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Short Communication

Urinary Metabolites of Rimantadine in Humans

Rimantadine (1; table 1)[1] is an agent used in the treatment (1) and prophylaxis (2) of influenza caused by the type A strain of virus. Rimantadine, which has an asymmetric center and is given as the racemate, is an investigational drug in the United States but has been routinely prescribed for many years in the USSR (3). Without giving data, Van Voris *et al.* (3) and Hayden *et al.* (4) have both reported that rimantadine is extensively metabolized in man to all possible hydroxylated metabolites (**2a**, **2b**, **3**, **4a**, and **4b**; table 1). Suprisingly, amantadine (5), an antiviral agent structurally similar to rimantadine, is virtually unmetabolized when given to humans (5).

This communication reports the determination of the urinary metabolites generated after a 200-mg single oral dose of rimantadine·HCl, containing 0.25 μCi/mg of methyl-labeled [^{14}C] rimantadine, to four male volunteers. The [^{14}C]rimantadine was synthesized from adamantane-1-carbonyl chloride and [^{14}C] methyl cadmium by Dr. A. Liebman, Isotope Synthesis Group, Hoffmann-La Roche Inc. Conversion to rimantadine using standard procedures yielded a product having radiochemical purity >97% and a specific activity of 54.9 mCi/mmol.

Urine samples were collected from each subject for a minimum of 72 hr post-dose. Urine volumes were measured. Two tenths to 1 ml of urine were mixed with 10 ml Aquasol (New England Nuclear, Boston, MA) containing 1% glacial acetic acid, and radioactivity was measured on a Beckman Model LS 3801 counter using the external channel ratio technique. These measurements indicated that 88, 75, 93, and 80% of the radioactivity was excreted in 72 hr for subjects 1, 2, 3, and 4, respectively.

Eight ml of individual urine samples (or 8 ml of representative pooled urine samples) were adjusted to pH 5.5 with 2 N HCl and incubated at 37°C for 18 hr with 80 μl of Glusulase (Dupont Pharmaceuticals, Wilmington, DE). One half ml of 5 N NaOH was added to 2-ml samples (individual and pooled, with and without the Glusulase treatment), and the samples were extractively benzoylated to ensure that all of the rimantadine and metabolites were extracted in a stable form. The procedure involved treatment of 2 ml of urine with 8 ml of a solution of cyclohexane saturated with triethanolamine/chloroform (2:1), followed by 100 μl of cyclohexane containing 2% pentafluorobenzoyl chloride (6). Samples were shaken at 30 strokes min^{-1} for 20 min and centrifuged at 1500 g, and 7.5 ml of the top layer were transferred to 16-ml screw-capped culture tubes. The organic solvents were removed under a stream of N_2(g) at 50°C (N-EVAP; Organomation Associates, South Berlin, MA), and the residues were reconstituted in 160 μl of heptane/2-propanol (95:5). These solutions were transferred to WISP vials equipped with conical inserts and were analyzed by LC-radioactivity monitoring, LC-MS, and TLC-radioactivity monitoring.

The LC-radioactivity monitoring system consisted of two Waters M6000A pumps, a Waters WISP autoinjector, a Waters model 720 system controller (Water Associates, Millipore Division, Milford, MA), and a Flo-one/Beta Model IC Radioactivity Monitor (Radiomatic Instruments and Chemical Co., Inc., Tampa, FL) to detect and quantitate radioactivity in the HPLC eluent. The analytical column was a prepacked Zorbax silica, 4.6 mm × 25 cm (Dupont Instruments, Wilmington, DE). The mobile phase consisted of hexane/2-propanol (95:5) at a constant flow of 1.5 ml min^{-1} for 10 min, followed by a linear gradient to hexane/2-propanol (90:10) over 20 min with a 5-min reequilibration at initial conditions.

