EXCRETION AND METABOLISM OF THE ANTIHYPERTENSIVE AGENT, RWJ-26240 (MCN-5691) IN DOGS

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ABSTRACT:
The excretion and metabolism of a 2-ethynylbenzenealkanamine analog, antihypertensive RWJ-26240 (McN-5691), in beagle dogs was investigated. Recoveries of total radioactivity in urine and feces in the 7 days after oral administration of 14C-RWJ-26240 (6 mg/kg dose) were 2.8% and 96.8% of the radioactive dose, respectively. Representative plasma, urine, and fecal samples were pooled and purified for metabolite profiling, isolation, and identification. Unchanged RWJ-26240 (<19% of the dose) plus 12 metabolites were isolated and identified from these samples using chromatography (TLC, HPLC), spectroscopy (NMR, MS), and derivatization techniques. Unchanged RWJ-26240 plus identified metabolites accounted for >75% of the sample radioactivity in plasma and feces. The formation of RWJ-26240 metabolites can be depicted by the following proposed pathways: 1) N-dealkylation, 2) O-demethylation, 3) phenyl hydroxylation, and 4) N-dealkylation. The first three pathways appeared to be quantitatively important. Steps which led to the production of four major metabolites (each >5% of the sample radioactivity). RWJ-26240 was extensively metabolized in the dog, and fecal excretion was the major route of elimination of RWJ-26240 and its metabolites.

The three main chemical classifications of calcium channel antagonists are 1,4-dihydropyridines such as nifedipine, benzothiazepines such as diltiazem, and arylalkylamines such as verapamil (Fleckenstein, 1983; Fleckenstein, 1984; Bean, 1984; TA-3090, 1988). RWJ-26240 (McN-5691), a 2-ethylbenzenealkanamine which was synthesized at The R.W. Johnson Pharmaceutical Research Institute, is a member of the arylalkylamine class (McN-5691, 1989; McN-5691, 1992; Carson et al., 1987). RWJ-26240 exerted antihypertensive activity in hypertensive rats and dogs, and coronary vasodilating activity with minimal negative inotropic activity in the Langendorff guinea pig heart (Carson and McNeil Lab, Inc., 1987; Brannan et al., 1986; Flaim et al., 1988; Flaim et al., 1991). Preliminary results from our laboratory on the disposition and metabolism of RWJ-26240 in Wistar rats and beagle dogs have been reported (Wu et al., 1991; Ng et al., 1987).

RWJ-26240 was extensively metabolized and excreted largely in feces in dogs and rats. Twelve metabolites of RWJ-26240 were identified in dogs and rats. Verapamil (Calan, G.D. Searle, Chicago, IL), an arylalkylamine analog, structurally similar to RWJ-26240, is a coronary vasodilator. It is rapidly metabolized and eliminated largely in feces of rats and dogs (McHlenny, 1971). Seven metabolites, four N/O-desmethyl and three oxidative N-dealkylated metabolites, were produced via three major pathways, O-demethylation, N-demethylation, and oxidative N-dealkylation. In comparison, both RWJ-26240 and verapamil were rapidly excreted and extensively metabolized in dogs and rats forming similar types of metabolites.

This paper describes the excretion, isolation, and identification of unchanged RWJ-26240 and 12 metabolites and the proposed metabolic pathways of RWJ-26240 in plasma, urine, and feces from dogs after oral administration of 14C-RWJ-26240.

