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## Note

# Gas chromatographic–mass spectrometric study of the urinary metabolism of trimipramine

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Trimipramine (10,11-dihydro-N,N-$\beta$-trimethyl-5*H*-dibenz[*b,f*]azepine-5-propanamine) is a tricyclic antidepressant that is structurally closely related to imipramine [1–3]. In addition to its antidepressive effect, trimipramine seems to be effective against gastric and duodenal ulcers [4,5]. The daily oral dose is 50–200 mg. The plasma kinetics of trimipramine and its metabolites desmethyltrimipramine, 2-hydroxytrimipramine and desmethyl-2-hydroxytrimipramine have been determined by gas chromatography (GC) with nitrogen–phosphorus detection and high-performance liquid chromatography with electrochemical detection [6–10]. Its elimination half-life after intravenous dosing is $23 \pm 1.9$ h. The oral bioavailability ranges from 17.8 to 62.7%, which is probably due to individual differences in first-pass metabolism [7]. Trimipramine plasma protein binding is high (94.9%). The pharmacological effect of trimipramine metabolites has not been studied. As desipramine, the major metabolite of the structural analogue imipramine, is still pharmacologically active, it seems to be likely that at least desmethyltrimipramine will also be active.

Little is known about the urinary metabolism of trimipramine in humans. In a study of urinary metabolism in rabbits and dogs by thin-layer chromatography (TLC), human metabolism was only briefly mentioned [11]. By hydrolysis and extraction of urine samples from patients with trimipramine overdose, Pfleger et al. [12] found two metabolites (V and X; see Fig. 1) in addition to the unchanged drug. After acetylation of the urine extracts with acetic anhydride, five deriva-

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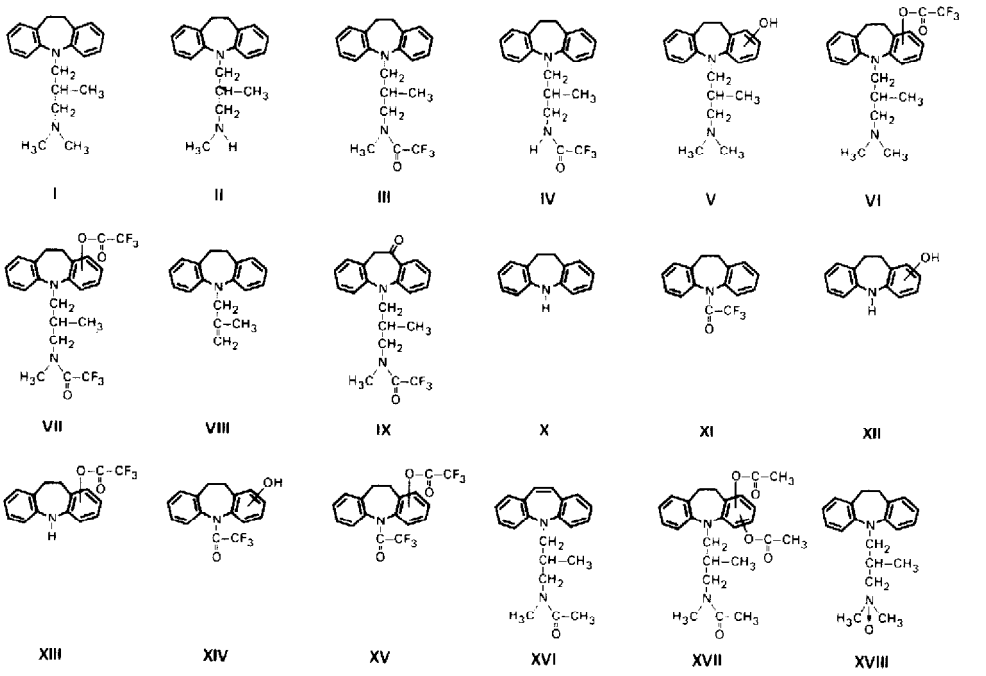


Fig. 1. Structures of trimipramine, its metabolites and derivatives.

tives and artifacts of trimipramine metabolites (acetylated analogues of VI, VII and XIII, XVI, XVII) were identified in addition.

