked with standard, there was a relative increase in the *cis* isomer (trace *D*).

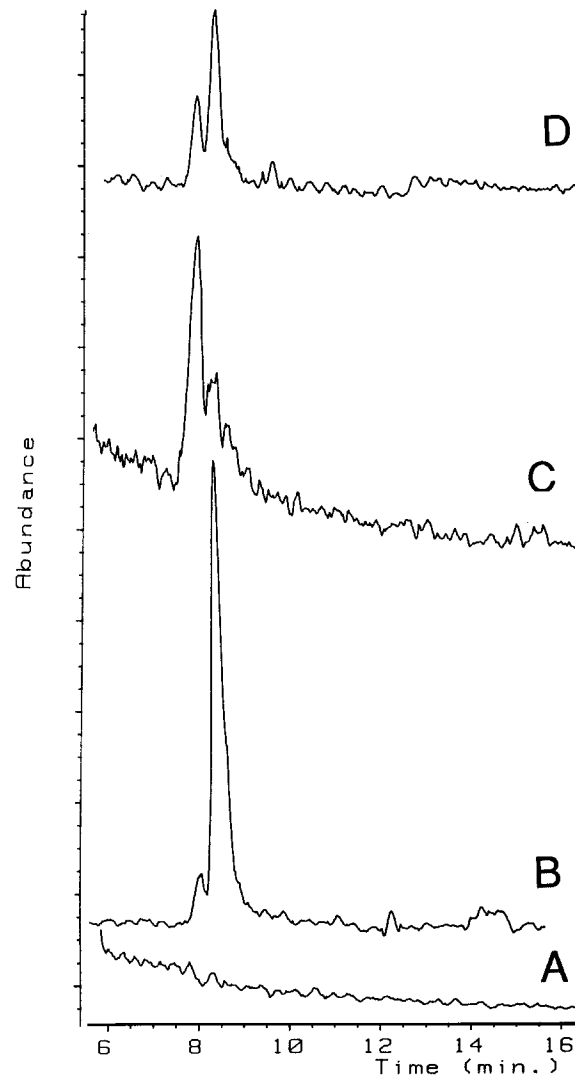


Fig. 2. Single-ion monitoring trace of bile obtained by LC/MS. *A*, control bile from patient not taking tamoxifen; *B*, blank bile spiked with 4-hydroxy-*N*-desmethyltamoxifen (the standard contained 85% *cis* form and 15% *trans* form); *C*, glucusase treated bile from patient receiving chronic tamoxifen treatment; *D*, the same bile as shown in trace *C*, supplemented with authentic 4-hydroxy-*N*-desmethyltamoxifen (85% *cis* form and 15% *trans* form). The instrument was set to monitor at 374 *m/z*, which corresponds to the (M+1)<sup>+</sup> ion of 4-hydroxy-*N*-desmethyltamoxifen.

Effluent from the peak eluting ahead of the tamoxifen peak (Fig. 1, shaded area, trace *E*) was collected, and analyzed by GC/MS as described in "Materials and Methods." The mass fragmentogram of this material (retention time, 11.92 min) was nearly identical to that of the authentic 4-hydroxy-*N*-desmethyltamoxifen standard (retention time, 11.94 min) (Fig. 3).

## DISCUSSION

We analyzed bile from a patient receiving chronic tamoxifen treatment using a chromatographic system which measures both hydroxylated and desmethylated tamoxifen metabolites (10). Notably, the sample processing, which involved mixing with an equal volume of acetonitrile, gave total recovery of such chemically diverse compounds (10). Thus, this analytical procedure includes both hydrophilic and hydrophobic metabolites of tamoxifen.

In addition to 4-hydroxytamoxifen, another major peak was demonstrated in bile. This material was absent in bile from patients not exposed to tamoxifen (Fig. 1, *trace A*) and decreased following drug withdrawal (Fig. 1, *shaded area, traces E and F*) suggesting that the material is derived from tamoxifen. It was identified as 4-hydroxy-*N*-desmethyltamoxifen. This metabolite appears to exist as a mixture of *cis* and *trans* isomer in bile. The identification was based on (a) coelution with authentic standard on reversed-phase chromatography and formation of fluorescent material after photoactivation (Fig. 1), (b) a molecular ion ( $M + 1$ )<sup>+</sup> of 374 *m/z* as determined with LC/MS (Fig. 2), (c) a fragmentogram identical to that of the authentic standard, as obtained by GC/MS (Fig. 3).

