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Mass Spectral Identification of the Metabolites of α,α-Dimethyl-4-(α,α,β,β-tetrafluorophenethyl)-benzylamine (MK-251), a Novel Antiarrhythmic Agent, in Various Species†

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Abstract—The identification of a number of metabolites of the novel antiarrhythmic agent, α,α-dimethyl-4-(α,α,β,β-tetrafluorophenethyl)benzylamine (MK-251), is presented. The compound is extensively metabolized by dog, monkey, baboon and man. Similar metabolic profiles were obtained for all species. Isolation and purification were accomplished by solvent extraction and chromatographic (column, gas and thin-layer) procedures. Gas chromatography, derivatization, infrared, nuclear magnetic resonance and particularly combined gas chromatography low and high resolution mass spectrometry techniques were employed to characterize the metabolites. The major urinary and plasma metabolites were identified as 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]-2-propanol and its glucuronide conjugate. Other metabolites characterized were: the *N*-glucuronide of MK-251; 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]propene; 2-nitro-2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]propane; α,α-dimethyl-4-(α,α,β,β-tetrafluorophenethyl)benzyl methyl ether; and 4-(α,α,β,β-tetrafluorophenethyl)acetophenone. The *O*-methyl ether metabolite represents the first instance of *in vivo* alkylation of a tertiary alcohol. Tentative identification was made for the *N*-hydroxy analog of MK-251 and for the glycol analog of 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]-2-propanol. The observed pharmacological response appears to result mainly from MK-251 and not from the four metabolites.

Introduction

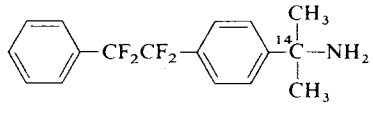
α,α-DIMETHYL-4(α,α,β,β-TETRAFLUOROPHENETHYL)BENZYLAMINE (MK-251), which is currently in clinical trial, has been found to prevent certain types of experimentally induced ventricular arrhythmias.[1] The synthesis and structure activity relationships of MK-251 and various analogs have been described.[2] A description of the antiarrhythmic, cardiovascular actions and hemodynamic safety of MK-251 in comparison with quinidine, lidocaine and procaine amide has been presented.[1,3] The physiological disposition[4,5] and method of analysis[6] of MK-251 have been reported previously. This report emphasizes the structural identification of the [^{14}C]MK-251 urinary and plasma metabolites obtained from various species using mass spectrometry, particularly on line gas chromatography high resolution mass spectrometry.

Experimental

ANIMAL STUDIES

In all studies [α ^{14}C]MK-251 was administered as the isethionate salt to fasted individuals or animals. All doses were calculated as the free base.

Metabolite isolation was performed on the urine or plasma obtained from animals and humans that received the following doses. Beagle dogs of either sex weighing 7–13 kg, female rhesus monkeys (3–4 kg) and female anubis baboons (4–5 kg) received the drug orally at a dose of 5 mg kg^{-1}. Six male human volunteers weighing 61–80 kg received 10 mg (17 μCi) of compound. Blood was sampled from the jugular, femoral or cephalic veins and immediately centrifuged to separate the plasma. Urine specimens were collected at 24 h intervals.



MK-251

INSTRUMENTATION

Gas chromatography

A Packard gas chromatograph model 7400 equipped with a flame ionization detector (f.i.d.), a ^{63}Ni electron capture detector (e.c.d.) and a proportional counter (p.c.) was used. Glass columns, 183 cm × 4 mm (i.d.), were prepared using the following material: Column A—2% PPE-20 on Chromosorb W-AW-DMCS (80/100 mesh, Supelco, Inc.); Column B—1% OV-17 on Supelcoport (80/100 mesh, Supelco, Inc.); and Column C—1% QF-1 on Chromosorb W-AW-DMCS (80/100 mesh, Applied Science Laboratories). Detector and injection port temperatures were operated

† Abbreviation: BSA = bis-(trimethylsilyl)acetamide.

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about 30 °C higher than column temperature. Carrier flow rates ranged from 45–86 ml min^{-1}.

Mass spectrometry

An LKB-9000S combined gas chromatograph mass spectrometer was used for the separation and identification of MK-251 and its metabolites. Samples were analyzed using probe or g.c. inlet systems. In g.c.m.s. analyses a 183 cm × 3 mm (i.d.) glass column containing 1% OV-17 on Supelcoport (80/100 mesh, Supelco, Inc., column D) or 2% PPE-20 on Chromosorb (W-AW-DMCS; Column E) was used. Spectra were obtained at an ionization energy of 20 and/or 70 eV with an accelerating voltage of 3.5 kV and a source temperature of 270 °C. Column operating conditions are stated in the text. The flash heater and separator temperatures were 30 °C higher than column temperature. The flow rate of the helium carrier gas was 30 ml min^{-1}.

All high resolution mass spectra ($m/\Delta m \approx 10\,000$) were obtained on an Associated Electrical Industries model MS-902 double focusing mass spectrometer under g.c.m.s. conditions with a DS-30 data system. The g.c. inlet operating parameters were as follows: 183 cm × 3 mm (i.d.) glass column packed with 1% OV-210 on 80/100 mesh Supelcoport. Column, source and injection port temperatures were 160, 250 and 270 °C, respectively. The carrier gas (He) flow rate was 30–33 ml min^{-1}. Total ion current profiles (Fig. 1) were obtained at 20 eV and 100 μamp trap current with automatic switching to 70 eV during data acquisition. Scan rates of 34 sec per decade were used in order to achieve on-line m.s. computer data acquisition. The reported measured mass values represent mean values obtained from four to six analyses for each of the metabolites and synthetic reference compounds.

