J. Pharm. Pharmacol. 1999, 51: 53–59
Received May 26, 1998
Accepted September 7, 1998

© 1999 J. Pharm. Pharmacol.

Identification of Urinary Metabolites of Clemastine after Oral Administration to Man

MAN HO CHOI, BYUNG HWA JUNG AND BONG CHUL CHUNG

Bioanalysis and Biotransformation Research Center, Korea Institute of Science & Technology, PO Box 131, Cheongryang, Seoul, Korea

Abstract

The metabolism of clemastine, 2-{2-[1-(4-chlorophenyl)-1-phenylethoxy]ethyl}-1-methylpyrrolidine, has been studied in three adult male volunteers after a single oral dose of 20 mg as the fumarate. After enzymatic hydrolysis solvent extracts of urine were derivatized with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide-ammonium iodide and analysed by gas chromatography-mass spectrometry. The structures of metabolites were determined on the basis of electron impact and chemical ionization mass spectra and the identities of some (e.g. carbinol, 4-chlorobenzophenone and 4-chlorophenylstyrene) were confirmed by use of authentic standards.

The principal route of metabolism of clemastine in man involves direct oxidation, *O*-dealkylation (fission of the ether bond), aromatic hydroxylation, aliphatic oxidation, alcoholic dehydration, and then enzymatic hydrolysis. Of the total amount of metabolites excreted in the urine 35% was carbinol (metabolite M3, major metabolite), 15% was M1, 17% was M2, 11% was M4, 9% was M5, 8% was M6 and 5% was M7.

Clemastine (2-{2-[1-(4-chlorophenyl)-1-phenylethoxy]ethyl}-1-methylpyrrolidine), an ethanolamine-type antihistamine, is rapidly and almost completely absorbed from the gastrointestinal tract. Peak plasma concentrations of the drug are obtained within 2–5 h of a single oral dose (Clarke & Nicholson 1978). After oral administration of clemastine fumarate, the antihistaminic effect of the drug (as measured by suppression of the weal response induced by intradermal injection of histamine) is maximum within 5–7 h and persists for 10–12 h (in some individuals up to 24 h) (Gerald & Pharm 1995). Despite widespread use of clemastine since its introduction in 1967 there are few reports on the pharmacokinetics and metabolism of the clemastine in man.

Studies on the detection of clemastine metabolites in rat by gas chromatography-mass spectrometry (GC-MS) have been reported (Göber et al 1988, 1989; Maurer & Pfleger 1988; Fröhlich et al 1996). The metabolites identified are products of fission of the ether bond, aromatic and aliphatic oxidation, alcohol dehydration, decarboxylation, *N*-oxidation and *O*-methylation. Several methods are available for the determination of clemastine in biological fluids (Tham et al 1978; Bedair et al 1988). These methods are based on the derivatization of clemastine into 4-chlorophenylstyrene or 4-chlorobenzophenone by use of acidic reagents and determination of the derivatives by capillary gas chromatography. Nevertheless, there have been no reports concerning the metabolism of clemastine in man.

We describe the identification, by GC-MS, of the metabolites of clemastine found in the urine of man after oral administration of the drug to three healthy volunteers.

Correspondence: B. C. Chung, Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul, Korea.

Materials and Methods

Materials

Clemastine fumarate was purchased from Sigma (St Louis, MO) and carbinoxamine, used as internal standard, was supplied by Choong Wae (Seoul, Korea). 4-Chlorobenzophenone (4-chlorophenylphenylmethanone) was purchased from Aldrich

(St Louis, MO). Carbinol (4-chloro-(α-methyl-α-phenyl)benzenemethanol; Lednicer & Mitscher 1980; Mayo et al 1989) and 4-chlorophenylstyrene (1-chloro-4-(1-phenylethenyl)benzene; Ebnöther & Weber 1976) were synthesized and their identities confirmed by mass spectrometry. Their purities, determined by mass spectrometry in electron impact (EI) mode (electron energy 70 eV), were 99·8% and 99·5%, respectively. Serdolit AD-2 resin (particle size 0·1–0·2 mm) was supplied by Serva (Heidelberg, Germany) and washed with acetone, methanol and distilled water before use. Glucuronidase (sulphatase-free, activity 200 units mL^{-1}) was purchased from Boehringer Mannheim (Germany). The silylating agents, MSTFA (*N*-methylsilyltrifluoroacetamide) and NH_4I were obtained from Sigma.

