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W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

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ZHOU XIN, CUI KAIRONG, WU MOUTIAN:
Structural Elucidation of the Metabolite of Tamoxifen in Human Urine
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Zhou Xin, Cui Kairong and Wu Moutian

Structural elucidation of the metabolite of tamoxifen in human urine

China Doping Control Center, National Research Institute of Sports Medicine, Beijing, China

ABSTRACT

The major metabolite of tamoxifen [(Z)-1-(4-[2-dimethylaminoethoxy]phenyl)-1,2-diphenyl-but-1-ene] was suggested to be 4-hydroxy-3-methoxytamoxifen [(E)-1-(4-[2-dimethylamino-ethoxy]phenyl)-1-(4-hydroxy-3-methoxyphenyl)-2-phenyl-but-1-ene] ((E)-SMT). This compound was synthesised, identified by GC/MS, LC/MS and NMR and equilibrates quickly into a mixture of (E) and (Z) isomers. Different GC retention times demonstrate that (E)- and (Z)-SMT and the urinary metabolite have different structures. Furthermore the molecular weight of the metabolite was studied using different derivatisation techniques.

INTRODUCTION

Tamoxifen (Fig.1) is a potent non-steroidal antiestrogenic drug and has been banned in male athletes by IOC and WADA since 2000. Previous studies on the metabolism of tamoxifen in man suggested that the major urinary metabolite is 4-hydroxy-3-methoxy-tamoxifen ((E)-SMT).[1]-[3]

EXPERIMENTAL

Urine sample collection and preparation

The urine samples were collected from two volunteers before and after oral administration of 10 mg and 20 mg of tamoxifen from Nolvadex. 5 ml aliquots of the urine samples were applied to an RP-C18 column, enzymatically hydrolyzed with ß-glucuronidase (3 hrs at 55℃, pH 6.8), then extracted with t-butyl methyl ether (TBME) at pH 8.8, dried in a N_2 stream at 40

℃ and derivatized with different reagents: (a) 49μl of N-methyl-N-trimethylsilyl-trifluoro-acetamide (MSTFA) +1μl of N-trimethylsilylimidazole at 70℃ for 30 min; (b) 50μl of N,N-bis-trifluoroacetamide (MBTFA) at 70℃ for 30 min; (c) 50mg of anhydrous K_2CO_3, 50μl of CH_3I and 50μl of anhydrous acetone at 60℃ for 3 hrs followed by transferring the supernatant which was dried in a N_2 stream and reconstituted in ethyl acetate.

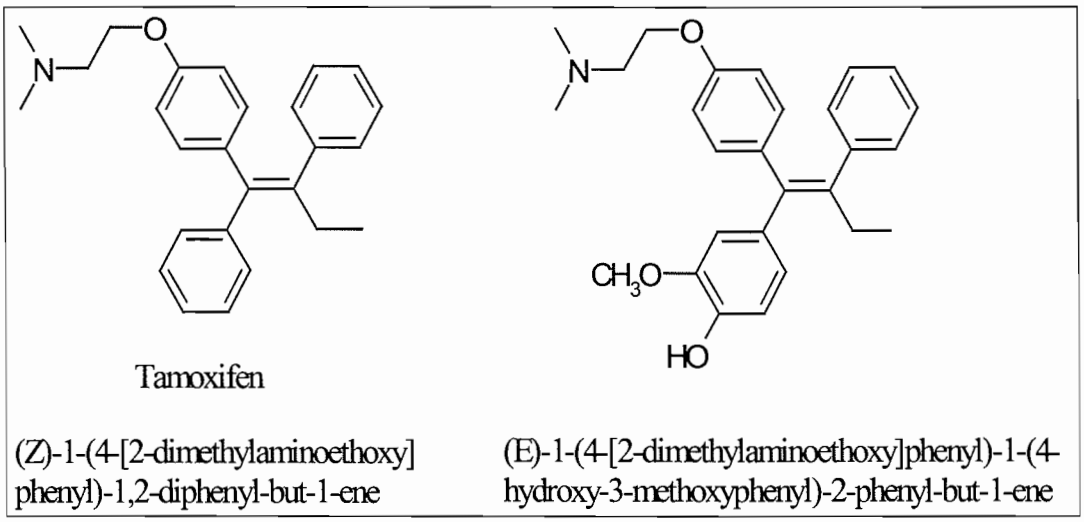
Instrumentation

GC/MSD Agilent 6890 / Agilent 5973

GC/MS Hewlett-Packard 5890 / Finnigan TSQ7000 for electron and chemical ionisation

GC/MS Agilent 6890 / Finnigan MAT 95XL

Always used were HP-1 columns (17m×200μm×0.11μm) and the oven temperature program was: 0 min 180℃, + 3.3℃/min, 0 min 231℃, +30℃/min, 2 min 310℃.