The LC-MS system consisted of an ISCO μLC-500 pump, a Rheodyne injector with a 5-μl loop, a 1 mm × 25 cm silica column (Brownlee Labs, Santa Clara, CA), and a modified Finnigan 1015 mass spectrometer equipped with a high speed pumping system (a Varian VHS-6 diffusion pump was used in the source region and a Varian VHS-4 diffusion pump was used in the analyzer region). The effluent from the LC was connected to the mass spectrometer using 36 in. of 60 μm (i.d.) flexible fused quartz tubing from SGE (Austin, TX). The last 18 in. of the quartz tubing were fit through 18 in. of stainless steel tubing heated by passage of an electrical current of 10 A at 5 V. The flow of hexane/2-propanol (90:10) was 125 μl min^{-1}. The ion source temperature was 300°C, and the mass spectrometer was tuned to give the maximum response consistent with reasonable peak shape and unit resolution. A hollow (0.25 in. i.d.) stainless steel tube connected at one end to a Balzer 18 m^3 hr^{-1} forepump was introduced into the high vacuum source region through a 0.5-in. solid probe inlet and was coupled inside the vacuum housing to the ion source to provide additional pumping.

The TLC plates (silica gel, product 60F-254; E. Merck, Darmstadt, FRG) were developed either in system 1 [chloroform/ethyl acetate/ethanol (70:30:1)] or system 2 [hexane/2-propanol (90:10)]. Radioactivity was detected using a Packard model 7201 radioactivity monitor.

Analysis of the experimental data showed that four distinct radioactive species were present, which corresponded to rimantadine and three chemically distinct metabolites. Fig. 1 shows the HPLC radiochromatogram containing the four radioactive peaks. The identity of each peak was discerned from the analysis of MS (LC-MS, EIMS,[2] and GC-CIMS) and NMR data.

Peak I (R_T 6.4 min). The material comprising the peak had an R_F of 0.6 in TLC system 1 and had chromatographic, mass spectral, and NMR properties identical to authentic derivatized rimantadine.

The LC-MS data from this peak (fig. 2) showed an MH^+ ion at m/z 374, the protonated molecular weight of derivatized intact rimantadine, and an ion of less intensity corresponding to the loss of HF (m/z 354). An EIMS (Vacuum Generators 7070) of

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[1] The proper nomenclature (IUPAC name) for all compounds listed in table 1 is as follows: **1**, α-methyltricyclo [3.3.1.1^{3,7}] decane-1β-methanamine; **5**, tricyclo [3.3.1.1^{3,7}] decan-1β-amine; **2a**, 1β-(1-aminoethyl)-tricyclo [3.3.1.1^{3,7}]decan-2α-ol; **2b**, 1β-(1-aminoethyl)-tricyclo [3.3.1.1^{3,7}]decan-2β-ol; **3**, 3α-(1-aminoethyl)-tricyclo [3.3.1.1^{3,7}]decan-1β-ol; **4a**, 1β-(1-aminoethyl)-tricyclo [3.3.1.1^{3,7}]decan-4α-ol; **4b**, 1β-(1-aminoethyl)-tricyclo [3.3.1.1^{3,7}]decan-4β-ol. Note that the absolute stereochemistry of the 1-aminoethyl substituent is not designated.

Send reprint requests to: Dr. E. K. Fukuda, Department of Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110.

[2] Abbreviations used are: EI, electron ionization; CI, chemical ionization.

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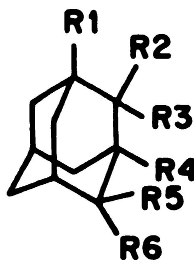
TABLE 1

Structures of rimantadine, rimantadine metabolites, and amantadine



Name	Designation	R1	R2	R3	R4	R5	R6
Rimantadine	**1**	$CH(CH_3)NH_2$	H	H	H	H	H
Amantadine	**5**	NH_2	H	H	H	H	H
See footnote 1	**2a**	$CH(CH_3)NH_2$	OH	H	H	H	H
See footnote 1	**2b**	$CH(CH_3)NH_2$	H	OH	H	H	H
See footnote 1	**3**	$CH(CH_3)NH_2$	H	H	OH	H	H
See footnote 1	**4a**	$CH(CH_3)NH_2$	H	H	H	H	OH
See footnote 1	**4b**	$CH(CH_3)NH_2$	H	H	H	OH	H