Infrared spectrometry

Spectra were obtained on a Perkin–Elmer model 621 spectrometer with a beam condenser. All samples were analyzed by preparing the sample in a KBr pellet.

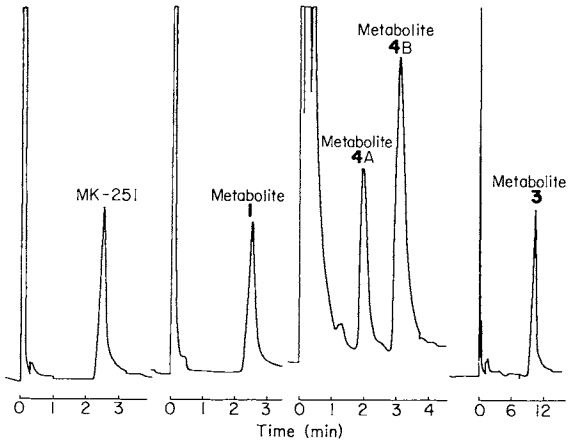


FIG. 1. Total ion current profiles for the isolated metabolites of MK-251. The metabolites were chromotographed on a 1% OV-210 column operated at 160 °C. In each instance the retention time of the synthetic material was identical to the respective metabolite.

Nuclear magnetic resonance

Spectra were measured in $CDCl_3$ or deuterated benzene on a Varian Associates HA-100 instrument equipped with a Fourier transform accessory.

Thin-layer chromatography

Analtech Silica Gel-G plates (0.25 mm thickness), which were prewashed before use by developing them in methanol + ethyl acetate (1 : 1), were employed. The chromatographic solvent systems used in the isolation steps are identical to those reported previously.[5]

The material which was applied to the plates at room temperature was eluted with methanol. All concentration steps were carried out under reduced pressure at a temperature of 45 °C or lower. Radioactive regions were located with a Packard radiochromatogram scanner model 7201 and/or by autoradiographic techniques following exposure of the plates to Medical X-ray film.

ISOLATION AND PURIFICATION OF METABOLITES

As a general method to show similarity between the metabolic profiles of the various species the following procedure was used. The pH of the urinary specimens was adjusted to 8 followed by extraction of the radioactive components into benzene and ethyl acetate. MK-251 was quantitatively extracted under these conditions. The organic phase was shaken with 0.1 N HCl to remove the basic components. The basic metabolites were subsequently reextracted into benzene at an alkaline pH. Initial thin-layer chromatography (t.l.c.) of the benzene extracts in solvent systems A and B indicated the presence of at least four radioactive fractions. The R_f values were similar for the various species.

Since the details of metabolite isolation have been reported previously,[5] only the salient aspects of the isolation procedures are stated below.

MONKEY AND BABOON URINE

The extraction pattern of urinary radioactivity was obtained before and after Ketodase treatment on pooled aliquots of 0–48 h monkey and baboon urines. Forty percent of the monkey urinary radioactivity was extracted into benzene at pH 8; this value increased to 67% after Ketodase. Similar data were obtained with the baboon urines. Overall, greater than 90% of the urinary radioactivity was extracted into benzene and ethyl acetate. In order to obtain sufficient quantities of metabolites for characterization, the entire pooled urine specimens from monkey and baboon were chromatographed separately on prewashed XAD-2 columns. The columns were eluted with water followed by methanol. Greater than 80% of the radioactivity was in the methanol fraction. This procedure was repeated to remove additional traces of extraneous salts. The methanol eluates (monkey and baboon) were concentrated to near dryness. The residue was dissolved in pH 8 buffer and then extracted with benzene

(5 times, 2 volumes). Following benzene extraction, the aqueous phase was adjusted to pH 5, treated with Ketodase and again extracted with benzene at pH 8. Overall, 65 to 70% of the baboon and monkey urinary radioactivity was extracted into benzene. Both the monkey and baboon benzene extracts (non-Ketodase) gave rise to five radioactive regions following t.l.c. using solvent system C at R_f values of 0.63, 0.56, 0.24, 0.14 and 0.04. Similar data were obtained from the extracts after Ketodase treatment; four radioactive regions at R_f values of 0.67, 0.31, 0.06 and 0.0—the major component at $R_f = 0.31$. Since the t.l.c. profiles of the monkey and baboon extracts were similar, the appropriate regions from the t.l.c. plates were combined, eluted and rechromatographed in the same system. Three regions were isolated; fraction 1 (0.0–0.12), fraction 3 (0.21–0.34) and fraction 5 (0.58–0.63).

Fraction 3 was further purified using t.l.c. techniques with solvent systems A, D, E, H, I and L. Only one radioactive peak was observed. Following final purification in solvent system H, the isolated metabolite was partitioned between heptane and water. Twenty-one mg of a white residue were obtained when the material in the heptane extract was subjected to a liquid chromatographic purification step on a silica gel column to remove traces of a yellow-colored component. This metabolite was labeled **1**.

Fraction 5, which did not show an increase in extractable radioactivity after Ketodase treatment, was chromatographed on t.l.c. plates in solvent systems E and J. Two radioactive regions were observed. The respective regions were eluted and rechromatographed in solvent systems J and H. The isolated lower radioactive fraction was labeled metabolite **4** and the upper fraction labeled metabolite **3**. Metabolite **3** was subjected to four additional t.l.c. purification steps using solvent systems E, J, H and K. Only one radioactive component was observed. Preliminary g.c. analysis indicated only one radioactive peak (~95% purity) and several trace components. Upon further purification using liquid chromatographic columns, 1 mg of a residue was obtained.