Materials and Methods

Chemicals and Materials. 14C-RWJ-26240. 14C-[2-(3,4-dimethoxyphenyl)ethyl]-5-methoxy-N,a-dimethyl-2-(phenylethynyl)benzenepranamine was synthesized at The R.W. Johnson Pharmaceutical Research Institute (Spring House, PA) with a radiochemical purity >97% (radio TLC and HPLC). (Weaner, unpublished results). The compound was used at a specific activity of 1.6 μCi/mg for the dog. RWJ-36539 (M-1), RWJ-36552 (M-4), RWJ-37086 (M-2), and RWJ-37087 (M-8) (fig. 1) were also synthesized at The R.W. Johnson Pharmaceutical Research Institute (Carson et al., 1988; Carson and McNeil Lab, Inc., 1987; Brannan et al., 1986; Carson, unpublished results). Homoveratic acid, Diazald, and N-ethyl-N-nitro-N-nitrosoguanidine were purchased from Aldrich Chemical (Milwaukee, WI). Glusulase was obtained from Endo Laboratories (Wilmington, DE). Formula-989 (scintillation solution) was purchased from New England Nuclear (Boston, MA). HPLC grade solvents were obtained from Fisher Scientific (Fair Lawn, NJ), and glass distilled solvents were purchased from Burdick and Jackson Laboratories (Muskegon, MI).

Animal Experiments. 14C-RWJ-26240 was administered by gavage to male and female beagle dogs (3 of each sex, weight 10.2–12.8 kg, Charles River Breeding Laboratory, Kingston, NY) as a single 6 mg/kg (as free base in corn oil) dose. Plasma samples were obtained for 24 hours after dosing. Urine and fecal samples were collected over a 7-day period. Each collected sample was assayed for total radioactivity and analyzed by TLC and HPLC.

Analytical Equipment. Total radioactivity in each sample was determined in a Tracor Analytic 81 liquid scintillation counter (Tracor Instruments, Lebanon, NJ). Thin-layer radiochemical analyses were performed using 5 × 20 cm silica gel GF plates (250 micron gel thickness, Analtech, Inc., Newark, DE). TLC plates were analyzed using a Radiomatic chromatograph Imaging System (BID 100) (Bioscan, Inc., Washington, DC). The HPLC was used as a CRT-based gradient liquid chromatograph (Beckman Instrument Co., Fullerton, CA) connected to a radiactive flow detector (Ramona) (DNUS Service Corp, Fairfield, New Jersey).
NJ). LiChrosorb RP-18 Hibar guard and analytical columns (10 micron, 25 cm × 4.6 mm i.d.) were used. Electron-impact mass spectra were obtained in a VG7035 (VG Micromass, Manchester, UK). Operating conditions for the spectrometer included: source temperature at 220°C; ionization potential at 70 eV; multiplier voltage at 1.8 KeV; electrometer setting at 10⁻² A/V; mass ranges: 18–1000 m/z at 1 sec/decade. Chemical-ionization mass spectra were obtained in a Finnigan Model 3300/6100 (Finnigan, Inc., Sunnyvale, CA) by direct inlet. The mass spectrometer was operated at an electron energy of 100 eV (CI), source temperature of 100°C and reagent gas source pressure of 1000 microns for methane. NMR spectra were determined in deuteroacetonitrile or deuterochloroform in a Bruker Model WM360 (Bruker Instrument, Inc., Billerica, MA).

**Sample preparation.** Prior to chromatographic (TLC and HPLC) analyses, it was necessary to purify most of the samples. Plasma samples (1, 4, 1–24

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**Fig. 1.** Structures and a summary of mass spectrometric data for RWJ-26240 and its metabolites.
hour, 10 ml each) were each extracted with ethyl acetate, concentrated by evaporation, precipitated with acetonitrile, and centrifuged. The per cent of radioactive sample recovered after purification was >83%. The fecal extract (50 ml, 0–48 hour pool, 72.9% of the radioactive dose) was evaporated to dryness, reconstituted with methanol, and the precipitate was removed by filtration. The per cent of sample radioactivity recovered after purification was 100%. The urine samples (50 ml, 0–24 hour pool, before and after Glusulase hydrolysis 2.0% of the radioactive dose) were prepared by treating aliquots from the urine pool in the following manner: Each urine
 aliquot was adjusted to pH 5.1 with glacial acetic acid and buffered with 1M sodium acetate solution (5 ml). Glusulase (1% by volume) was added to this mixture and allowed to react overnight at 37 °C. The per cent of sample radioactivity recovered after purification was 98.5%.