In bile 4-hydroxy-*N*-desmethyltamoxifen appears as a mixture of *trans* and *cis* isomer, with the former compound as the prevailing species (Fig. 2). If 4-hydroxy-*N*-desmethyltamoxifen is excreted as the *trans* isomer, one must postulate an isomerization of this metabolite during collection or storage of bile. Others have reported on temperature-dependent isomerization of phenolic nonsteroidal estrogens and antiestrogens both in stock solution and in biological samples (13). We observed no

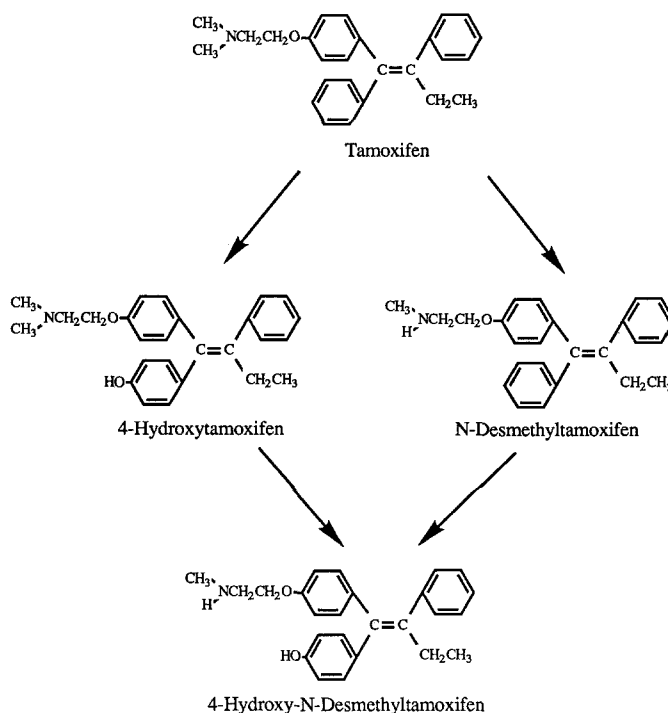


Fig. 4. Possible pathways for the formation of 4-hydroxy-*N*-desmethyltamoxifen.

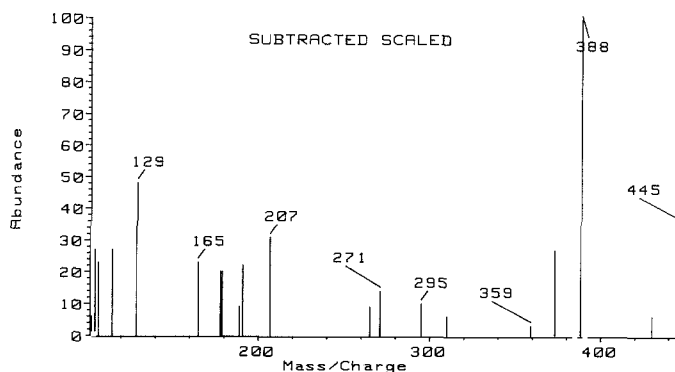
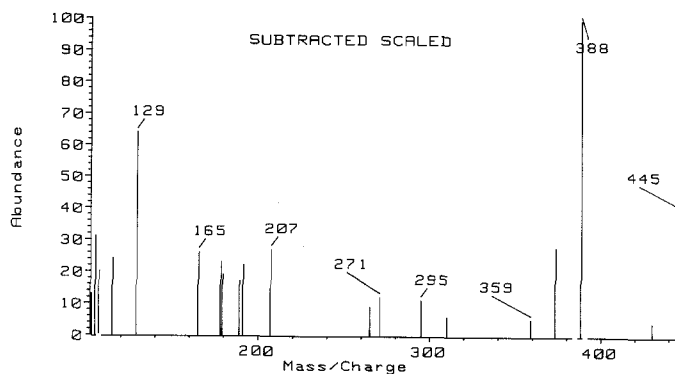