Tamoxifen

(Z)-1-(4-[2-dimethylaminoethoxy] phenyl)-1,2-diphenyl-but-1-ene

(E)-1-(4-[2-dimethylaminoethoxy]phenyl)-1-(4-hydroxy-3-methoxyphenyl)-2-phenyl-but-1-ene

Fig. 1 Chemical structures of tamoxifen and (E)-SMT

RESULTS AND DISCUSSION

Characterization of the synthetic SMT

HPLC/API-MS: Two peaks were detected in a ratio of about 1:1, presenting the same quasi-molecular ion (M+1) at m/z 418. The (E)-SMT readily equilibrates to a mixture of Z- and E-isomers in a few hours at room temperature. Allan B.Foster et al.[4] suggested that the isomerization occurs even on storage of (Z)-4-hydroxy-tamoxifen in solid phase.

GC/MS: The TMS derivatives of the two isomers could be separated by GC/MS and present two peaks with M^+ = 489. Since the Z-isomer is slightly higher than the E-isomer in the

^{1}H-NMR integration results, the peak with the bigger integration area was hypothesised to correspond to the TMS derivative of the Z-isomer (Fig. 2).

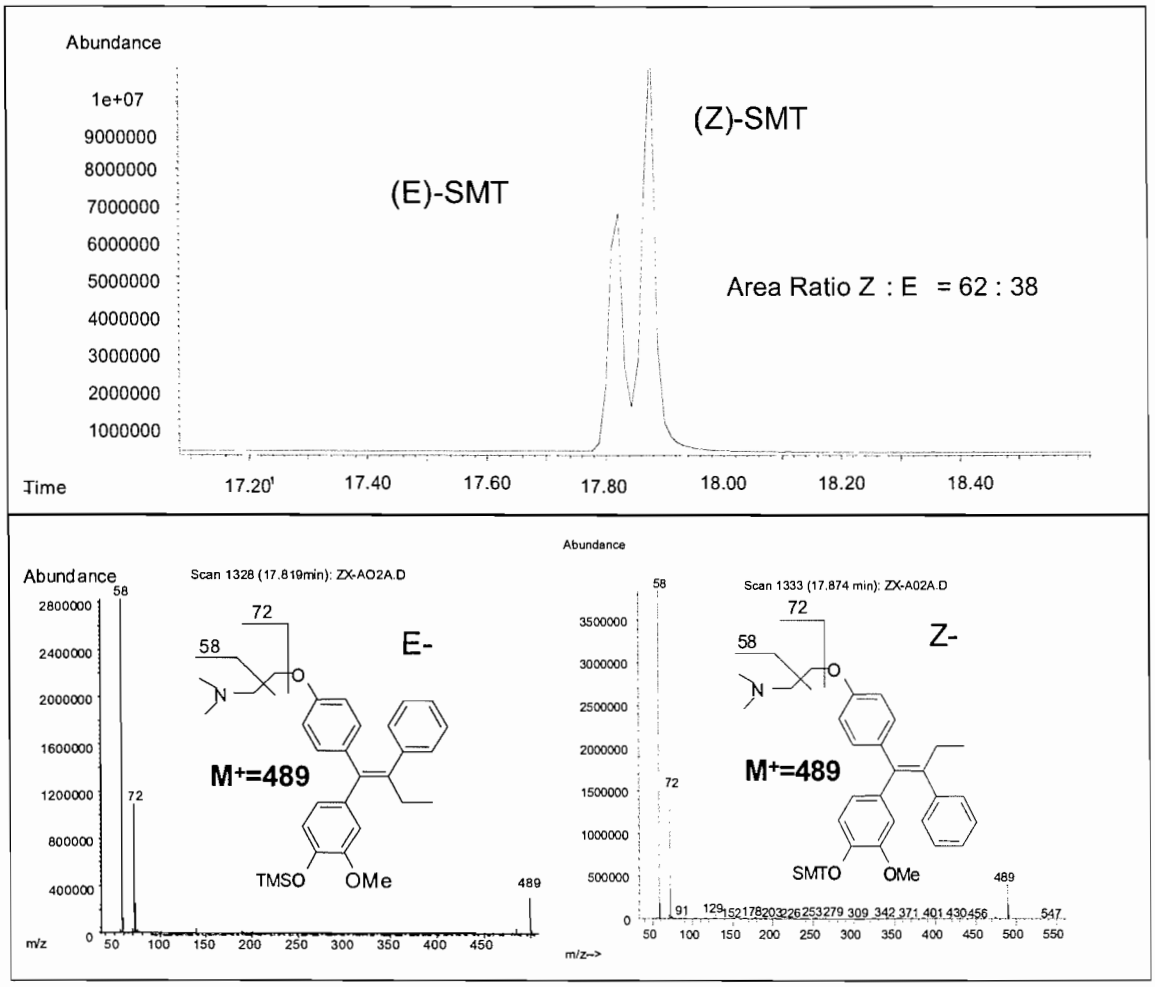


Fig. 2 TIC and EI-MS of mono-TMS derivatives of the synthetic SMT