Drug administration

Clemastine fumarate was given to three healthy male volunteers, 32, 38 and 41 years and 71, 73 and 75 kg, respectively, as a single oral 20-mg dose, in a gelatine capsule, in the morning. Blank urine was collected before drug administration and urine was collected for 72 h after the dose of drug was taken; urine was immediately stored at 4°C until analysis.

Instrumentation

Gas chromatography-mass spectrometry was performed with a Hewlett-Packard 5890 series II/5989B instrument. A cross linked Ultra-1 capillary column (length 17 m, i.d. 0·2 mm, film thickness 0·11 μm, Hewlett-Packard, CA) was inserted directly into the ion source. Helium, flow rate 0·45 mL min^{-1}, was employed as carrier gas. Samples were injected in split (1 : 10) mode. The injector and detector temperatures were 280 and 300°C, respectively. The initial temperature of the column oven was 100°C; this was maintained for 1·5 min and subsequently increased by 8°C min^{-1} to 320°C. Positive chemical ionization (PCI) was used for confirmation of the molecular ion; the reagent gas was methane.

Sample preparation

Isolation of the metabolites was based on a method currently used in the procedure to detect doping with anabolic steroids (Schänzer & Donike 1993). Aqueous Serdolit AD-2 slurry was placed in a disposable syringe (i.d. 0·9 cm, bed height 3 cm) and the column were washed with 10 mL distilled water. Urine (30 mL) was then passed through the columns, followed by a second wash with distilled water (20 mL). Adsorbed compounds were eluted with three consecutive portions of methanol (5 mL) and the methanol fractions were evaporated under reduced pressure at 45°C. The residue and some traces of water were resolved in 2 mL phosphate buffer (0·2 M, pH 7·0). To hydrolyse conjugated forms, β-glucuronidase (100 μL) was added to the phosphate buffer solution and the solution was heated at 55°C for 1 h. After cooling to room temperature the solution was made basic by addition of potassium carbonate (50 mg), and diethyl ether (5 mL) was added. The mixture was mechanically shaken (10 min) and centrifuged (2400 rev min^{-1}, 5 min) and the organic phase transferred to a test tube. The ether was evaporated to dryness by rotary evaporation. The hydrolyzed buffer solution was extracted with diethyl ether (3 × 5 mL) to enhance the efficiency of recovery of the extraction procedure. The ether fraction was evaporated to dryness and the residue was dried in a vacuum desiccator over P_2O_5-KOH for at least 30 min before derivatization.

Derivatization

The residue from extraction of the urine was trimethylsilylated by addition of a mixture of MSTFA and NH_4I (1000 : 2, v/w; 50 μL) and heating at 60°C for 20 min.

Results

Urine samples over 6–24 h were pooled for each of the three volunteers. Results from GC-MS analysis of the urinary metabolites of clemastine in man are shown in Figure 1. The metabolite peaks were found by comparing the chromatograms obtained from pre- and post-dose urine extracts. Table 1 lists the amounts of urinary metabolites excreted by the three volunteers, as a mean percentage of the total amount of metabolites detected, and the retention time and relative retention time of each metabolite. Urine samples were analysed by GC-MS to characterize the metabolites; the quantity of each metabolite was assessed by comparing peak intensities in a GC-MS chromatogram. Seven metabolites (M1–M7) were detected after enzymatic hydrolysis of the urinary extract. The parent drug was not detected in this study.

Because the metabolite 4-chlorobenzophenone (M2) has not previously been reported, its identity was confirmed by comparison with an authentic standard. The molecular weights of the metabolites were confirmed from the spectra of the compounds obtained by chemical ionization MS; each meta-

Table 1. GC-MS characteristics and excretion pattern of metabolites of clemastine after oral administration.