**Sample Analysis/Total Radioactivity.** Feces from day 1 to day 7 were extracted twice with approximately three volumes of methanol and filtered. The fecal residue samples were combusted in a Packard Tri-carb Model 306 sample oxidizer with a Permafluor V/Carbo-Sorb II (1:1, v/v) mixture to trap and count the 14CO2. Duplicate aliquots of plasma (0.1 ml), untreated (day 1 to day 7) and Glusulase-treated (day 1) urine (0.1 to 1 ml), fecal extracts (0.1
to 1 ml) and purified metabolites in methanol were assayed directly for total radioactivity in 10 ml of Formula-989\(^*\) by liquid scintillation spectrometry. The fecal extract (0–48 hour pool) and urine (0–24 hour) were used for metabolite isolation and identification.

**TLC.** Thin-layer radiochromatographic analysis of the samples was performed using an acidic solvent system, chloroform/methanol/glacial acetic acid (55/5/5, v/v/v) and a basic solvent system, methylene chloride/methanol/ammonium hydroxide (90/5/0.5, v/v/v). Each TLC plate was developed to a height of 14 cm above the spot or band where the sample was originally applied. Each sample was cochromatographed with RWJ-26240, RWJ-36359 (M-1), and RWJ-37086 (M-2) as reference compounds and visualized with short wavelength ultraviolet light after each plate was air dried. Each TLC was analyzed by radiochromatogram imaging.

**HPLC.** Each HPLC analysis was performed at 280 nm using the following conditions: The mobile phases of gradient elution used for each 50–100 thin-layer radiochromatogram and radioactive counts.

**Metabolite Isolation and Identification.** After sample purification, individual metabolites were isolated using HPLC peak collection methods. Unresolved HPLC metabolite peaks were further separated using TLC zone scraping methods. The TLC and HPLC procedures were identical to those used in metabolite profiling. All metabolites were characterized by desorption CI (CH\(_4\)) mass spectrometry. Mass spectra of the isolated metabolite to be represented by each metabolite, along with the per cent of radioactive component (10% of the radioactive dose) (table 2). The CI-MS data representative of RWJ-26240 (table 1). TLC biotransformation profiles in the basic solvent system (fig. 2) were obtained for three sample pools: plasma, urine, and fecal extract. An HPLC biotransformation profile (fig. 3) was obtained for the fecal extract pool which revealed most of the identified metabolites. EI-MS of RWJ-26240 and its metabolites revealed weak molecular ions; however, their CI-MS (methane) exhibited intense protonated molecular ions along with prominent fragmentations. Unchanged RWJ-26240 was detected in trace amounts (<0.1% of the radioactive dose) in urine and accounted for less than 36% of the radioactive dose at 0–48 hour (table 2). Identified metabolites and the per cent of sample radioactivity expressed in three structural classes: RWJ-26240 analogs (M-1 to M-10), diphenylacetylene analogs (M-11) and homoveratric acid (M-12). The per cent of sample radioactivity represented by each metabolite, along with the per cent of radioactive dose values, are summarized in table 2. Identified metabolites and RWJ-26240 accounted for greater than 75% of the radioactive dose in plasma and fecal samples (table 2). Isolation and identification of unchanged RWJ-26240 and individual metabolites are described below.

**Unchanged RWJ-26240** was observed in significant amounts in plasma (36% of the sample radioactivity at 4 hour) and feces (19% of the radioactive dose at 0–48 hour) (table 2). It was identified on the basis of TLC, HPLC, MS, and NMR data compared with those of an authentic sample (Carson et al., 1988). The CI-MS spectrum and \(^1\)H NMR spectrum of the isolated RWJ-26240 are presented in fig. 1 and table 3, respectively.