Metabolite **4** (see above) gave rise to only one radioactive peak upon t.l.c. analysis in several systems. The metabolite, when partitioned with heptane at pH 1, 6 and 11 remained quantitatively in the organic solvent. Upon liquid column purification on a silica gel G column, 2 mg of radioactive material were isolated.

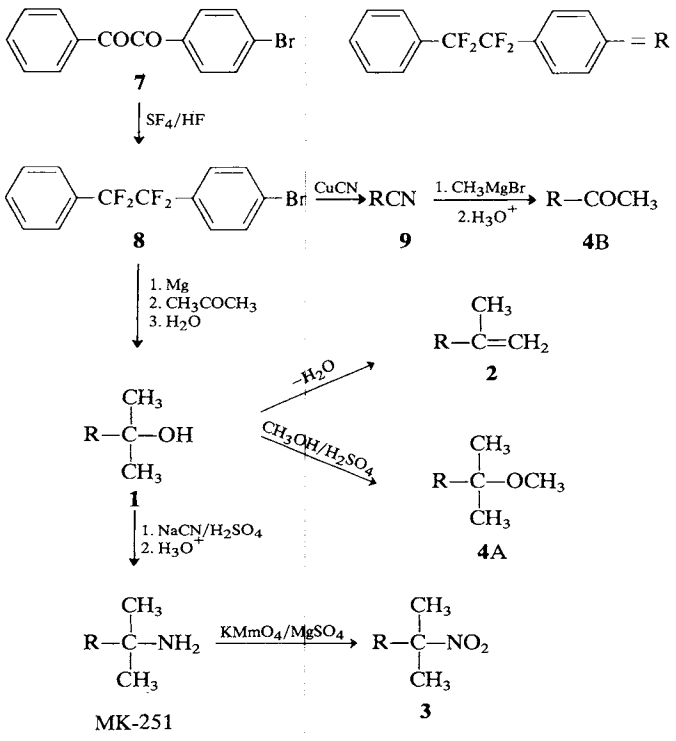
Fraction 1, which represented highly polar metabolites and was obtained after Ketodase treatment, afforded five metabolites ($\mathbf{5}_1$–$\mathbf{5}_5$) following t.l.c. purification in systems A, I and H and nine column chromatographic steps.

HUMAN URINE

Less than 1% of the dose was present as unchanged MK-251 in the 0–48 h urine specimens.[5] Metabolite isolation was performed only on the urine of one subject since preliminary t.l.c. data of the benzene extracts from each subject produced similar results. The procedure employed was similar to that described for monkey and baboon urine. Following XAD-2 column chromatography, 6% of the eluted radioactivity was extracted into benzene; an additional 66% was extracted following Ketodase treatment. Aliquots of the pooled benzene extract when analyzed by g.c.p.c. using column B (170 °C, 86 ml min^{-1}) gave rise to radioactive peaks which corresponded to the retention times of authentic MK-251, metabolites **1** and **3** and the *N*-acetyl analog of MK-251. Following derivatization of the extract with diazomethane, trifluoroacetic anhydride and/or bis-(trimethylsilyl)acetamide (BSA), other radioactive peaks were detected by the radioactivity monitor. Using the purification techniques of t.l.c., sample partitioning between organic and aqueous solvents at various pH values and column chromatography, eight radioactive metabolites were isolated, namely **6** and $\mathbf{6}_1$–$\mathbf{6}_7$.

SYNTHESIS OF METABOLITES

The synthetic reaction sequences for the metabolites are presented in Scheme 1. 2-[4-($\alpha,\alpha,\beta,\beta$-Tetrafluorophenethyl)phenyl]-propanol-2 (**1**) was an intermediate



SCHEME 1. Reaction sequences for the chemical synthesis of the metabolites.

in the synthesis of MK-251. It was obtained from the reaction of acetone with the Grignard reagent prepared under forcing conditions from 4-bromo-$\alpha,\alpha,\alpha',\alpha'$-tetrafluorobibenzyl (**8**). Compound **8** in turn resulted from the hydrogen fluoride-catalyzed reaction of the known 4-bromobenzil (**7**) with sulfur tetrafluoride. The facile acid-catalyzed dehydration of the

carbinol (**1**) yielded 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]-propene (**2**). α,α-Dimethyl-4-(α,α,β,β-tetra-fluorophenethyl)benzyl methyl ether (**4A**) was prepared in good yield by the sulfuric acid-catalyzed condensation of the carbinol (**1**) with methanol. The aryl bromide (**8**) was converted to the corresponding nitrile (**9**) with cuprous cyanide. Reaction of the nitrile (**9**) with methyl Grignard reagent followed by aqueous acid work-up afforded 4-(α,α,β,β-tetrafluorophenethyl)acetophenone (**4B**). MK-251 resulted from acid hydrolysis of the formamide obtained from the Ritter reaction of the carbinol. Oxidation of MK-251 with neutral permangate in aqueous acetone yielded 2-nitro-2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]propane (**3**).

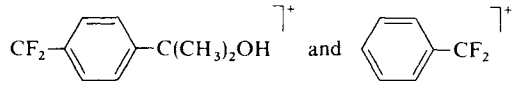
All of these compounds are crystalline and were characterized[2] by m.p., i.r. and n.m.r. spectra, and satisfactory analytical data (±0.4% for C, H, F and/or N).