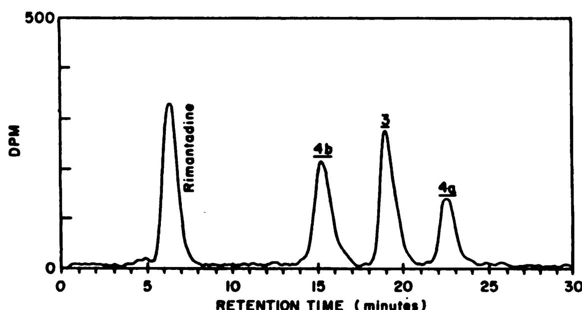


FIG. 1. *HPLC-radiochromatogram of extracted experimental urine after Glusulase treatment from a volunteer given radioactive rimantadine.*

an ethyl acetate extract of the TLC spot showed ions at m/z 373 ($M^{+\cdot}$, 2% of base peak), m/z 238 (C_6F_5-C(=0)NH-$\overset{+}{C}$H-CH_3, 1%), m/z 195 (C_6F_5-$\overset{+}{C}$=0, 15%), m/z 135 (adamantyl cation, 100%), m/z 107 (m/z 135 − C_2H_4, 7%), and m/z 93 (m/z 135 − C_3H_6, 15%). The formation of the latter three ions from the adamantyl cation has been described (7). A GC-CIMS analysis (Finnigan 1015, methane PCI at 0.5 torr, 4 feet by 2 mm glass column packed with 3% SP 2250 on 100–120 mesh GCQ at 215°C) gave peaks at m/z 374 (MH^+, 100%), m/z 372 (MH^+ − H_2, 13%), m/z 354 (MH^+ − HF, 6%), m/z 163 (adamantyl-$\overset{+}{C}$H-CH_3, 78%), and m/z 135 (adamantyl cation, 90%).

The NMR spectrum (Varian XL400, $CDCl_3$, 400 MHz, 27°C temperature, pulse width equivalent to a 40° flip angle, 2-sec pulse-repetition rate) of the material in the peak showed a partially resolved doublet at δ5.66 (1H, J = 10 Hz; N*H*), a partially resolved eight-peak pattern (doublet of quartets) centered at δ3.92 (1H, J = 7 and 10 Hz; HN-C*H*-CH_3), a singlet at δ2.00 (3H, R4 protons), a complex series of peaks at δ1.44 to δ1.72 (remaining methylene protons plus impurities), and a doublet centered at δ1.12 (*AX*, 3H, J = 8 Hz; HN-CH-C*H*$_3$). For reference, the δ1.2 to δ1.8 region in the NMR spectrum of authentic derivatized rimantadine consists of three singlets at δ1.68, δ1.58, and δ1.48 (6H; R2, and R3 protons) and a quartet centered at δ1.58 [*AB*, 6H, J = 36 Hz (geminal coupling); R5 and R6 protons].

Based on the above, the material comprising the peak was identified as derivatized rimantadine. In the four subjects, intact rimantadine accounted for 22 ± 8% of the dose (mean ± SD; table 2). Another 10 ± 3% of the dose was conjugated intact rimantadine (table 2).

Peak II (R_T 15.5 min). The material comprising this peak had an R_F of 0.3 in TLC system 1 and an R_F of 0.2 in TLC system 2 and had chromatographic, mass spectral, and NMR properties different than either derivatized authentic **3** obtained from Dr. P. Manchand, Hoffmann-La Roche (Nutley, NJ) or derivatized authentic **2a/2b** (mixture)[3] obtained from Dr. I. Sims, Hoffmann-La Roche (Nutley, NJ). Authentic **4a/4b** was not available. The purity of the pentafluorobenzoyl derivatives of authentic **3** and **2a/2b** were checked by TLC.