**M-1** was isolated from the plasma and fecal extract using HPLC and TLC. The CI-MS (fig. 1) revealed an apparent protonated molecular ion at m/z 444 (14 amu less than RWJ-26240) along with prominent fragment ions at m/z 292, 165, and 151, indicating the isolated metabolite to be N-desmethyl RWJ-26240. The representative mass spectra for CI and EI of M-1 are presented in fig. 4. The \(^1\)H NMR spectrum (table 3) exhibited no resonance signal for N-methyl at 62.20. **M-1** was confirmed as N-desmethyl RWJ-26240 by comparison of TLC, NMR, and MS of the isolated material with those of a synthetic sample (RWJ-36359) (Carson et al., 1988; Carson, unpublished results). **M-1** was found in significant amounts (16% of the sample radioactivity) in plasma only (table 2).

**M-2** was isolated by HPLC and TLC from fecal extract as a major component (10% of the radioactive dose) (table 2). The CI-MS data

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**TABLE 1**

Percentages of administered dose excreted in urine and feces from male and female dogs after a single oral dose of \(^{14}\)C-RWJ-26240 (6 mg/kg)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Males (% Dose)</th>
<th>Females (% Dose)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>2.33</td>
<td>2.29</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>24–48</td>
<td>0.54</td>
<td>0.41</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>48–72</td>
<td>0.19</td>
<td>0.17</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>72–96</td>
<td>0.11</td>
<td>0.13</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>96–168</td>
<td>0.06</td>
<td>0.06</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Total (0–168)</td>
<td>3.23</td>
<td>3.06</td>
<td>2.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.17</td>
<td>2.15</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.39</td>
<td>0.67</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.28</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.14</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.09</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.08</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.90</td>
<td>3.33</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.81</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**RESULTS**

A total of 96.8% and 2.8% of the radioactive dose was excreted in feces and urine, respectively, during the 7 days after oral administration of \(^{14}\)C-RWJ-26240 (table 1). TLC biotransformation profiles in the basic solvent system (fig. 2) were obtained for three sample pools: plasma, urine, and fecal extract. An HPLC biotransformation profile (fig. 3) was obtained for the fecal extract pool which revealed most of the identified metabolites. EI-MS of RWJ-26240 and its metabolites revealed weak molecular ions; however, their CI-MS (methane) exhibited intense protonated molecular ions along with prominent fragmentations. Unchanged RWJ-26240 was detected in trace amounts (<0.1% of the radioactive dose) in urine and accounted for less than 36% of the radioactive sample and 19% of the radioactive dose in plasma and fecal extract, respectively. RWJ-26240 plus 12 metabolites were isolated, quantified, and identified (fig. 1, table 2). Their structures and mass spectra data are presented in fig. 1. RWJ-26240 metabolites can be categorized into three structural classes: RWJ-26240 analogs (M-1 to M-10), diphenylacetylene analogs (M-11) and homoveratric acid (M-12). The per cent of sample radioactivity represented by each metabolite, along with the per cent of radioactive dose values, are summarized in table 2. Identified metabolites and RWJ-26240 accounted for greater than 75% of the radioactive dose in plasma and fecal samples (table 2). Isolation and identification of unchanged RWJ-26240 and individual metabolites are described below.

Unchanged RWJ-26240 was observed in significant amounts in plasma (36% of the sample radioactivity at 4 hour) and feces (19% of the radioactive dose at 0–48 hour) (table 2). It was identified on the basis of TLC, HPLC, MS, and NMR data compared with those of an authentic sample (Carson et al., 1988). The CI-MS spectrum and \(^1\)H NMR spectrum of the isolated RWJ-26240 are presented in fig. 1 and table 3, respectively.