NMR: Most signals of the NMR and relationships observed in ^{1}H, ^{13}C, NOESY, H,HCOSY, DEPT, HMQC and HMBC have been assigned. The signals of the Z-isomer are usually present in pairs with those of the E-isomer, so the signals could be divided into two groups.

Tab. 1 ^{1}H-NMR and ^{13}C-NMR data of the synthetic SMT

	^{1}H-NMR (600MHz, $CDCl_3$)		^{13}C-NMR(600MHz, $CDCl_3$)	
	E-	Z-	E-	Z-
①CH_3 (-CH_2CH_3)	t, 2.2H, δ 0.931ppm	t, 3H, δ 0.92ppm	δ 13.663ppm	δ 13.607ppm
②CH_3 (-$N(CH_3)_2$)	s, 4.2H, δ 2.29ppm	s, 6H, δ 2.36ppm	δ 45.896ppm	δ 45.851ppm
③CH_2 (-CH_2CH_3)	q, 1.5H, δ 2.479ppm	q, 2H, δ 2.481ppm	δ 29.156ppm	δ 29.128ppm
④CH_2 (=N-CH_2-)	t, 1.4H, δ 2.66ppm	t, 2H, δ 2.76ppm	δ 58.288ppm	δ 58.353ppm
⑤CH_3 (-OCH_3)	s, 2.1H, δ 3.83ppm	s, 3H, δ 3.43ppm	δ 55.896ppm	δ 55.593ppm
⑥CH_2 (-CH_2-O-)	t, 1.4H, δ 3.93ppm	t, 2H, δ 4.10ppm	δ 65.630ppm	δ 65.873ppm

In the NOESY spectrum, the relationship between the signal generated by (CH_2, -CH_2CH_3) and the signal resulting from the B2-H was observed in one group of signals (Fig. 3), suggesting they should be assigned to the E-isomer.

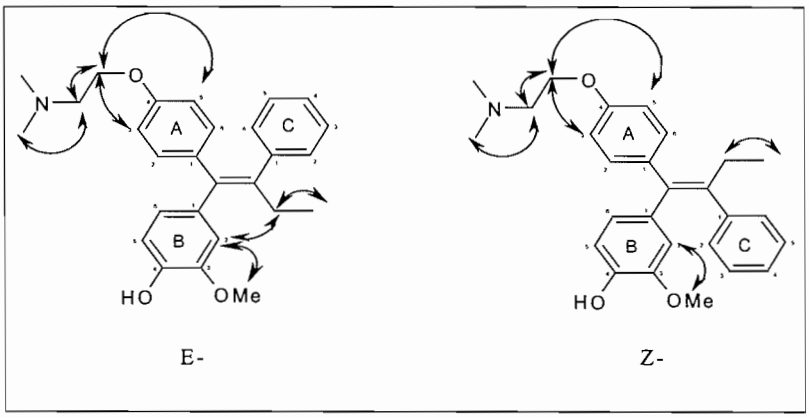


Fig 3: The relationship observed in the NOESY spectrum.