The LC-MS analysis of this peak (fig. 2) indicated that it was a hydroxylated rimantadine metabolite. An intense MH^+ − H_2O ion at m/z 372 and a very small MH^+ ion at m/z 390 (protonated molecular ion of derivatized hydroxylated rimantadine) were observed. An EI mass spectrum of an ethyl acetate extract of the TLC spot showed ions at m/z 389 ($M^{+\cdot}$, 3%), m/z 238 (C_6F_5 − C(=0)NH − $\overset{+}{C}$H-CH_3, 4%), m/z 195 (C_6F_5 − $\overset{+}{C}$ = 0, 20%), m/z 167 ($C_6F_5^+$, 5%), m/z 151 (hydroxy-adamantyl cation, 100%), m/z 133 (m/z 151 − H_2O, 20%), m/z 107 (m/z 133 − C_2H_2, 9%), m/z 105 (m/z 133 − C_2H_6, 10%), m/z 93 (m/z 133 −

[3] Properties of pentafluorobenzoylated **2a/2b** are as follows: R_F in TLC system 1 = 0.4, EIMS showed ions (>30% relative abundance) at m/z 371 ($M^{+\cdot}$-H_2O, 70% of base peak), m/z 239 (70%), m/z 221 (100%), and m/z 195 (60%). The GC-CIMS analysis (m/z >100 and >10% relative abundance) gave two chromatographic peaks. The first chromatographic peak gave mass spectral peaks at m/z 372 (100%), m/z 178 (12%), and m/z 161 (10%). The second chromatographic peak gave mass spectral peaks at m/z 372 (MH^+ − H_2O, 100%), m/z 222 (90%), m/z 178 (13%), m/z 169 (40%), and m/z 161 (25%). The NMR spectrum, reflecting the diastereomeric mixture, showed two partially resolved doublets centered at δ5.84 and δ6.8 (*AX*, 1H; N*H*), two partially resolved eight-peak patterns (doublet of quartet) centered at δ4.0 and δ4.31 (1H; HN-C*H*-CH_3), two singlets at δ3.74 and δ3.84 (*H*-C-OH), a complex series of peaks at δ1.4 to δ2.2 (remaining methylenes and the three bridgehead protons at δ1.93), and a complex series of peaks centered at δ1.22 (side chain methyl).

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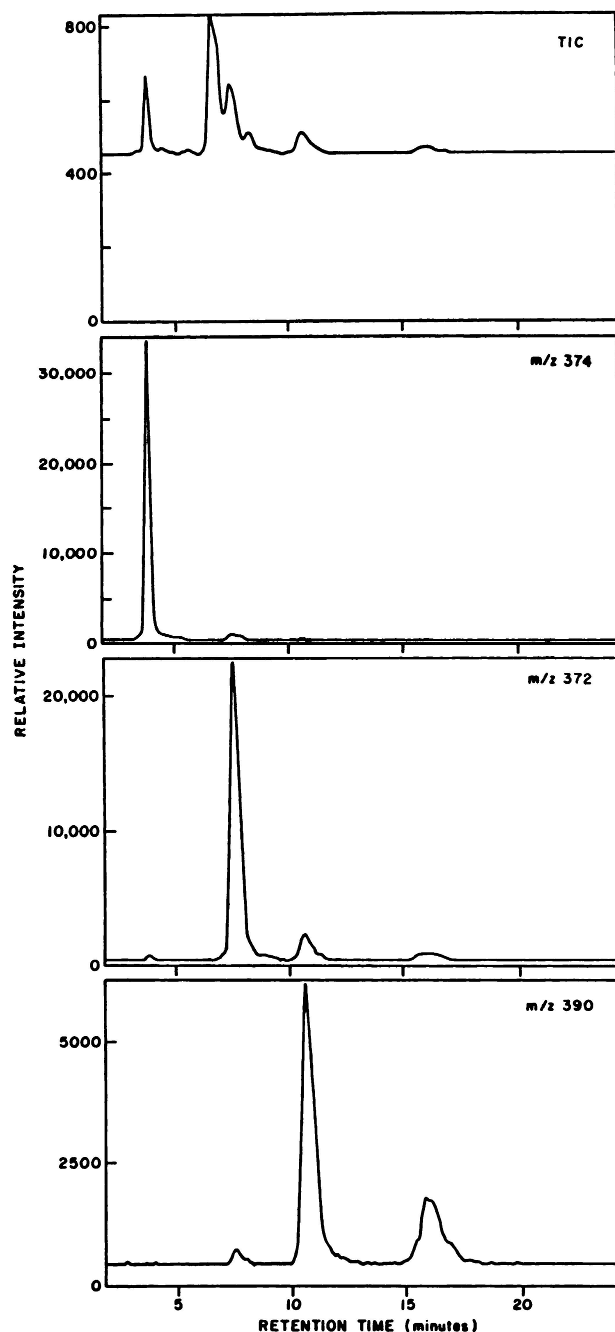