Results and discussion

The mass spectrum of MK-251 (Table 1) serves as a model of the fragmentation pattern for the metabolites. An extremely weak molecular ion is observed at *m/e* 311 (~0.2% rel. int.) and is accompanied by a weak $[M-1]^+$ peak. The base peak occurs at *m/e* 296 and results from the loss of a methyl radical from the molecular ion. Similar observations have been noted with a number of other α,α-dimethyl substituted compounds in this series. The weak peak at *m/e* 183 results from the loss of $C_6H_5CF_2$ from the $[M-1]^+$ ion. The second most abundant ion (~20% rel. int. at *m/e* 169) is formed by the loss of the $C_6H_5CF_2$ radical from the base peak. This homolytic cleavage of the CF_2—CF_2 bond also results in the formation of the *m/e* 127 ion $[C_6H_5CF_2]^+$. Other ion identities are given in Table 1.

MONKEY AND BABOON METABOLITES

Preliminary mass spectral data obtained from a g.c.m.s. run of a crude sample of metabolite **1** indicated that the compound had an apparent molecular weight of 312, no nitrogen, and a functional group that reacted with trifluoroacetic anhydride to form a derivative that deacylated (loss of CF_3COOH) readily to the corresponding olefin (mol. wt. = 294). Based upon these data and the observed fragmentation pattern of the low resolution mass spectrum, a tertiary alcohol was postulated as the metabolite and a sample of synthetic material was prepared. Table 2 presents a comparison of the g.c. high resolution mass spectral data for the isolated metabolite **1** and the synthetic reference sample of the suspected carbinol metabolite, namely, 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]-2-propanol. The molecular weight was established as 312.1150 corresponding to an empirical formula of $C_{17}H_{16}OF_4$. The fragmentation pattern is consistent with this series of compounds. The base peak at *m/e* 297 results from the loss of a methyl radical from the molecular ion. The abundant ions at *m/e* 185 and 127 result from homolytic cleavage of the CF_2—CF_2 bond giving rise to the



species, respectively. The ion at *m/e* 167 is the same as that ion observed for MK-251. Other ion identities are presented in Table 2.

Upon derivatization with trifluoroacetic anhydride, the fragmentation patterns of the derivatives were identical for both the synthetic carbinol and metabolite **1**. A molecular ion was observed at 408 and the ions at 127, 167, 281 and 294 result from cleavages described for the underivatized sample giving rise to the following fragment ions: $[C_7H_5F_2]^+$, $[C_{10}H_9F_2]^+$,

TABLE 1. High resolution mass spectrum of MK-251

Measured *m/e*	Calculated *m/e*	Empirical formula	Relative intensity	Ion identity
—	311.1297	$C_{17}H_{17}NF_4$	0.2	$[M]^+$
310.1231	310.1219	$C_{17}H_{16}NF_4$	0.9	$[M-H]^+$
296.1070	296.1062	$C_{16}H_{14}NF_4$	100	$[M-CH_3]^+$
294.1009	294.1032	$C_{17}H_{14}F_4$	1.6	$[M-NH_3]^+$
292.1343	292.1313	$C_{17}H_{17}NF_3$	1.4	$[M-F]^+$
277.1070	277.1078	$C_{16}H_{14}NF_3$	2.1	$[296-F]^+$
258.1104	258.1094	$C_{16}H_{14}NF_2$	9.8	$[277-F]^+$
256.1047	256.1064	$C_{17}H_{14}F_2$	4.2	$[294-F_2]^+$
183.0855	183.0860	$C_{10}H_{11}NF_2$	1.4	$[310-\phi CF_2]^+$
169.0708	169.0703	$C_9H_9NF_2$	19.8	$[296-\phi CF_2]^+$
168.0657	168.0625	$C_9H_8NF_2$	3.0	$[169-H]^+$
167.0669	167.0672	$C_{10}H_9F_2$	3.9	$[183-NH_2]^+$
153.0484	153.0516	$C_9H_7F_2$	1.5	
141.0509	141.0516	$C_8H_7F_2$	2.8	
127.0360	127.0359	$C_7H_5F_2$	9.2	$[\phi CF_2]^+$
126.0279	126.0281	$C_7H_4F_2$	1.4	$[127-H]^+$
77.0382	77.0391	C_6H_5	2.4	$[\phi]^+$
58.0664	58.0657	C_3H_8N	11.5	$[(CH_3)_2NH_2]^+$
42.0356	42.0344	C_2H_4N	10.2	

TABLE 2. A comparison of the g.c. high resolution mass data for metabolite **1** and synthetic **1**