Metabolite	Name	Retention time (min)	Relative retention time	Molecular ion	Total (% urinary excretion)
M1	1-Chloro-4-(1-phenylethenyl)-benzene	8·28	0·67	214	15·26
M2	4-Chlorophenyl-phenyl methanone	9·35	0·71	216	17·33
M3	4-Chloro-(α-methyl-α-phenyl) benzenemethanol	10·81	0·82	304[a]	35·83
M4	ζ-[1-(4-Chlorophenyl)ethenyl]-phenol	11·02	0·91	302[a]	11·31
M5	ζ-[1-(4-Chlorophenyl)ethenyl]-phenol	12·09	0·92	302[a]	9·25
M6	1-(4-Chlorophenyl)-1-phenyl-1,2-ethandiol	13·17	1·07	392[a]	8·04
M7	1-(4-Chlorophenyl)-hydroxy-phenylethanal	14·53	1·18	318[a]	4·66

Relative retention time was relative to carbinoxamine (13·21 min). [a]Molecular weight as trimethylsilylated compounds. ζ denotes the specific position of the hydroxyl group was not defined. Urine was collected for 72 h.

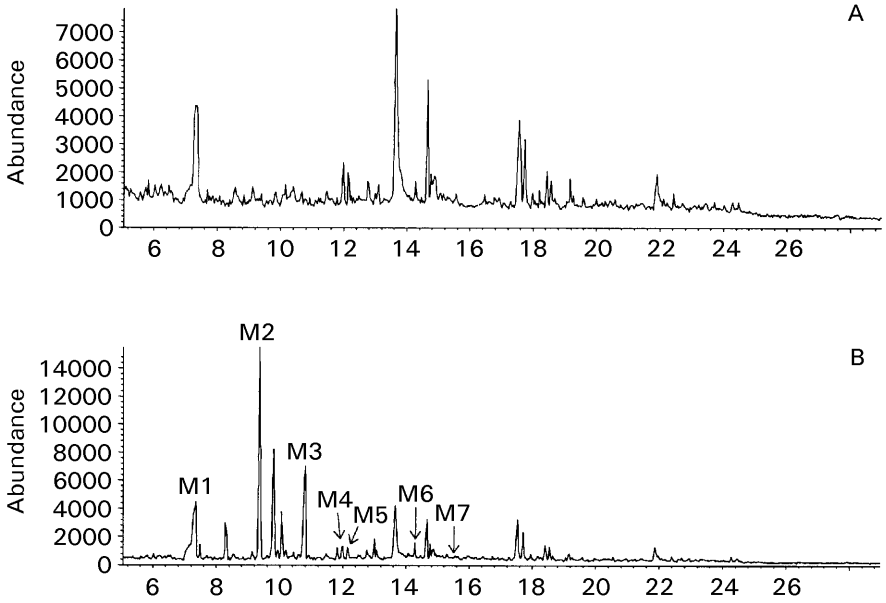

Figure 1. Extracted ion chromatograms of m/z 77 (EI mode) obtained from an extract of urine collected before administration of clemastine (A), and an extract of urine collected between 6 and 24 h after administration of clemastine fumarate (20 mg) (B).

bolite was characterized by electron impact GC-MS (Figures 2 and 3).

Metabolite 1 (M1)

To confirm the identity of this metabolite, 4-chlorophenylstyrene was synthesized by reaction of carbinol with 6 N HCl. The EI mass spectrum of the metabolite (Figure 2, M1) was identical with that of the authentic sample (molecular ion at m/z 214, fragment ions at m/z 199, 179, 89 and 76; data not shown). This metabolite accounted for (approx.) 15% of total excreted urinary metabolites.

Metabolite 2 (M2)

The mass fragmentation of this metabolite correspond to that of authentic 4-chlorobenzophenone (molecular ion at m/z 216, fragment ions at 181, 139, 111 and 105). On the basis of peak abundance metabolite M2 was estimated to constitute (approx.) 17% of total excreted urinary metabolites.