M-1 was isolated from the plasma and fecal extract using HPLC and TLC. The CI-MS (fig. 1) revealed an apparent protonated molecular ion at m/z 444 (14 amu less than RWJ-26240) along with prominent fragment ions at m/z 292, 165, and 151, indicating the isolated metabolite to be N-desmethyl RWJ-26240. The representative mass spectra for CI and EI of M-1 are presented in fig. 4. The \(^1\)H NMR spectrum (table 3) exhibited no resonance signal for N-methyl at 62.20. M-1 was confirmed as N-desmethyl RWJ-26240 by comparison of TLC, NMR, and MS of the isolated material with those of a synthetic sample (RWJ-36359) (Carson et al., 1988; Carson, unpublished results). M-1 was found in significant amounts (16% of the sample radioactivity) in plasma only (table 2). M-2 was isolated by HPLC and TLC from fecal extract as a major component (10% of the radioactive dose) (table 2). The CI-MS data
indicated an intense protonated molecular ion at \( m/z \) 444, along with prominent fragment ions at \( m/z \) 292, 222, 165, and 151 (fig. 1), which suggested that \( M-2 \) is 5-O-desmethyl RWJ-26240. The \( ^1\)H NMR spectrum of \( M-2 \) (table 3) revealed a lack of a O-methyl group at the 5-position of RWJ-26240. The metabolite was derivatized separately with diazomethane and diazoethane. The CI-MS and \( ^1\)H NMR of the methylated derivative was identical to those of RWJ-26240 (fig. 1). The CI-MS and \( ^1\)H NMR data for \( M-2 \) ethyl ether are presented in fig. 1 and table 3. \( M-2 \) was confirmed as O-desmethyl RWJ-26240 by comparison of TLC, HPLC, NMR, and MS comparison with a synthetic sample (RWJ-37086) (Carson et al., 1988; Carson, unpublished results). \( M-2 \) further formed as a conjugate in urine (fig. 2).

\( M-3 \) was isolated in minor quantities (<5% of the radioactive dose) from feces. The CI-MS displayed a protonated molecular ion at \( m/z \) 460 together with diagnostic fragment ions at \( m/z \) 308, 165, and 151, which indicated that the minor metabolite was a hydroxylated N-desmethyl RWJ-26240 (fig. 1). \( M-3 \) was derivatized individually with diazomethane and diazoethane. The CI-MS of the methylated derivative provided an intense protonated molecular ion at \( m/z \) 474 (fig. 1). Informative fragment ions at \( m/z \) 322, 208, 165, and 151 suggested the

![TLC biotransformation profiles of dog plasma, urine, and fecal extract samples.](image-url)
loss of an N-methyl group and the appearance of an O-methyl group in the diphenylacetylene moiety (fig. 1). The CI-MS and $^1$H NMR data of M-3 ethyl ether are presented in fig. 1 and table 3, respectively. The NMR data shows a pattern of AA$^9$BB$^9$ quartet resonances at $d_{6.88}$ and $d_{7.41}$ and confirmed the 4-hydroxyphenyl ethynyl substitution of this metabolite.

M-4 was isolated in significant amounts (6% of the radioactive dose) from the fecal extract (table 2) and detected in trace amounts in the plasma and urine on the basis of TLC data. The direct CI-MS (fig. 1) analysis of the isolated compound provided two apparent adduct molecular ions at m/z 472 (MC$_5$H$_{11}$) and m/z 444 (MH$^+$), along with several diagnostic fragments at m/z 306, 208, 151, and 137. The MS data indicated that the metabolite is an O-desmethyl metabolite of RWJ-26240. The $^1$H NMR spectrum of M-4 is presented in table 3, which indicated a lack of a 4-methoxy group by comparison of the chromatographic (TLC, HPLC) and spectroscopic data (NMR, MS) of the isolated component with those of a synthetic sample, RWJ-36552 (Carson et al., 1988; Carson, unpublished results). M-4 was further derivatized as methyl and ethyl ethers. Their MS and NMR data are shown in fig. 1 and table 3, respectively. M-4 methyl ether was identical to RWJ-26240.

M-5 was isolated in small amounts (<5% of the radioactive dose) from the fecal extract (table 2). The CI-MS of the isolated metabolite showed an intense protonated molecular ion at m/z 474, along with several prominent fragment ions at m/z 322, 222, 165, and 151 (fig. 1), which indicated the addition of a hydroxy group to the diphenylacetylene moiety. Treatment of M-5 with diazomethane led to the formation of a methyl ether which gave an intense protonated molecular ion at m/z 488 and informative fragment ions in CI-MS (fig. 1). M-5 was also derivatized as an ethyl ether with diazoethane. The CI-MS data suggested the structure of metabolite 5 ethyl ether (fig. 1). The $^1$H NMR data (table 3) of metabolite 5 ethyl ether indicated addition of an O-ethyl group at $d_{4.00}$ (q. O-CH$_2$CH$_3$) and $d_{1.35}$ (t.}

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**FIG. 3.** HPLC biotransformation profile of dog fecal extract (0–48 hours).