In a case of acute poisoning with trimipramine, several previously unknown metabolites and derivatives of trimipramine could be detected by a gas chromatographic–mass spectrometric (GC–MS) screening procedure [13]. This finding prompted us to study the urinary metabolism of trimipramine in patients who had received a therapeutic dose. As, in general, the GC properties of trifluoroacetyl derivatives are superior to those of the acetyl analogues, urine extracts were analysed with and without trifluoracetylation.

## EXPERIMENTAL

### *Clinical studies*

Urine samples (24 h) were collected after oral doses of $3 \times 25$ mg of trimipramine (Stangyl®) per day for at least one week to four patients (two males, two females) with endogenous depression. Laboratory tests were normal with respect to liver and kidney function. Urine was pooled and stored at $-20°$ prior to analysis.

### *Reagents and chemicals*

Pure samples of trimipramine, desmethyltrimipramine, didesmethyltrimipramine and 2-hydroxytrimipramine were generously provided by Rhône-Poulenc (Norderstedt, F.R.G.). Trimipramine N-oxide was synthesized by reaction of hydrogen peroxide with trimipramine at pH 9. All reagents, of analytical-reagent

grade or better, were purchased from commercial sources and used without further purification.

*Extraction*

Volumes of 20 ml of urine were extracted twice at pH 3 and subsequently at pH 10 with dichloromethane–isopropanol (9:1) (Nanograde® compounds, Mallinckrodt, St. Louis, MO, U.S.A.). The organic solvent was removed with a stream of dry nitrogen. The residue was dissolved in 100 $\mu$l methanol and a 1–3 $\mu$l aliquot was used for GC–MS analysis. Similarly, urine was extracted after incubation (at 37°C) with 0.5 ml of glucuronidase–sulphatase (Merck, Darmstadt, F.R.G.) at pH 5.5 for 30 min and after hydrolysis with hydrochloric acid for 30 min at 100°C. Extracts were analysed directly and after trifluoroacetylation with trifluoroacetic anhydride (Merck) in diethyl ether (Nanograde, Mallinckrodt) at room temperature.

*Instrumentation*

Mass spectra were run on a Model 4021 gas chromatograph–mass spectrometer with an Incos data system (Finnigan, San José, CA, U.S.A.). For GC, a 25 m × 0.32 mm I.D. fused-silica capillary column (SE-54) (Macherey & Nagel, Düren, F.R.G.) was used with an injection port temperature of 290°C and a column temperture programmed from 75 to 300°C at 25°C/min. The column was directly coupled to the mass spectrometer. Kováts retention indices were determined by calibration of the column with a mixture of $C_{12}$–$C_{22}$ *n*-alkanes. The ion source pressure was $4 \cdot 10^{-5}$ Pa in the electron-impact (EI) mode and $3 \cdot 10^{-3}$ Pa in the chemical ionization (CI) mode using methane. The multiplier voltage was 1200 V.

All samples were run in the EI (70 eV) and CI modes. Structure elucidation was based on reference mass spectra (NBS library of Finnigan-MAT, Incos Data System, version 5.5-E), mass spectra of reference compounds, determination of the molecular ion by CI, the fragmentation pattern and the formation of the corresponding derivatives after trifluoroacetylation of the extracts.

Compound XVIII ($R_F$ 0.10) could be identified after TLC of urine extracts on silica $F_{254}$ rapid plates (ICN Biomedical, Eschwege, F.R.G.) with toluene–ethanol–25% aqueous ammonia (80:20:1).