Metabolite 3 (M3)

To confirm the identity of this metabolite carbinol was synthesized by Grignard reaction of 4-chlorobenzophenone with methylmagnesium bromide. The EI mass spectrum of this metabolite in the extract after TMS derivatization (Figure 2, M3) was identical with that from authentic carbinol (molecular ion at m/z 304, fragment ions at m/z 289, 215, 178 and 103, data not shown). On the basis of peak abundance M4 was estimated to constitute (approx.) 36% of total excreted urinary metabolites.

Metabolite 4 and 5 (M4 and M5)

The EI mass spectra of these metabolites in the extract after TMS derivatization (Figure 3, M4 and

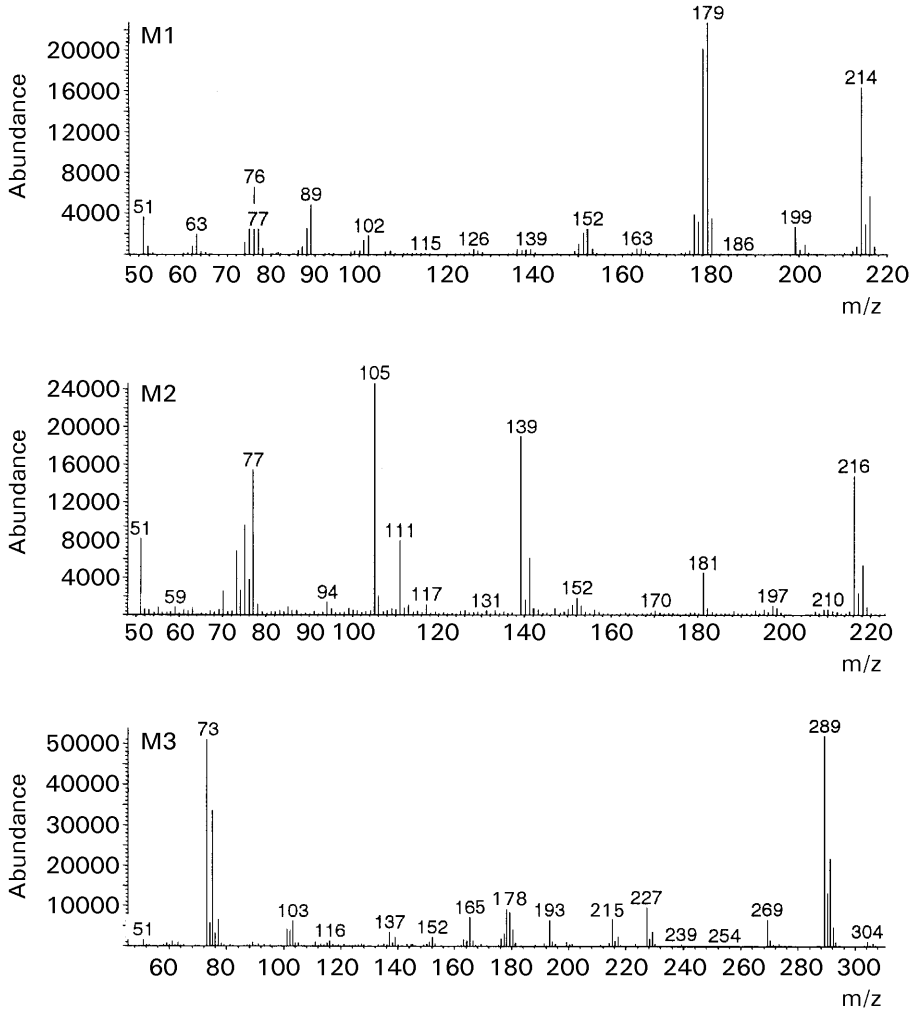

Figure 2. Electron impact (EI) mass spectra of the urinary metabolites (M1–M3) of clemastine.

M5) were identical (molecular ion at m/z 302, fragment ions at 287 $[M^{+\cdot}\text{-}CH_3]^+$, 165 $[M^{+\cdot}\text{-}TMSO\text{-}C_6H_4]^+$, and 111 $[C_6H_4Cl]^+$. The substitution on the benzene ring has not yet been determined but because their mass spectra were identical it is assumed that they are *meta* and *para* substituted isomers on the basis of their GC retention times and previous reports (Maurer & Pfleger 1988; Göber et al 1989). These metabolites were estimated to account for (approx.) 11% and 9% of total urinary excreted metabolites, respectively.