**TABLE 2**

<table>
<thead>
<tr>
<th>Sample Content</th>
<th>Plasma (4-hr)</th>
<th>Urine (0–24 hr)</th>
<th>Fecal Extract (0–48 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2% Dose</td>
<td>73% Dose</td>
</tr>
<tr>
<td>Sample</td>
<td>Dose</td>
<td>Sample</td>
<td>Dose</td>
</tr>
<tr>
<td>I: RWJ-26240</td>
<td>36</td>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td>M-1 (RWJ-36359)</td>
<td>16</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>M-2 (RWJ-37086)</td>
<td>20</td>
<td>30</td>
<td>0.6</td>
</tr>
<tr>
<td>M-3</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>M-4 (RWJ-36552)</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>M-5</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>M-6</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>M-7</td>
<td>–</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>M-8 (RWJ-37087)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-9</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>M-10</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>M-11</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>M-12 (homoveratric acid)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* + presents at <5% of total $^{14}C$ in sample.
  – none detected.
O-CH$_2$CH$_3$) and a lack of a 4-H resulted in the formation of a singlet resonance at 6.99 from 3-H and 6-H. Therefore, 4-hydroxyphenyl RWJ-26240 was assigned for the structure of M-5.

M-6 was isolated in minor quantities (<5% of the radioactive dose) from fecal extract (table 2). The CI-MS analysis of the isolated metabolite exhibited an intense protonated molecular ion at m/z 430 along with diagnostic fragment ions at m/z 278, 165, and 151. The 1H NMR spectrum (fig. 1) revealed a lack of both N-methyl and 5-O-methyl groups. M-6 and RWJ-37087, a synthetic sample (Carson et al., 1988; Carson, unpublished results), showed differences in TLC R$_f$ values; however, both metabolites showed similar MS data (fig. 1) and 1H NMR data (table 3). M-6 methyl ether was identical to N-desmethyl RWJ-26240 (M-1, RWJ-36359) on the basis of their identical MS, NMR, HPLC, and TLC data.

M-7 was isolated in significant quantities (4% of the radioactive dose) by HPLC and TLC from the fecal sample (table 2). The CI-MS spectrum (fig. 1) showed an intense adduct-molecular ion at m/z 474, 16 amu more than RWJ-26240, together with several prominent fragment ions at m/z 322, 222, 165, and 151. Both M-5 and M-7 displayed similar fragment ions (fig. 1). Treatment of M-7 with diazomethane and diazoethane yielded M-7 methyl and ethyl ethers, respectively. The CI-MS data of M-7 methyl and ethyl ethers were similar to the corresponding ethers of M-5 (fig. 1). Therefore, M-5 and M-7 appeared to be positional isomers with different hydroxy substitution. Analysis of M-7 ethyl ether by 1H NMR (table 3) showed the addition of an O-ethyl group and a pair of well-defined AA’BB’ quartets for four phenyl protons at the 2’, 3’, 5’, and 6’-positions of the diphenylacetylene moiety. Therefore, M-7 was tentatively identified as 4-hydroxyphenyl ethynyl RWJ-26240.

M-8 was isolated in small amounts (<5% of the radioactive dose) from the fecal extract (table 2). The CI-MS spectrum (fig. 1) showed a protonated molecular ion at m/z 430, along with characteristic ions at m/z 278, 165, and 151. The 1H NMR spectrum (fig. 1) revealed a lack of both N-methyl and 5-O-methyl groups. M-8 and RWJ-37087, a synthetic sample (Carson et al., 1988; Carson, unpublished results), were shown to be identical on the basis of their HPLC, TLC, MS, and NMR data. The isolated metabolite was further derivatized as an ethyl ether. The metabolite 8 ethyl ether structure was confirmed by CI-MS (fig. 1) and 1H NMR data (table 3).