Fig. 3. Identification of the unknown metabolite with GC/MS. The effluent from the reversed-phase column, corresponding to the marked peak in *trace E*, Fig. 1, and authentic 4-hydroxy-*N*-desmethyltamoxifen were subjected to GC/MS as described in the text. Both compounds showed a retention time of 11.9 min upon gas chromatography. *Top*, mass fragmentogram of unknown metabolite; *bottom*, authentic 4-hydroxy-*N*-desmethyltamoxifen.

time-dependent conversion to the *cis* isomer upon storage of bile. Thus, it is conceivable that 4-hydroxy-*N*-desmethyltamoxifen may be excreted as a mixture of both isomers.

Since both the *cis* and *trans* form of 4-hydroxy-*N*-desmethyltamoxifen may exist *in vivo* (5), stereoselective metabolism should be considered to take place during biotransformation of tamoxifen. Furthermore, isomerization of active metabolites may have pharmacodynamic implications. It has been established that antiestrogens may change their inhibitory profile after isomerization. The *trans* isomer of tamoxifen is a general estrogen antagonist and the *cis* isomer may act as an agonist (14, 15).

The chromatographic peaks corresponding to 4-hydroxytamoxifen and the two isomers of 4-hydroxy-*N*-desmethyltamoxifen appeared after glucuronase treatment of bile, and were nearly absent in nontreated bile (Fig. 1). This finding shows that these hydroxylated metabolites of tamoxifen are excreted and exist as conjugates in bile, probably as glucuronides.

Excretion of conjugated 4-hydroxy-*N*-desmethyltamoxifen and other hydroxylated tamoxifen metabolites into bile delivers these compounds to the intestine, where they are exposed to the intestinal microorganisms. The conjugated metabolites can be degraded by  $\beta$ -glucuronidases or sulfatases derived from bacteria in the gut, and may become reabsorbed and thereby undergo enterohepatic circulation (16). Enterohepatic circulation has been demonstrated for tamoxifen (6) and should be considered for the biliary metabolite, 4-hydroxy-*N*-desmethyltamoxifen.

The existence of 4-hydroxy-*N*-desmethyltamoxifen in human bile points to the possibility that this metabolite is present in tissues as well. The tamoxifen metabolite termed M2 by Borgna and Rochefort, which was found to be a major metabolite in rat uterus (17) may be identical to 4-hydroxy-*N*-desmethyltamoxifen, and significant amount of conjugated 4-hydroxy-*N*-desmethyltamoxifen is formed from tamoxifen by rat hepatocytes in suspension (9). After local percutaneous administration

of 4-hydroxytamoxifen to patients, 4-hydroxy-*N*-desmethyltamoxifen is demonstrated in breast tissue and plasma (5).

There are indications that hydroxylated metabolites of tamoxifen, including 4-hydroxy-*N*-desmethyltamoxifen, play an important role in the mechanism of action of tamoxifen (4). *trans*-4-Hydroxytamoxifen has more than 100 times higher affinity to the estrogen receptor than *trans*-tamoxifen, and a similar *in vitro* affinity has been reported for 4-hydroxy-*N*-desmethyltamoxifen (4).

Possible routes of formation of 4-hydroxy-*N*-desmethyltamoxifen in humans are depicted in Fig. 4. It is conceivable that this metabolite is formed by hydroxylation in the 4-position of *N*-desmethyltamoxifen and/or by *N*-demethylation of 4-hydroxytamoxifen. Both these possible intermediates have been identified in human material (3).

4-Hydroxy-*N*-desmethyltamoxifen has been demonstrated in breast tissue following local administration of the radioactive precursor 4-hydroxytamoxifen. Preliminary data suggest that the concentration of a tamoxifen derivative, tentatively identified as 4-hydroxy-*N*-desmethyltamoxifen, is higher than that of 4-hydroxytamoxifen in serum of several patients receiving chronic p.o. tamoxifen treatment.<sup>4</sup> The unequivocal identification of this serum component by mass spectrometry has been difficult because only small amounts are present in serum. Our present efforts are directed towards improving the detection limit of our LC/MS system so that the distribution of 4-hydroxy-*N*-desmethyltamoxifen in human biological material other than bile can be investigated.