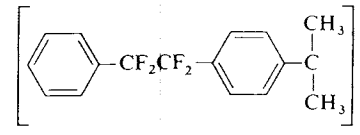
Empirical formula	Calculated *m/e*	Measured *m/e*		Ion identity
		Synthetic	Metabolite	
$C_{17}H_{16}OF_4$	312.1137	312.1115	312.1150	$[M]^+$
$C_{17}H_{15}OF_4$	311.1059	311.1069	311.1035	$[M-H]^+$
$C_{16}H_{13}OF_4$	297.0902	297.0899	297.0918	$[M-CH_3]^+$
$C_{16}H_{12}OF_4$	296.0824	296.0829	—	
$C_{17}H_{15}F_4$	295.1110	295.1091	295.1087	$[M-OH]^+$
$C_{17}H_{14}F_4$	294.1032	294.1034	294.1034	$[M-H_2O]^+$
$C_{17}H_{16}OF_3$	293.1153	293.1148	293.1149	$[M-F]^+$
$C_{17}H_{14}F_2$	256.1064	256.1062	256.1066	
$C_{17}H_{14}$	218.1096	218.1099	218.1092	
$C_{14}H_9F_2$	215.0672	215.0684	215.0674	
$C_{10}H_{11}OF_2$	185.0778	185.0779	185.0770	$[M-\phi CF_2]^+$
$C_9H_8OF_2$	170.0543	170.0540	170.0538	$[297-\phi CF_2]^+$
$C_{10}H_{11}F_2$	169.0829	169.0816	169.0783	
$C_9H_7OF_2$	169.0465	169.0462	169.0464	
$C_{10}H_9F_2$	167.0672	167.0673	167.0679	
$C_9H_5F_2$	151.0359	151.0362	151.0369	
$C_8H_7F_2$	141.0516	141.0516	141.0501	
$C_7H_5F_2$	127.0359	127.0358	127.0355	$[\phi CF_2]^+$
$C_7H_4F_2$	126.0281	126.0279	126.0279	$[127-H]^+$
C_7H_5	77.0391	77.0387	77.0387	$[\phi]^+$
C_3H_7O	59.0497	59.0498	59.0485	$[C(CH_3)_2OH]^+$
C_2H_3O	43.0184	43.0206	43.0203	

$[C_{12}H_{10}O_2F_5]^+$ (M-127) and $[C_{17}H_{14}F_4]^+$ (M−CF_3COOH), respectively.

The thin-layer chromatographic properties of the metabolite were identical in six solvent systems (A, D, E, H, I and L) with the properties of authentic material.

The n.m.r. spectrum of metabolite **1** was identical with authentic reference material. Replacement of the amino group by the hydroxy group results in a 0.07 ppm downfield shift of the geminal methyl signals and a barely detectable change in the position of the disubstituted ring protons (~0.01–0.02 ppm). Comparisons of the i.r. spectra of the metabolite with authentic carbinol, underivatized and as the trifluoroacetyl derivative, supported the identity of the metabolite.

Metabolite **3** was unequivocally characterized as the nitro analog of MK-251, namely, 2-nitro-2-[4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)phenyl]propane, by the following data. Upon g.c. analysis of the metabolite, one radioactive peak was detected at a retention time considerably longer than MK-251 (7.6 min vs 2.0 min) indicating a compound more polar than MK-251. All attempts to derivatize the metabolite with a variety of reagents were unsuccessful. Solvent partitioning with petroleum ether and aqueous phases at pH 1, 7 and 11 established that the metabolite was a neutral lipophilic molecule. The i.r. spectrum, when compared with spectra of related reference compounds, indicated that the nucleus was intact; however, a strong absorption band at 1540 cm^{-1} was observed, and is consistent with a sterically hindered nitro group. Mass spectral analysis confirmed that the nucleus



was intact; however, no molecular ion was observed at low resolution via direct probe or via the g.c. inlet. Under the assumption that the metabolite was the nitro analog, the metabolite was reduced with $SnCl_2$+HCl for 1 h at 80 °C. An extract of the reaction mixture when subjected to g.c. analysis gave rise to three peaks at T_r values of 2.0 (major), 2.8 (minor) and 6.0 (minor) min. Authentic MK-251 and metabolite **3** exhibited T_r values of 2.0 and 7.6 min, respectively. Mass spectral analysis of the 2.0 min component confirmed the identity as MK-251. These data give further support for the presence of the nitro group on the benzyl carbon. High resolution mass analysis of metabolite **3** (Table 3) established the empirical formula of the major fragment ions. An extremely weak molecular ion was observed; however, no mass measurements were possible. The intense peak at *m/e* 295 had the measured mass of 295.1119 corresponding to an empirical formula of $C_{17}H_{15}F_4$ $[M—NO_2]^+$; the calculated mass is 295.1110. These data give strong support for the structure of the metabolite. The proposed metabolite was chemically synthesized and used as a reference for comparison with the metabolite, using t.l.c., g.c., i.r. and m.s. analyses (Table 3). In all instances the results obtained with the metabolite were identical to those obtained with the synthetic material. The identities of the abundant ions observed following

TABLE 3. A comparison of the g.c. high resolution data for metabolite **3** and synthetic **3**

Empirical[a] formula	Calculated *m/e*	Measured *m/e*		Ion identity
		Synthetic	Metabolite	
$C_{17}H_{15}OF_4$	311.1059	311.1070	311.1075	$[M-NO]^+$
$C_{17}H_{15}F_4$	295.1110	295.1109	295.1119	$[M-NO_2]^+$
$C_{17}H_{14}F_4$	294.1032	294.1031	294.1041	$[M-HNO_2]^+$
$C_{17}H_{15}F_3$	276.1126	276.1114	276.1107	$[295-F]^+$
$C_{17}H_{14}F_3$	275.1048	275.1042	275.1044	
$C_{17}H_{14}F_2$	256.1064	256.1063	256.1063	$[275-F]^+$
$C_{14}H_9F_4$	215.0672	215.0674	215.0673	
$C_{10}H_{11}F_2$	169.0829	169.0820	169.0821	
$C_9H_7OF_2$	169.0465	169.0465	169.0463	
$C_{10}H_{10}F_2$	168.0751	168.0725	168.0724	$[295-\phi CF_2]^+$
$C_{10}H_9F_2$	167.0672	167.0671	167.0669	$[294-\phi CF_2]^+$
$C_9H_7F_2$	153.0516	153.0518	153.0514	
$C_9H_5F_2$	151.0359	151.0361	151.0355	
$C_7H_5F_2$	127.0359	127.0358	127.0358	$[\phi CF_2]^+$
$C_7H_4F_2$	126.0281	126.0278	126.0272	
C_6H_5	77.0391	77.0381	77.0390	$[\phi]^+$

[a] No molecular ion was observed; however, a peak at *m/e* 322 corresponding to M−F (322.1061) was observed upon probe analysis of both synthetic and metabolite **3**.

g.c. high resolution m.s. analysis are presented in Table 3.