M-9 was isolated in minor quantities (<5% of the radioactive dose) from the fecal sample (table 2). In the MS spectrum (fig. 1), an apparent protonated molecular ion at m/z 460 was observed, along with several diagnostic ions at m/z 308, 208, 165, and 151. Diazoethane and diazoethanol treatment of the isolated metabolite formed the methyl and ethyl ethers, respectively. They were characterized on the basis of MS data (fig. 1). The MS data suggested the structure of M-9 to be a hydroxy-N-desmethyl RWJ-26240 analogue with a hydroxyl group attached to a phenyl ring of the diphenylacetylene moiety. In the 1H NMR spectrum (table 3), 3-H and 6-H were observed as a singlet at 6.92, similar to those observed for M-5; therefore, M-9 was tentatively identified as 4-hydroxy-N-desmethyl RWJ-26240.

M-10 was isolated in minor amounts (<5% of the radioactive dose) by HPLC from the fecal extract (table 2). The structure of M-10 was tentatively elucidated on the basis of MS and NMR data. M-10 and M-9 showed differences in TLC R$_f$ values; however, both metabolites showed similar MS data (fig. 1), with an intense protonated molecular
ion at \textit{m/z} 460 and three prominent ions at 308, 165, and 151. In addition, an important ion at \textit{m/z} 222 observed in the spectrum of M-10, as opposed to the \textit{m/z} 208 of M-9, indicated that M-10 was a hydroxylated-O-desmethyl RWJ-26240. Derivatization of M-10 with diazoethane led to the production of a diethyl ether. M-10 diethyl ether was confirmed by the MS data (fig. 1). In the $^1$H NMR spectrum (table 3), $2''$-H, $3''$-H, $5''$-H, and $6''$-H were observed as a pair of AA'BB' quartets at 6.75 and 7.35, similar to those of M-7 (table 2). Therefore, M-10 was tentatively assigned as the 4-hydroxyphenyl ethynyl-O-desmethyl RWJ-26240 structure.

M-11 was isolated in minor quantities (<5% of the radioactive dose) from fecal extract (table 2). The CI-MS spectrum (fig. 1) of M-11 displayed three apparent adduct-molecular ions at \textit{m/z} 320 (MC$_3$H$_5$), 308 (MC$_2$H$_5$), and 280 (MH$^+$), together with two fragment ions at \textit{m/z} 263 and 222. Comparing the TLC and MS data of M-11 with a synthetic sample confirmed its identity (Carson et al., 1988; Carson, unpublished results).

M-12 was isolated as a nonradioactive product from the urine sample and further derivatized with diazomethane. The CI-MS (fig. 1) of the isolated metabolite and its methyl ester exhibited protonated

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mass_spectra.png}
\caption{CI and EI mass spectra of M-1.}
\end{figure}
molecular ions at m/z 197 and 211, respectively, accompanied by significant ions at m/z 179 and 151. The TLC and MS data for M-12 methyl ester were identical to those of an authentic methyl homovera-trate. The quantity of M-12 present in the urine sample is still unknown.