FIG. 2. *Total ion current (TIC) and reconstructed ion current profile tracings from the LC-MS analysis of a portion of an extractive pentafluorobenzoylation residue from urine of a volunteer administered rimantadine.*

C_3H_4, 9%), *m/z* 91 (*m/z* 133 − C_3H_6, 10%), *m/z* 81 (*m/z* 133 − C_4H_4, 10%), and *m/z* 79 (*m/z* 133 − C_4H_6, 8%). The GC-CIMS analysis gave peaks at *m/z* 390 (MH^+, 10%), *m/z* 372 (MH^+ − H_2O, 100%), *m/z* 195 (C_6F_5-$\overset{+}{C}$=O, 6%), *m/z* 179 (hydroxy adamantyl-$\overset{+}{C}$H-CH_3, 56%), *m/z* 161 (*m/z* 179 − H_2O, 82%), *m/z* 151 (hydroxy adamantyl cation, 98%), and *m/z* 133 (*m/z* 151 − H_2O, 20%).

The NMR spectrum of the material in the peak showed a partially resolved doublet at δ5.70 (1H; N*H*), a partially resolved eight-peak pattern centered at δ3.92 (1H; HN-C*H*-CH_3), a singlet at δ3.82 (1H; HO-C-*H*), a singlet at δ2.0 (3H; R4 protons), a complex series of peaks at δ1.2 to δ2.0 (remaining methylene protons plus impurities), and a doublet centered at δ1.14 (*AX*, 3H, J = 8 Hz; NH-CH-CH_3). Included in the complex series of peaks was a quartet centered at δ1.68 (*AB*, 4H, J = 12 Hz; R5 and R6 protons) and three doublets at δ1.96, δ1.88, and δ1.34 (*AB*, 2H, J = 12 Hz; R2 and R3 protons). The integrated signals for the bridgehead and methylene protons were less than expected, probably due to interferences from residual water and ethyl acetate resonances. Based on the above and the data that will be presented on peaks III and IV, the material comprising peak II was identified as **4b**. In the four subjects, this metabolite accounted for 13 ± 7% of the dose ~~(table 2).~~ Another 1.6 ± 0.5% of the dose was conjugated **4b**.

Peak III (R_T 19.2 min). The material comprising the peak had an R_F of 0.2 in TLC system 1 and had chromatographic, mass spectral, and NMR properties identical to derivatized authentic **3**.

The LC-MS analysis of this peak (fig. 2) indicated that it was a hydroxylated rimantadine metabolite. An intense MH^+ ion at *m/z* 390 and ions corresponding to [MH^+ − H_2O] and [MH^+-H_2-H_2O] at *m/z* 372 and *m/z* 370, respectively, of about a third the intensity of the MH^+ ion were observed. An EI mass spectrum of an ethyl acetate extract of the spot showed ions at *m/z* 389 ($M^{+\cdot}$, 2%), *m/z* 239 (C_6F_5-$\dot{C}$(OH)NH-$\overset{+}{C}$H-CH_3, 100%), *m/z* 220 (*m/z* 239 − F, 20%), *m/z* 195 (C_6F_5-$\overset{+}{C}$=O, 40%), *m/z* 167 ($C_6F_5^+$, 15%), *m/z* 151 (hydroxy adamantyl cation, 55%), *m/z* 133 (*m/z* 151 − H_2O, 30%), *m/z* 107 (*m/z* 151 − C_2H_4, 15%), *m/z* 93 (*m/z* 151 − C_3H_6, 35%), and *m/z* 79 (*m/z* 151 − C_4H_8, 19%). The intense ion at *m/z* 239 in the EI mass spectrum can be rationalized as the result of direct ionization at the hydroxy group with subsequent elimination of oxygenated adamantyl. Direct ionization would be made possible by the lower ionization potential of the tertiary hydroxy group (8) compared with a carbonyl adjacent to a strongly electron-withdrawing group. The GC-CIMS analysis gave peaks at *m/z* 390 (MH^+, 80%), *m/z* 372 (*m/z* 390 − H_2O, 80%), *m/z* 239 (C_6F_5-$\dot{C}$(OH)NH-$\overset{+}{C}$H-CH_3, 90%), *m/z* 179 (hydroxy adamantyl-$\overset{+}{C}$H(CH_3), 100%), *m/z* 161 (*m/z* 179 − H_2O, 55%), and *m/z* 151 (hydroxy adamantyl cation, 45%).