HRMS: mass 417.231819, calculated mass 417.23394 (3.4 ppm), formula $C_{27}H_{31}NO_3$.

Comparison of the synthetic SMT and the urinary metabolite by GC/MS

The GC retention times of (E)-SMT and (Z)-SMT (17.80 and 17.84 min) are different from that of the urinary metabolite (17.55min) (Fig. 4). In addition, two urine samples from IDAS Kreischa and the Romanian Doping Control Laboratory were also analyzed and compared in the same way. The outcome of the comparison is the same.

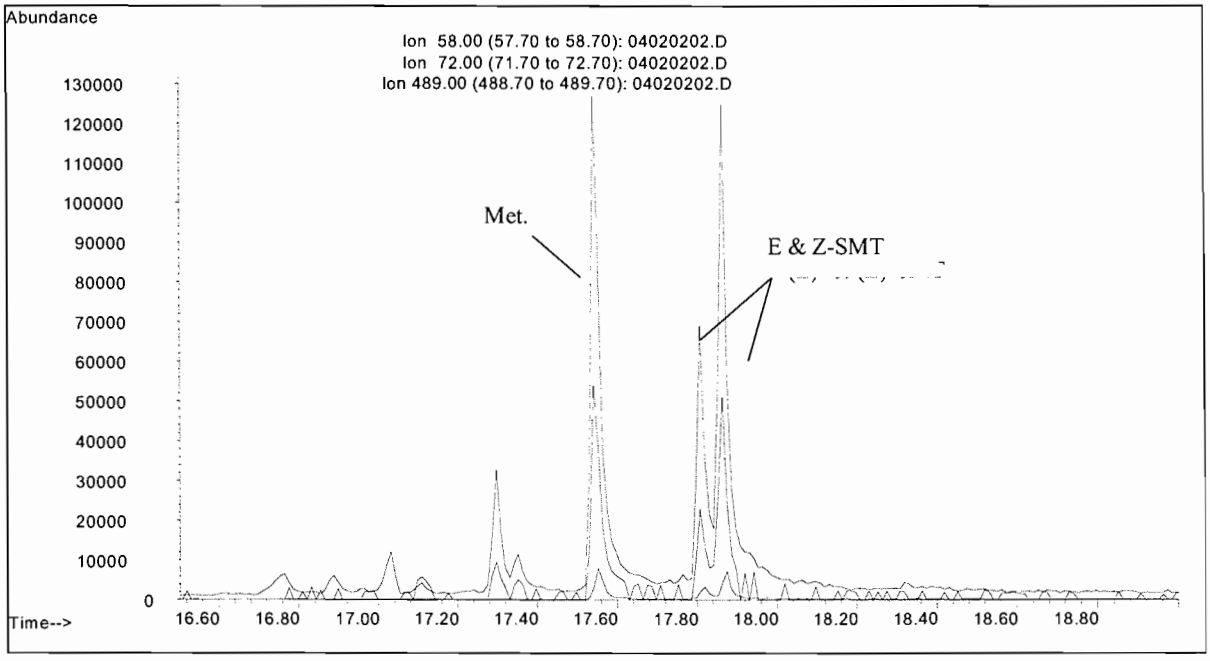


Fig. 4 Comparison of the GC retention times: a urine from an excretion study spiked with synthetic SMT.

Further structural elucidation

Urinary metabolite (Met) derivatized with three different reagents: Three derivatives of Met were prepared and analyzed. The three presumable molecular ions M^+ at m/z 489, 513, 431 have been detected after MSTFA, MBTFA and CH_3I derivatization (Fig. 5).