## REFERENCES

1. Patterson, J. S. 10 years in breast cancer. A review. *In*: J. G. M. Klijn, R. Pariedaens, and J. A. Foekens (eds.), *Hormonal Manipulation of Cancer: Peptides, Growth Factors, and New (Anti) Steroidal Agents*. New York: Raven Press, 1987.
2. Jordan, V. C., Allen, K. E., and Dix, C. J. Pharmacology of tamoxifen in laboratory animals. *Cancer Treat. Rep.*, **64**: 745-759, 1980.
3. Furr, B. J. A., and Jordan, V. C. The pharmacology and clinical uses of tamoxifen. *Pharmacol. Ther.*, **25**: 127-205, 1984.
4. Robertson, D. W., Katzenellenbogen, J. A., Long, D. J., Rorke, E. A., and Katzenellenbogen, B. S. Tamoxifen antiestrogens. A comparison of the activity, pharmacokinetics, and metabolic activation of the *cis* and *trans* isomers of tamoxifen. *J. Steroid Biochem.*, **16**: 1-13, 1982.
5. Mauvais-Jarvis, P., Baudot, N., Castaigne, D., Banzet, P., and Kuttenn, F. *trans*-4-Hydroxytamoxifen concentration and metabolism after local percutaneous administration to human breast. *Cancer Res.*, **46**: 1521-1525, 1986.
6. Fromson, J. M., Pearson, S., and Bramah, S. The metabolism of tamoxifen (ICI 46474). Part I: In laboratory animals. *Xenobiotica*, **3**: 693-709, 1973.
7. Wilking, N., Appelgren, L.-E., Carlström, K., Pousette, Å., and Theve, N. O. The distribution and metabolism of <sup>14</sup>C-labelled tamoxifen in spayed female mice. *Acta Pharmacol. Toxicol.*, **50**: 161-168, 1982.
8. Fromson, J. M., Pearson, S., and Bramah, S. The metabolism of tamoxifen (ICI 46474). Part II. In females patients. *Xenobiotica*, **3**: 711-714, 1973.
9. Parr, I. B., McCague, R., Leclercq, G., and Stoessel, S. Metabolism of tamoxifen by isolated rat hepatocytes. *Biochem. Pharmacol.*, **36**: 1513-1519, 1987.
10. Lien, E. A., Ueland, P. M., Solheim, E., and Kvinnsland, S. Determination of tamoxifen and four metabolites in serum by low-dispersion liquid chromatography. *Clin. Chem.*, **33**: 1608-1614, 1987.
11. Bakke, O. M., and Scheline, R. Analysis of simple phenols of interest in metabolism. II. Conjugate hydrolysis and extraction methods. *Anal. Biochem.*, **27**: 451-462, 1969.
12. Kemp, J. V., Adam, H. K., Wakeling, A. E., and Slater, R. Identification and biological activity of tamoxifen in human serum. *Biochem. Pharmacol.*, **32**: 2045-2052, 1983.
13. Katzenellenbogen, J. A., Carlson, K. E., and Katzenellenbogen, B. S. Facile geometric isomerization of phenolic non-steroidal estrogens and antiestrogens: limitations to the interpretation of experiments characterizing the activity of individual isomers. *J. Steroid Biochem.*, **22**: 589-596, 1985.
14. Jordan, V. C., Haldeman, B., and Allen, K. E. Geometric isomers of substituted triphenylethylenes and antiestrogen actions. *Endocrinology*, **108**: 1353-1361, 1981.
15. Katzenellenbogen, B. S., Norman, M. J., Ecker, R. L., Peltz, S. W., and Mangel, W. F. Bioactivities, estrogen receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxytamoxifen isomers in MCF-7 human breast cancer cells. *Cancer Res.*, **4**: 112-119, 1984.
16. Woolf, T. F., and Jordan, R. A. Basic concepts in drug metabolism: Part I. *J. Clin. Pharmacol.*, **27**: 15-17, 1987.
17. Borgna, J.-L., and Rochefort, H. Hydroxylated metabolites of tamoxifen are formed *in vivo* and bound to estrogen receptor in target tissues. *J. Biol. Chem.*, **256**: 859-868, 1981.

<sup>4</sup> E. A. Lien, unpublished data.