Metabolite 6 (M6)

The EI spectrum of the bis-TMS derivative of M6 is shown in Figure 3 (M6). This derivatized metabolite gave molecular ion at m/z 392 and fragment ions at m/z 377 $[M^{+\cdot}\text{-}CH_3]^+$, 303 $[M^{+\cdot}\text{-}TMSO]^+$, 215 $[M^{+\cdot}\text{-}2TMSO]^+$ and 201 $[M^{+\cdot}\text{-}TMSO\text{-}CH_2TMSO]^+$. This metabolite constituted (approx.) 8% of total excreted urinary metabolites.

Metabolite 7 (M7)

A weak peak eluting at 15·628 min was observed by selected ion monitoring at m/z 303. The mass spectrum of its TMS enol ether derivative (Figure 3, M7) had a molecular ion at 318 and fragment ions at m/z 303 $[M^{+\cdot}\text{-}CH_3]^+$, 289 $[M^{+\cdot}\text{-}CHO]^+$ and 201 $[M^{+\cdot}\text{-}CHO\text{-}TMSO]^+$. This metabolite was estimated to account for (approx.) 5% of total excreted urinary metabolites.

Discussion

The urinary metabolic pathway proposed for clemastine in man is presented in Figure 4. It is

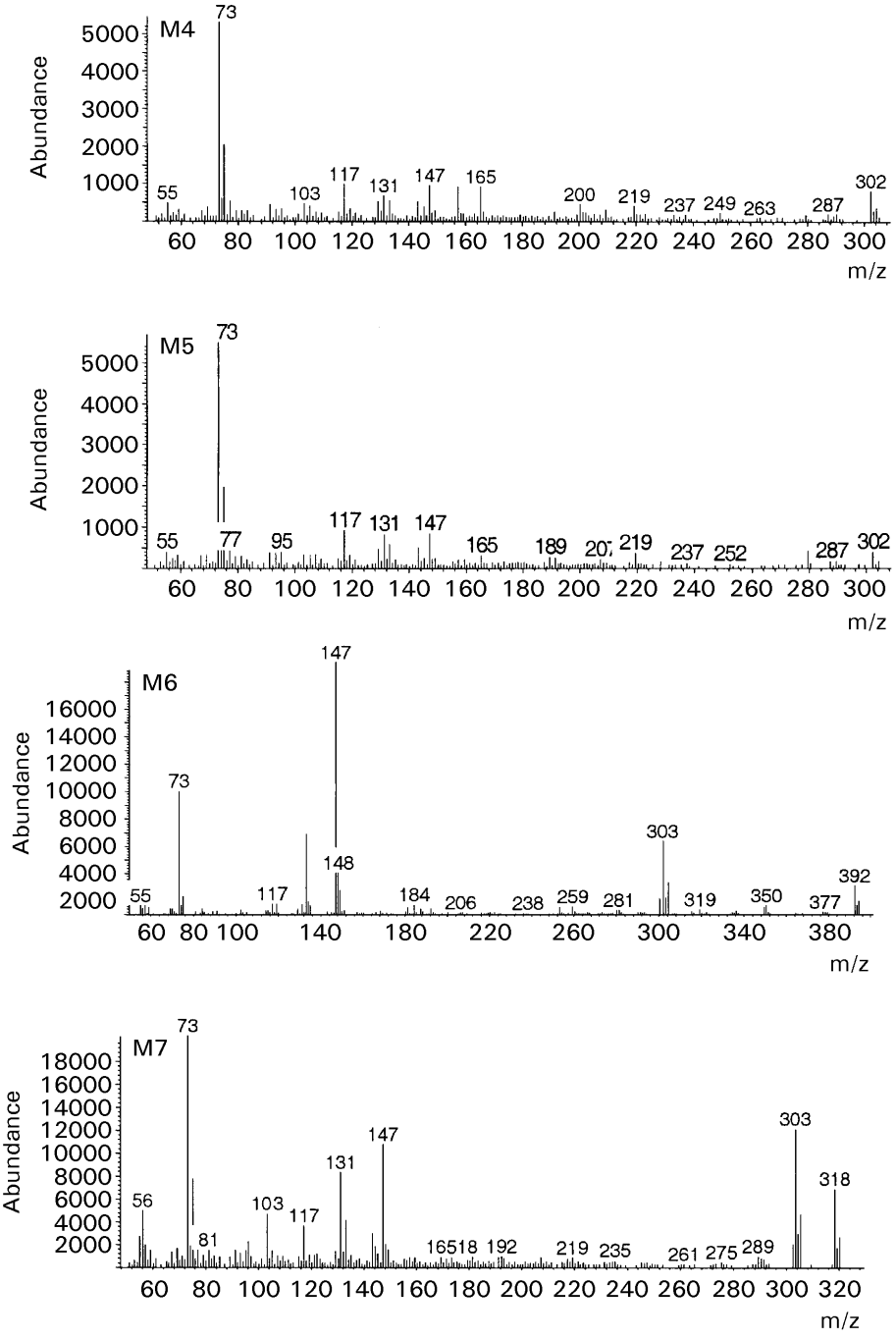

Figure 3. Electron impact (EI) mass spectra of urinary metabolites (M4–M7) of clemastine.