The NMR spectrum of the material in the peak showed a partially resolved doublet at δ5.68 (*AX*, 1H, J = 8 Hz; N*H*), a partially resolved eight-peak pattern centered at δ4.02 (1H; HN-C*H*-CH_3), a singlet at δ2.25 (2H; R4 protons), a complex series of peaks at δ1.4 to δ1.8 (remaining methylene protons plus impurities), and a doublet centered at δ1.15 (*AX*, 3H, 8 Hz; HN-CH-CH_3). Included in the complex series of peaks is a quartet centered at δ1.66 (*AB*, 4H, J = 12 Hz; R5 and R6 protons). An unresolved multiplet adjacent to the H_2O peak at δ = 1.45 was presumably due to four methylene protons. The remaining "missing" resonances due to another four methylene protons were presumed to be underneath the H_2O peak. NMR assignments were aided by comparison with authentic derivatized **3** and with the published NMR spectra of 1,3-disubstituted adamantanes (9).

Based on the above, the material comprising the peak was identified as derivatized **3**. In the four subjects, **3** accounted for

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TABLE 2

Urinary Excretion[a] of rimantadine and its metabolites in four healthy male volunteers

Subject	Rimantadine	3	4b	4a
		% of dose		
1	15.89 (10.70)[b]	14.26 (7.77)	17.60 (1.47)	6.56 (5.75)
2	33.40 (7.30)	5.23 (7.37)	2.09 (1.02)	0.87 (3.53)
3	21.12 (14.57)	12.43 (8.37)	16.61 (1.62)	5.51 (4.05)
4	17.94 (7.71)	12.27 (5.66)	16.59 (2.31)	5.63 (4.37)
Mean ± SD	22 ± 8 (10 ± 3)	11 ± 4 (7 ± 1)	13 ± 7 (1.6 ± 0.5)	4.6 ± 2.6 (4.4 ± 0.9)

[a] 0–72 hr post-dose.

[b] Number in parentheses is the additional percentage of the dose released on treatment with Glusulase.

11 ± 4% of the dose (table 2). Another 7 ± 1% of the dose was conjugated **3**.

Peak IV (R_T 22.7 min). The material comprising the peak had an R_F of 0.3 in system 1, an R_F of 0.1 in system 2, and chromatographic, mass spectral, and NMR properties different than either derivatized authentic **3** or derivatized authentic **2a/2b**.

The LC-MS analysis of this peak (fig. 2) indicated that it was hydroxylated rimantadine metabolite. An intense MH^+ ion at m/z 390 and less intense ions corresponding to the loss of H_2O (m/z 372) and H_2O and H_2 (m/z 370) were observed. An EI mass spectrum of an ethyl acetate extract of the spot showed ions at m/z 389 $M^{+\cdot}$, 5%), m/z 238 (C_6F_5-C(=O)NH-$\overset{+}{C}$H-CH_3, 15%), m/z 195 (C_6F_5 − $\overset{+}{C}$=O, 40%), m/z 167 ($C_6F_5^+$, 10%), m/z 151 (hydroxy adamantyl cation, 100%), m/z 133 (m/z 151 − H_2O, 80%), m/z 105 (m/z 133 − C_2H_4, 15%), m/z 91 (m/z 133 − C_3H_6, 40%), and m/z 79 (m/z 133 − C_4H_3, 20%). The GC-CIMS analysis gave peaks at m/z 390 (MH^+, 100%), m/z 372 (MH^+ − H_2O, 50%), m/z 195 (C_6F_5-$\overset{+}{C}$=O, 5%), m/z 179 (hydroxy adamantyl-$\overset{+}{C}$-CH_3, 70%), m/z 161 (m/z 179 − H_2O, 30%), m/z 151 (hydroxy adamantyl cation, 75%), and m/z 133 (m/z 151 − H_2O, 35%).