The i.r. analysis of metabolite **4**, which was isolated and purified as described previously, suggested a mixture by the presence of both intact methyl group absorption (2960 and 2865 cm^{-1}) and the intense absorption at 1690 and 1740 cm^{-1} indicating a carbonyl function. The aromatic nucleus appeared to be intact. Subsequent g.c. analysis on columns B and C confirmed the presence of two radioactive metabolites. Low resolution mass data (g.c.m.s.) of the first component (metabolite **4**A) gave rise to ions at the following a.m.u. values: 326 (apparent molecular ion), 325, 311, 295, 199, 184, 169, 167 and 127. High resolution data for these ions are presented in Table 4. These data are consistent with the methoxy compound, namely, α,α-dimethyl-4-(α,α,β,β-tetrafluorophenethyl)benzyl methyl ether. The second component (metabolite **4**B) was identified as 4-(α,α,β,β-tetrafluorophenethyl)-acetophenone. The major fragment ions following g.c.m.s. of the metabolite, occurred at *m/e* 296, 281, 253, 169, 154, 141 and 127. The m.s. and g.c. characteristics of a synthetic sample were identical to those obtained for this metabolite. High resolution data (Table 5) for these ions give support for the structure.

The gas chromatogram for metabolite $\mathbf{5}_1$ showed two peaks when scanned for the drug-related *m/e* 127 ion or for radioactivity. The first component exhibited an apparent molecular ion at *m/e* 339; the ions in order of decreasing peak intensity occurred at the following a.m.u. values: 212 (100%), 296 (98.6%), 127 (87.6%), 169 (77.7%), 168 (66.5%), 324 (65.1%),

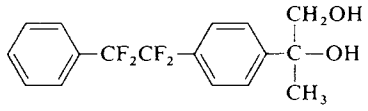
TABLE 4. A comparison of the g.c. high resolution data for metabolite **4**A and synthetic **4**A

Empirical formula	Calculated *m/e*	Measured *m/e*		Ion identity
		Synthetic	Metabolite	
$C_{18}H_{18}OF_4$	326.1294	326.1250	—	$[M]^+$
$C_{18}H_{17}OF_4$	325.1215	325.1254	325.1257	$[M-H]^+$
$C_{17}H_{15}OF_4$	311.1059	311.1047	311.1048	$[M-CH_3]^+$
$C_{18}H_{18}OF_3$	307.1310	307.1296	307.1319	$[M-F]^+$
$C_{17}H_{15}F_4$	295.1110	295.1112	295.1109	$[M-OCH_3]^+$
$C_{17}H_{14}F_4$	294.1032	294.1019	294.1027	
$C_{11}H_{13}OF_2$	199.0934	199.0924	199.0931	$[M-\phi CF_2]^+$
$C_{10}H_{10}OF_2$	184.0700	184.0702	184.0695	$[199-CH_3]^+$
$C_{10}H_{11}F_2$	169.0829	169.0827	169.0829	
$C_9H_7OF_2$	169.0465	169.0464	169.0463	
$C_{10}H_{10}F_2$	168.0751	168.0738	168.0737	
$C_{10}H_9F_2$	167.0627	167.0656	167.0674	$[199-HOCH_3]^+$
$C_9H_7F_2$	153.0516	153.0505	153.0512	
$C_8H_7F_2$	141.0516	141.0522	141.0516	
$C_7H_5F_2$	127.0359	127.0366	127.0357	$[\phi CF_2]^+$
$C_7H_4F_2$	126.0281	126.0289	126.0282	$[127-H]^+$
C_6H_5	77.0391	77.0403	77.0388	$[\phi]^+$

TABLE 5. A comparison of the g.c. high resolution data for metabolite 4B and synthetic 4B

Empirical formula	Calculated *m/e*	Measured *m/e*		Ion identity
		Synthetic	Metabolite	
$C_{16}H_{12}OF_4$	296.0824	296.0827	296.0828	$[M]^+$
$C_{15}H_9OF_4$	281.0589	281.0585	281.0580	$[M-CH_3]^+$
$C_{16}H_{12}OF_3$	277.0840	277.0843	277.0829	$[M-F]^+$
$C_{16}H_{12}OF_2$	258.0856	258.0862	258.0859	$[277-F]^+$
$C_{14}H_9F_4$	253.0640	253.0674	253.0651	$[M-COCH_3]^+$
$C_{15}H_9OF_2$	243.0621	243.0611	243.0621	$[258-CH_3]^+$
$C_{14}H_8F_2$	214.0594	214.0591	214.0579	
$C_{10}H_9OF_2$	183.0621	183.0609	183.0608	
$C_9H_7OF_2$	169.0465	169.0462	169.0458	$[M-\phi CF_2]^+$
$C_8H_4OF_2$	154.0230	154.0229	154.0228	$[169-CH_3]^+$
$C_8H_7F_2$	141.0516	141.0515	141.0515	
$C_7H_5F_2$	127.0359	127.0353	127.0351	$[\phi CF_2]^+$
$C_7H_4F_2$	126.0281	126.0279	126.0285	$[127-H]^+$
C_6H_5	77.0391	77.0379	77.0377	$[\phi]^+$
C_2H_3O	43.0184	43.0198	—	

167 (55.6%), 294 (46.7%), 339 (27.2%) and 297 (25.9%). This minor component is tentatively identified as the formamide derivative of MK-251 based upon direct comparison of the low resolution spectrum to that of authentic material. The formamide is the immediate precursor in the synthesis of MK-251. The second component gave rise to peaks at 328 (apparent molecular ion), 313, 297, 296, 185, 169 and 127. These data suggest a vicinal diol, namely



The characteristic losses of M−15, M−32 are observed. Insufficient material prevented high resolution analysis (see metabolite $\mathbf{5}_3$ below).