## RESULTS

EI mass spectra are summarized in Table I. The basic mass spectral fragmentation pattern of trimipramine is depicted in Fig. 2. The main fragmentation steps were $\alpha$-cleavage at the site of the amino functions and cleavage of the C–N bond accompanied by a hydrogen shift. Analogous fragmentation steps were observed in the mass spectra of trimipramine derivatives. CI with methane yielded the typical $[M+41]^+$, $[M+29]^+$ and $[M+1]^+$ ions, but no fragment ions.

In underivatized urine extracts (with and without glucuronide cleavage and hydrochloric acid hydrolysis), I, II, V, VIII, X and XII were identified. After trifluoroacetylation, III, IV, VIa, VIb, VII, IX, XI, XIII, XIV and XV were detected

TABLE I

KOVÁTS RETENTION INDICES AND ELECTRON-IMPACT MASS SPECTRA OF TRIMIPRAMINE, ITS METABOLITES AND DERIVATIVES

| Compound | Kováts retention index | *m/z* (intensity %)* |
|---|---|---|
| I | 2130 | $M^{+\cdot}$ 294 (8), 250 (6), 249 (34), 234 (12), 220 (4), 208 (21), 194 (4), 193 (18), 165 (4), 99 (19), 91 (3), 84 (13), 58 (100) |
| II | 2270 | $M^{+\cdot}$ 280 (18), 250 (7), 249 (52), 248 (12), 234 (21), 220 (11), 208 (100), 195 (82), 194 (58), 167 (10), 165 (8), 91 (12), 72 (19), 70 (26) |
| III | 1950 | $M^{+\cdot}$ 376 (13), 234 (1), 220 (1), 209 (18), 208 (100), 194 (8), 193 (34), 167 (6), 165 (5), 140 (7), 91 (6) |
| IV | 1860 | $M^{+\cdot}$ 362 (14), 360 (2), 209 (12), 208 (100), 194 (6), 193 (27), 167 (2), 165 (3), 139 (4), 125 (11), 111 (19), 97 (26), 83 (28), 69 (46), 57 (80) |
| V | 2800 | $M^{+\cdot}$ 310 (13), 266 (6), 265 (40), 264 (11), 250 (16), 236 (4), 224 (22), 211 (10), 210 (6), 209 (12), 195 (3), 180 (4), 167 (2), 165 (2), 99 (8), 84 (12), 58 (100) |
| VIa | 1830 | $M^{+\cdot}$ 406 (10), 361 (52), 346 (12), 332 (1), 320 (12), 305 (2), 264 (6), 232 (1), 208 (6), 180 (6), 167 (1), 165 (2), 99 (9), 84 (9), 69 (10), 58 (100) |
| VIb | 1835 | $M^{+\cdot}$ 406 (8), 362 (6), 361 (40), 346 (12), 332 (1), 320 (11), 306 (2), 264 (3), 224 (1), 208 (9), 193 (3), 180 (3), 167 (1), 165 (2), 99 (10), 84 (8), 58 (100) |
| VII | 2760 | $M^{+\cdot}$ 488 (7), 321 (12), 320 (100), 306 (1), 305 (12), 234 (1), 208 (6), 195 (6), 182 (4), 180 (3), 165 (2), 140 (8), 97 (3), 91 (2), 69 (11), 55 (10) |
| VIII | 1710 | $M^{+\cdot}$ 249 (70), 234 (15), 208 (39), 194 (100), 167 (12), 165 (12), 152 (8), 116 (11), 91 (25), 65 (9) |
| IX | 2120 | $M^{+\cdot}$ 390 (8), 250 (2), 234 (1), 223 (11), 222 (100), 194 (12), 180 (8), 165 (3), 140 (4), 91 (3), 69 (3) |
| X | 1650 | $M^{+\cdot}$ 195 (96), 194 (100), 193 (10), 192 (9), 191 (8), 180 (37), 167 (11), 165 (8), 152 (6), 139 (3), 96 (50), 83 (21) |
| XI | 1460 | $M^{+\cdot}$ 291 (17), 222 (6), 205 (2), 194 (20), 193 (7), 192 (18), 179 (12), 167 (3), 165 (4), 154 (14), 148 (11), 140 (4), 121 (18), 100 (31), 86 (100) |
| XII | 2700 | $M^{+\cdot}$ 211 (100), 210 (84), 196 (18), 182 (4), 181 (4), 167 (11), 105 (32), 77 (13) |
| XIII | 1490 | $M^{+\cdot}$ 307 (40), 209 (3), 194 (42), 192 (21), 181 (6), 180 (6), 168 (16), 167 (100), 166 (18), 141 (11), 70 (52), 69 (51) |
| XIV | 1530 | $M^{+\cdot}$ 307 (12), 194 (11), 193 (10), 181 (7), 168 (12), 167 (100), 165 (24), 152 (16), 140 (3), 69 (3) |
| XV | 1532 | $M^{+\cdot}$ 403 (9), 375 (1), 334 (2), 306 (10), 296 (7), 284 (6), 254 (7), 200 (6), 166 (7), 97 (10), 88 (100), 69 (15) |
| XVIII | Solid inlet | $M^{+\cdot}$ 310 (0.4), 308 (0.6), 294 (3), 293 (2), 249 (42), 235 (14), 220 (4), 208 (25), 194 (43), 193 (19), 167 (4), 165 (5), 130 (3), 116 (4), 98 (12), 91 (10), 84 (5), 77 (5), 58 (100) |