suggested that the pathway in man is successive *O*-dealkylation, direct oxidation, aromatic hydroxylation, aliphatic oxidation, and alcohol dehydration followed by enzymatic hydrolysis. Approximate proportions of metabolites in the 6–24-h urine fraction were evaluated by comparing peak intensities in a GC-MS chromatogram.

Previous reports indicate that the glucuronidation of carbinol was also the major route of metabolism of clemastine in rats (Göber et al 1989). Some minor metabolites found in rat urine and faeces, for example products of *N*-oxidation, the dihydrodiol mechanism and oxidation of aliphatic alcohol to carboxylic acid, were not found in this study, even in the fragmentograms of ions of m/z 392, 405 and 406 obtained in selected-ion monitoring (SIM) mode. 4-Chlorobenzophenone (M2), a substantial amount of which was detected in urine from man,

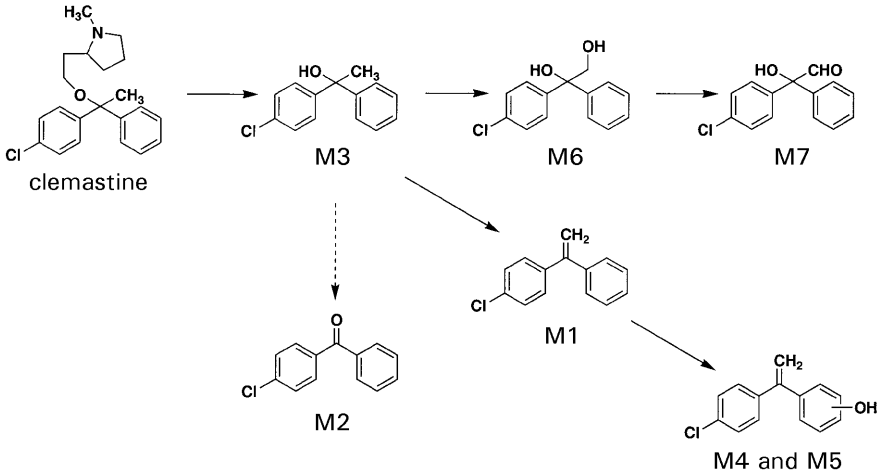

Figure 4. Proposed urinary metabolic pathways of clemastine in man.

has not been found in rat urine and faeces. In this experiment with man some of the metabolites have been identified as substances hydroxylated in the phenyl part of the molecule. Substances produced by hydroxylation of the phenyl part do not yield the 4-chlorobenzene ring upon oxidation.