The basic metabolite $\mathbf{5}_2$ and its trifluoroacetyl derivative exhibited slightly longer retention times than MK-251 or its derivative on columns B and C. The i.r. spectrum was similar to the spectrum of MK-251 in the 400–1300 cm^{-1} region; however, the metabolite also gave rise to absorption at 1340, 1400, 1545, 1660, 1740 and 2350 cm^{-1} suggesting the presence of an oxygen atom. High resolution g.c.m.s. was performed; no apparent molecular ion was observed. The major fragment ions had the following empirical formula: 296 ($C_{16}H_{14}NF_4$; 100%), 184 ($C_{10}H_{12}NF_2$; 1.1%), 169 ($C_9H_9NF_2$; 43.6%), 167 ($C_{10}H_9F_2$; 22.5%), 153 ($C_9H_7F_2$; 2.5%), 141 ($C_8H_7F_2$; 4.6%) and 127 ($C_7H_5F_2$; 17.2%). The identity of this metabolite is not known. Insufficient material prevented characterization of this minor metabolite.

Metabolite $\mathbf{5}_3$, a neutral metabolite, exhibited a broad peak at 15 min on column B (180 °C, 86 ml min^{-1}). Reaction of the metabolite with diazomethane caused no change in T_r value; however, following trimethylsilylation with BSA two radioactive peaks were detected at retention times of 9.0 and 9.5 min. Derivatization with trifluoroacetic anhydride also gave rise to two peaks. These data suggest the presence of more than one polar functional group. Nuclear magnetic resonance analysis on the underivatized metabolite established that one of the methyl groups of MK-251 was present as the alcohol; it also established the presence of an OH, NHOH or NH_2 moiety on the benzylic carbon. Infrared data confirmed the above observations. Analysis of the derivatized metabolite (BSA) by g.c.m.s. gave rise to two drug-related peaks as indicated by the 127 ion. This was confirmed by g.c.p.c. analysis. The first component (minor) represented the addition of one TMSi group. The second component (major) exhibited an apparent $[M-15]^+$ ion at *m/e* 457. Other characteristic ions were observed at *m/e* 369 (base peak), 242, 191, 183, 167 and 127. The peak at *m/e* 369 represents the loss of CH_2OTMSi from the molecular ion. This same loss has been demonstrated for the glycol metabolite of pronethalol[7] and the hydroxymethyl metabolite of methsuximide.[8] The peaks at *m/e* 242 and 127 result from the homolytic cleavage of the CF_2—CF_2 bond. The presence of a vicinal diol structure is established by the characteristic peak at *m/e* 191. Other investigators[7–9] previously established the structure of this ion as $[(CH_3)_3—Si—O=CH—O—Si(CH_3)_3]^+$, and reported it as the base peak in the spectra of the derivatized dihydrodiol metabolites of phenylhydantoin,[10] phenobarbital,[11] mephobarbital,[11] methsuximide[8] and glutethimide.[12] The aforementioned data give strong evidence that metabolite $\mathbf{5}_3$ is 2-hydroxy-2-[4-$\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)phenyl]-propanol. Synthetic material was unavailable for direct comparison.

HUMAN METABOLITES

MK-251 is extensively metabolized in man, less than 1% of the dose (10–500 mg) being excreted unchanged