*$M^{+\cdot}$ = molecular ion; base peak (100%) underlined.

in addition. The exact positions of the hydroxyl, trifluoroacetyl and keto functions of V–VII, IX and XII–XV could not be determined from the mass spectrum. The identification of the isomers VIa and b after trifluoroacetylation implies the existence of two isomers of V which were not separated under the GC conditions described here. Trifluoroacetylation of XII was not complete and led to a mixture

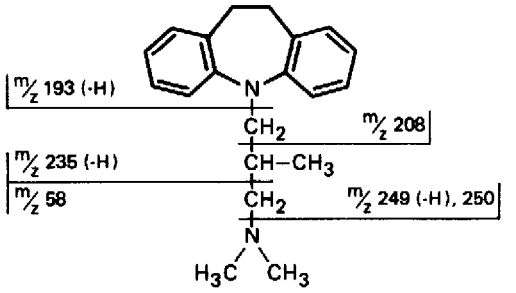


Fig. 2. Fragmentation pattern of trimipramine (molecular ion $m/z$ 294).

of mono- and bistrifluoroacetylated derivatives (XIII–XV). The same metabolites and derivatives were identified in a case of trimipramine overdose. Neither in the underivatized extracts, nor in the trifluoroacetylated extracts could analogues of XVI or XVII be detected. VIII was probably an artifact generated from trimipramine N-oxide (XVIII) by Cope elimination. Reference compound XVIII did not pass through the GC column unchanged but decomposed completely to VIII. The mass spectrum of XVIII (solid inlet) is given in Table I.

## DISCUSSION

In addition to the unchanged drug (I), four metabolites of trimipramine (II, V, X and XII) could be identified directly from their mass spectra. After trifluoroacetylation, derivatives (IV, VII, IX) of four further metabolites were detected in addition. Trifluoroacetylation of V led to the formation of VIa and b, which indicates that V probably consisted of two isomers which were not separated under the GC conditions used.

The GC–MS study of urinary extracts yielded evidence of the formation of nine metabolites after a therapeutic dose of trimipramine. Four of these were identified directly or indirectly by Pfleger et al. [12] in cases of trimipramine overdose. The metabolic degradation of trimipramine is similar to that of imipramine [10]. It includes N-demethylation, hydroxylation of the phenyl ring, oxidation of the C-10 atom, N-1 dealkylation and N-oxidation.

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