The metabolism of clemastine is similar to that of the structurally related antihistamine drugs chlorphenoxamine (Goenechea et al 1987; Köppel et al 1987) and setastine (Kapolai et al 1989)—both involve cleavage of the ether bond and 4-hydroxylation of the phenyl ring. Remarkably, clemastine metabolites M1 and M4 in man resemble minor insecticide metabolites such as those of Stauffer R-3828 (Chamberlain & Hopkins 1972) and DDT analogues (Francis et al 1976). Carbinol, resulting from *O*-dealkylation of clemastine, was a major metabolite of clemastine, representing 24% (approx.) of the total metabolites found. Quantification was performed for all urine samples for 0–72 h by GC-MS using carbinoxamine as internal standard. Excretion of each metabolite was estimated as a mean percentage of the total excreted urinary metabolites in the three samples. No parent compound was recovered unchanged, suggesting that metabolism was the sole route of elimination. The mean amount of the administered dose recovered in the urine within 72 h was 42·1%. Several minor peaks were present in the gas chromatograms, but their intensities were minimal, and we could not obtain full scan data for correct structural interpretation. The excretion pattern was similar for all volunteers.

References

Bedair, M. M., Korany, M. A., Lssa, A. S. (1988) Derivative spectrophotometric determination of clemastine fumarate in pharmaceutical preparations. Analyst 113: 1137–1138

Chamberlain, W. F., Hopkins, D. E. (1972) Metabolism of ^{14}C-labeled Stauffer R-3828 by a steer. J. Econ. Entomol. 66: 119–125

Clarke, C. H., Nicholson, A. N. (1978) Performance studies with antihistamines. Br. J. Clin. Pharmacol. 6: 31–35

Ebnöther, A., Weber, H. P. (1976) Synthese und absolute Konfiguration von Clemastin und seiner Isomeren. Helv. Chim. Acta 59: 2462–2468

Francis, A. J., Spanggord, R. J., Ouchi, G. I., Bramhall, R., Bohonos, N. (1976) Metabolism of DDT analogues by a *Pseudomonas* sp. Appl. Environ. Microbiol. 32: 213–216

Fröhlich, L., Pietzyk, B., Smolink, K., Göber, B. (1996) Untersuchungen zur biomimetischen Oxidation von Clemastinhydrogenfumarate. Pharmazie 51: 409–414

Gerald, K. M., Pharm, D. (1995) Drug Information, AHFS, Wisconsin, pp 16–17.

Goenechea, S., Rücker, G., Brazezink, H., Hoffmann, G., Neugebauer, M., Pyzik, T. (1987) Isolation and identification of chlorphenoxamine metabolites in human urine. Arzneim. Forsch. 37: 854–859

Göber, B., Al Mardini, M. A., Franke, P. (1988) Isolation and identification of clemastine metabolites in rat feces. Pharmazie 43: 558–559

Göber, B., Al Mardini, M. A., Franke, P. (1989) Clemastinmetaboliten aus Kot und Harn der ratte. Pharmazie 44: 847–851

Kapolai, L., Petocz, L., Fekete, M., Budai, G. (1989) Comparative pharmacological study of the new antihistaminic Lodrrix and its metabolites. Kiserletugyi Orvostud. 41: 98–107

Köppel, C., Tenczer, J., Arndt, I., Ibe, K. (1987) Urinary metabolism of chlorphenoxamine in man. Arzneim. Forsch. 37: 1062–1064

Lednicer, D., Mitscher, L. (1980) The Organic Chemistry of Drug Synthesis, John Wiley & Sons, New York, pp 32–33

Maurer, H., Pfleger, K. (1988) Screening procedure for the detection of alkanolamine antihistamines and their metabolites in urine using computerized GC/MS. J. Chromatogr. 428: 43–60

Mayo, D. W., Pike, R. M., Butcher, S. S. (1989) Microscale Organic Laboratory, John Wiley & Sons, New York, pp 201–206.

Schänzer, W., Donike, M. (1993) Metabolism of anabolic steroids in man: synthesis and use of reference substances for identification of anabolic steroid metabolites. Anal. Chim. Acta 275: 23–48

Tham, R., Norlander, B., Hägermark, Ö., Faansson, L. (1978) Gas chromatography of clemastine. Drug Research 28: 1017–1020