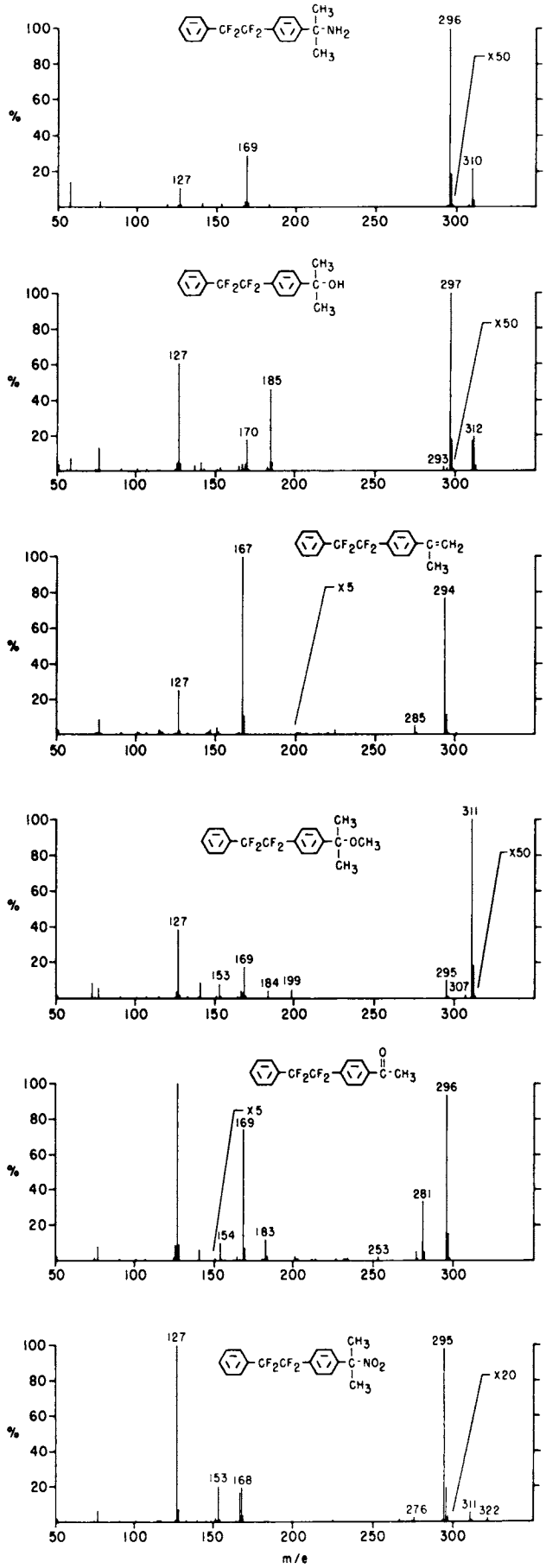
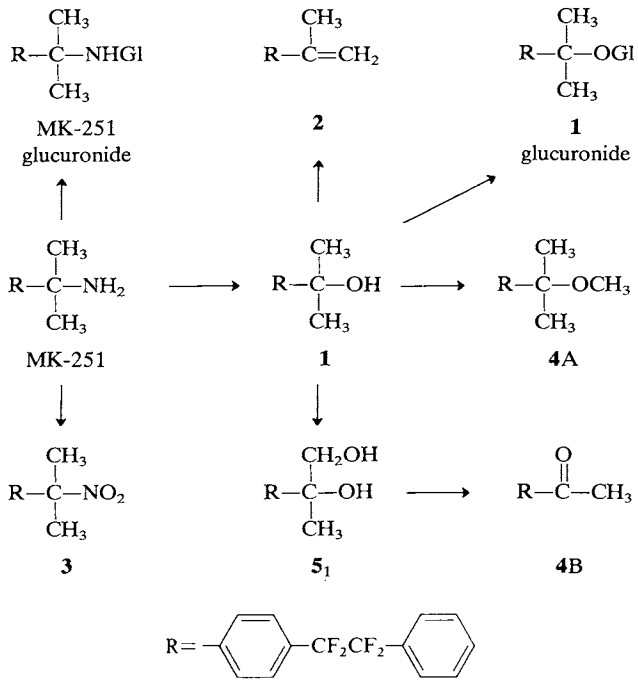


FIG. 2. Low resolution mass spectra of the metabolites isolated from human urine.

in the 0–48 h urine specimens.[5,6] The t.l.c. and g.c. characteristics of the isolated metabolites were similar to those obtained for the metabolites isolated from monkey and baboon urine. For sake of simplicity, only the mass spectral data for the isolated metabolites (see Experimental section) will be presented. In all instances, the g.c. and g.c.m.s. properties of the isolated metabolites (before and after derivatization) were identical to those of authentic reference material. Metabolite **6** was characterized as unchanged MK-251 based upon g.c., g.c.m.s. and derivatization properties. The low resolution spectrum (Fig. 2) was essentially identical to the data presented in Table 1. The mass spectra of metabolites $\mathbf{6}_1$, $\mathbf{6}_2$, $\mathbf{6}_3$ and $\mathbf{6}_4$ (Fig. 2) confirmed the identities of these metabolites as: $\mathbf{6}_1$, 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]-2-propanol; $\mathbf{6}_2$,4-(α,α,β,β-tetrafluorophenethyl)acetophenone;$\mathbf{6}_3$, 2-[4-(α,α,β,β-tetrafluorophenethyl)phenyl]-propene; and $\mathbf{6}_4$, 2-nitro-2-[4-(α,α,β,β-tetrafluorophenethyl)-phenyl]propane. Metabolite $\mathbf{6}_5$ is assigned the structure, α,α,-dimethyl-4-(α,α,β,β-tetrafluorophenethyl)-benzylhydroxylamine. The major fragment ions of the trimethylsilylated metabolite were observed at *m/e* values of 399 $[M]^+$, 384, 383, 368, 295, 294, 256, 194 and 127. Metabolite $\mathbf{6}_3$ may be an artifact of isolation since we have shown that under various g.c. conditions we can dehydrate the parent the carbinol metabolite to the corresponding olefin. Similar observations were made with the trifluoroacetyl derivative of metabolite $\mathbf{6}_1$.

In summary, the urinary metabolic profile of MK-251 is similar for the dog, monkey, baboon and man. The structures for the various metabolites are presented in Scheme 2.



SCHEME 2. Summary of the characterized urinary metabolites of MK-251.

Metabolites **1** and **2** have been detected in plasma samples from dog, monkey and man. Using techniques similar to those described, the major metabolite of MK-251 present in the urine and systemic circulation of dog, monkey, baboon and man was the carbinol analog of MK-251 (metabolite **1**) and its glucuronide conjugate. Little unchanged MK-251 (less than 15% of plasma radioactivity) was present in the plasma and a small percent as the *N*-glucuronide. To our knowledge the *O*-methyl ether metabolite represents the first reported instance of alkylation of a tertiary alcohol. Metabolites **1**, **2**, **3** and **4B** showed little activity when examined for their ability to modify or prevent the ventricular arrhythmia that is produced in dogs by the intracoronary injection of a sclerosing agent.

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