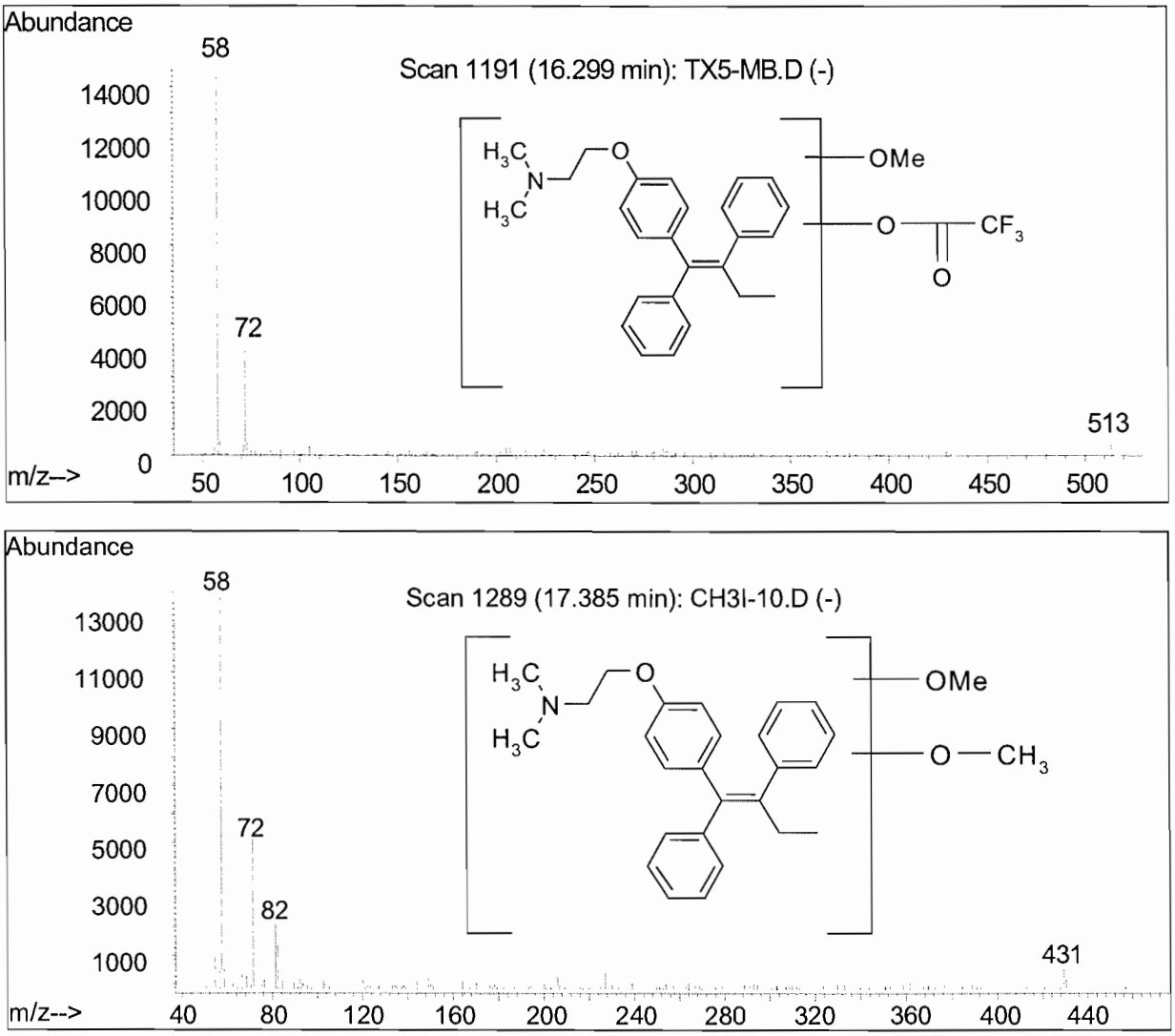


Fig. 5 Mass spectra of urinary metabolite (Met) derivatized with MBTFA and CH_3I

EI&CI: The TMS derivative of Met presents a molecular ion M^+ at m/z 489 in EI mode and $(M+1)^+$ at m/z 490 in CI mode.

HRMS: mass 489.2718, calc. mass 489.2694 (5.0ppm), formula $C_{30}H_{39}NO_3Si$.

CONCLUSION

The different GC retention times of the synthetic (E)-1-(4-[2-dimethylaminoethoxy]phenyl)-1-(4-hydroxy-3-methoxyphenyl)-2-phenyl-but-1-ene ((E)-SMT) and the urinary metabolite

(Met) indicate that they are not identical. EI-MS and CI-MS of the mono-TMS metabolite present the molecular ion M^+ at m/z 489 and the $(M+1)^+$ at m/z 490, and the mass spectra of the differently derivatized urinary metabolite show that the urinary metabolite has a molecular weight of 417 with a single derivatisable position. Hydroxylation and methoxylation may occur to the parent drugs *in vivo* metabolism, but the exact positions of the hydroxy and methoxy groups are still unknown. Further studies are in progress.

Acknowledgements

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