The NMR spectrum of the material in the peak showed a partially resolved doublet at δ5.65 (1H; N*H*), a partially resolved eight-peak pattern centered at δ3.98 (1H; HN-C*H*-CH_3), a singlet at δ3.82 (1H; HO-C-*H*), two doublets, centered at δ2.06 and δ1.45 (*AX*, 4H, 12 Hz; methylene protons), a singlet at δ1.96 (3H; R4 protons), a complex series of peaks at δ1.2 to δ1.8 (remaining methylene protons plus impurities), and a doublet centered at δ1.14 (3H; HN-CH-CH_3). The integrated signals for the methylene protons were less than expected, probably due to interferences from residual water and ethyl acetate resonances.

The EI and GC-CIMS data suggest that peak II and IV are both ring-hydroxylated metabolites. By elimination, they must be **4a** and **4b**. A striking difference between the epimers is their retention times. In the nonpolar HPLC solvent system used, peak II elutes over 7 min earlier than peak IV. A possible explanation for this is gained by inspecting molecular models. If peak II is **4b**, the hydroxyl proton can hydrogen bond to the carbonyl adjacent to the pentafluorophenyl ring, and the hydrogen-bonded species would be expected to be less polar than the nonhydrogen-bonded species. Such hydrogen bonding would not be possible with **4a**. Consistent with this assignment is the fact that the hydroxy metabolite comprising peak II loses water in the LC-MS and GC-MS analyses much more readily than that comprising peak IV. The hydrogen-bonded epimer would be expected to have a higher proton affinity than the nonhydrogen-bonded epimer (10), and protonation at this site will lead to water loss. For **4a**, protonation will occur predominately at the amide carbonyl, a site remote from the hydroxy. The NMR also supports the assignment. The **4b** isomer has a quartet (remaining R5 and R6 protons) centered at δ1.69. In the **4a** isomer, this quartet has collapsed to two separate doublets because of the chemical inequivalence of the R5 and R6 protons resulting from the interaction of the R6 protons with the R6 hydroxy (11). In addition, the substance comprising peak IV, with 4.6 ± 2.6% of the dose excreted intact (table 2) and 4.4 ± 0.9% of dose excreted conjugated (table 2), is conjugated to greater extent than that in peak II, which would be expected considering the greater steric crowding offered to the conjugating enzyme system by **4b** compared with **4a**.

No **2a/2b** metabolites were found, although the techniques used in this study were sufficiently sensitive to detect these metabolites at a level of 1% of the dose. This finding is contrary to that reported by Hayden and co-workers (3, 4). Although the introduction of an hydroxy substituent at the 2-position results in diastereomers that do have different chromatographic properties, no metabolite having identical chromatographic properties to authentic pentafluorobenzoyl **2a/2b** (R_F = 0.4, solvent system 1) was observed. In solvent system 1, the **2a** and **2b** diastereomers do not separate.

Conclusions. In summary, the urinary metabolites of rimantadine have been identified as conjugated and unconjugated rimantadine, **3**, **4a**, and **4b**. Contrary to previous reports, no **2a/2b** was found. In the 72-hr post-dose urine of four human volunteers, rimantadine and its metabolites account for a mean ± SD of 74 ± 10% of the dose.

Department of Drug Metabolism,
Hoffmann-LaRoche, Inc.

F. R. RUBIO
E. K. FUKUDA
W. A. GARLAND

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