Discussion

The excretion data revealed no sex differences in dogs. Of the radioactive dose, 96.8% and 2.8% was recovered in feces and urine, respectively, in the 7 days after oral administration of 14C-RWJ-26240. More than 87% of the dose was excreted in feces during the 48 hours. RWJ-26240 was extensively metabolized in dogs. Unchanged RWJ-26240 was found in less than 0.1% and 19% of the dose in the 0–24 hour urine and 0–48 hour fecal extract, respectively, and 36% of the sample in the 4 hour plasma. The plasma, urinary, and fecal metabolic profiles showed no sex differences in dogs. Unchanged RWJ-26240 and a total of 12 metabolites were isolated and identified in dogs on the basis of TLC, HPLC, MS, NMR, and methyl and ethyl derivatization techniques, and by comparison of synthetic samples (M-1, M-2, M-4, M-8, M-11, and M-12). EI-MS analysis of metabolites of RWJ-26240 (fig. 4) showed weak molecular ions; however, CI-MS exhibited intense protonated molecular ions and apparent adduct molecular ions (C$_2$H$_5$M$^+$) and prominent, as well as important, diagnostic ions for the structural elucidation of metabolites and their derivatives. The phenolic and carboxyl metabolites were derivatized to form methyl and ethyl ethers and esters for the further confirmation of metabolite structures. Particularly, treatment of phenolic metabolites with diazoethane formed metabolite ethyl ethers, i.e. M-2 and M-5 ethyl ethers, which facilitated the assignment of phenolic positions of metabolites using MS and NMR techniques. The formation of the metabolites of RWJ-26240 can be explained by the following four proposed pathways: 1) N-demethylation, 2) O-demethylation, 3) phenyl hydroxylation, and 4) oxidative N-dealkylation.

The proposed biotransformation pathways are presented in fig. 5. Pathway 1 produced an important circulating plasma metabolite M-1 (N-desmethyl-RWJ-26240) accounted for 16% of the sample and <4% of the dose in plasma and feces, respectively. M-1, an active metabolite, showed antihypertensive activity (10% of RWJ-26240) in dogs and rats (Carson et al., 1988; Carson, unpublished results). Pathway 2 formed two quantitatively important O-desmethyl metabolites, M-2 and M-4, present in major amounts in plasma (20% and <5% of the sample for M-2 and M-4, respectively) and feces (10% and 6% of the dose for M-2 and M-4, respectively). M-2 is also a major urinary metabolite accounting for 30% of the sample, which further formed as a conjugate. M-2 showed slightly antihypertensive activity (<10% of RWJ-26240) in dogs and rats (Carson et al., 1988; Carson, unpublished results). Pathway 2 in conjunction with pathway 1 resulted in the formation of two N/O-didesmethyl-RWJ-26240's, M-6 (<4% of the dose in feces) and M-8 (<5% of the sample in plasma, <4% of the dose in feces). Pathways 1 and 2 produced similar types of N/O-desmethyl metabolites, which were previously reported in the metabolism of Verapamil in rats and dogs (McIlhenny, 1971). Pathway 3 formed two phenyl hydroxylated metabolites, M-5 and M-7, in minor (<4% of the dose) and moderate (4% of the dose) amounts, respectively, in feces. Pathway 3 along with pathways 1 and 2 produced two hydroxylated N-desmethyl metabolites (M-3 and M-9) and a hydroxylated O-desmethyl metabolite (M-10), respectively. All three metabolites excreted in minor quantities (each <4% of the dose) in feces. Pathway 4 generated two oxidative N-dealkylated metabolites, M-11 present in feces and urine, and M-12 in plasma, urine, and feces. Each metabolite was present in minor amounts (<4% of the dose). Metabolites M-1, M-2, M-4, M-6, and M-8 represented more than 30% of the dose, suggesting that N/O-
demethylation pathways were the major routes of elimination of RWJ-26240 in dogs. Overall, unchanged RWJ-26240 plus identified metabolites accounted for >75% of the sample in plasma and feces. RWJ-26240 was extensively metabolized after oral administration and fecal excretion was the major route of elimination of RWJ-26240 and its metabolites. In rats, recovery of total radioactivity in urine and feces was 0.9% and 96.0% of the dose, respectively, in the 8 days after a single 50 mg/kg oral dose of 14C-RWJ-26240 (Wu et al., 1991; Ng et al., 1987). In a comparison of excretion and metabolism between dogs and rats, both showed similar excretion data. However, it appears that rats metabolized RWJ-26240 more extensively than dogs. Dogs excreted more unchanged RWJ-26240 (19% of the dose) than rats (3% of the dose) in the 0–48 hour feces. Both species produced the same metabolites (M-1 to M-10, and M-12) except M-11, an amine metabolite for the dog, but methyl ketone metabolite for the rat. Both amine and ketone metabolites formed via oxidative N-dealkylation pathway.

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