Further Studies on the Metabolism of Carbidopa, (−)-L-α-Hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic Acid Monohydrate, in the Human, Rhesus Monkey, Dog, and Rat

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Major urinary metabolites of carbidopa have been identified. Estimates were made based on the recovery of radioactivity or by glc analysis of pooled urine of the amounts of the urinary metabolites II (2-methyl-3′-methoxy-4′-hydroxyphenylpropionic acid), III (2-methyl-3,4-dihydroxyphenylpropionic acid), IV (3,4-dihydroxyphenylacetone), V [2-methyl-3-(3′-methoxy-4′-hydroxyphenyl)lactic acid], VI [2-methyl-3-(3′,4′-dihydroxyphenyl)lactic acid], and VII (3-hydroxy-α-methylphenylpropionic acid). Metabolite II represented ~10% of the urinary radioactivity in both man and monkey and 16% in the dog. Metabolite III represented 10, 17, and 19% of the urinary radioactivity in man, monkey, and dog. Metabolite IV represented <5% of the urinary radioactivity in human and dog. Metabolite VII represented ~10% of the urinary radioactivity in man and monkey. The corresponding figure for the rat was ~20%. In the dog, compounds V and VI represented <5% of the urinary radioactivity. It was concluded that the loss of the hydrazine functional group (probably as molecular nitrogen) represents the major metabolic pathway for carbidopa.

This paper describes metabolism studies of carbidopa (I), (−)-L-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic acid monohydrate,† a potent inhibitor of extracerebral aromatic amino acid decarboxylase (Figure 1).

Carbidopa enhances the efficiency of Dopa in increasing brain dopamine levels and is used in conjunction with Dopa for the treatment of Parkinson's disease.‡

Preliminary absorption–excretion studies pertaining to carbidopa-^{14}C have been reported previously.[1,2] After 20 mg/kg (oral) the dog, monkey, and rat excreted 66, 40, and 16% of the dose of radioactivity in the urine, respectively. An average 50% of an oral dose (50 mg) of carbidopa-^{14}C was excreted in the urine of humans. Analysis of expired air indicated that there was little decarboxylation of carbidopa-*1*-^{14}C either in the rat or the human. In the present report, additional metabolites are identified and quantitative data on the biotransformation of carbidopa in several mammalian species, *viz.* human, rhesus monkey, dog, and rat, are given.

Data are presented to support a mechanism for the formation of the principal metabolites of carbidopa by the loss of nitrogen rather than the elimination of hydrazine from the molecule.

Experimental Section

In the human study carbidopa-2-^{14}C (specific activity after recrystallization, 0.483 μCi/mg) was administered in capsules. For the animal experiments, male Holtzman rats (130–140 g), pure bred beagle dogs of either sex (7–9.9 kg), and female Rhesus monkeys (3–4 kg) were given either carbidopa-*1*-^{14}C or carbidopa-*2*-^{14}C diluted in an aqueous HCl solution with the necessary amount of carrier drug. In the rat (four animals) and dog (four animals) studies, urine and feces were collected separately in stainless steel metabolism cages following a single oral dose (20 mg/kg) of carbidopa-^{14}C, the urine being received in Dry Ice cooled containers. Urine from monkeys (four animals) was collected by catheterization which was performed prior to placing them in restraining chairs and dosing orally with carbidopa-2-^{14}C. Urine from humans (three normal volunteers) who received 50-mg capsules of labeled carbidopa was collected at definite time intervals and frozen.

Radioassay Procedure. Radioactivity was measured by a liquid scintillation technique using either a Packard 3375 or a Packard 3310 spectrometer. The scintillation cocktail employed consisted of dioxane (3 l.), naphthalene (300 g), 2,5-diphenyloxazole ("PPO," 21 g), 1,4-bis(5-phenyl-2-oxazolyl)benzene ("POPOP," 0.9 g), and Cab-O-Sil (90 g). An internal standard (toluene-^{14}C) was employed to determine efficiency; samples were counted for 20 min.

Samples of urine were added directly to the polyethylene counting vial containing 20 ml of scintillation medium.

Spectra. Absorption data in the visible region were obtained with a Beckman Model DK2A. Fluorometric analyses were performed with an Aminco-Bowman spectrofluorometer.

Thin-Layer Chromatography (Tlc). Glass plates (8 × 2 in.) coated with silica gel (250 μ) were purchased from Analtech (Wilmington, Del.). Prior to use they were washed with methanol. Solvent systems used in the development of the plates were benzene–methanol (4:1), benzene–acetic acid (4:1), and acetic acid–acetone–benzene–butanol–water (equal parts). Radioactive areas on the plates were determined by the use of a Packard radiochromatogram scanner. Visualization was achieved by spraying with either Folin's reagent or *p*-dimethylaminobenzaldehyde in 1 *N* H_2SO_4 (2% solution).

Combined Gas–Liquid Chromatography–Mass Spectroscopy (Glc–MS). Mass spectra were obtained with an LKB Model 9000 combined gas chromatograph—mass spectrometer using OV-1, QF-1, or SE-30 columns. The metabolites were trimethylsilylated with bis(trimethylsilylacetamide) (BSA) and BSA-d_{18} (the deuterated analog).

Gas–Liquid Chromatography (Glc). A Model F&M 5750 equipped with a flame ionization detector was used for glc on coiled glass columns (0.25 in. inside diameter × 6 ft) packed with 3% OV-17 or 3% SE-30 on Gas Chrom Q (80–100 mesh). The column, detector, and flash heater temperatures were 170–200, 210, and 220°. The carrier gas helium was used at a flow rate of 60 ml/min.

Radioactivity Monitoring of Glc Effluents. A Barber Coleman Model 5000 gas chromatograph equipped with a radioactivity monitor was used for this purpose.

Isolation of 2-Methyl-3-(3′-methoxy-4′-hydroxyphenyl)lactic Acid (V) and 2-Methyl-3-(3′,4′-dihydroxyphenyl)lactic Acid (VI). As shown in Figure 2, dog urine was hydrolyzed with Glusulase and then sequentially extracted with benzene and ether. The ether extract after further purification by extraction with pH 10 buffer and thin-layer chromatography yielded compounds V and VI.

Isolation and Determination of 2-Methyl-3′-hydroxyphenylpropionic Acid (VII) from Urine. As shown in Figure 3 urine from human, monkey, and rat was hydrolyzed with Glusulase and then extracted with ether. The ether extract after further

† This systematic name is presently preferred by Chemical Abstracts. Alternate names used in previous communications are L-α-methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid and L-(−)-α-hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate. The (−)-L form has been referred to in the biological literature as MK-486, HMD, and (incorrectly) as α-hydrazino-α-methyl-Dopa. MK-485 has been used as a designation for the racemic form.

‡ Due to a typographical error, the compound was incorrectly described as increasing rather than decreasing dosage requirement of L-Dopa.[2]

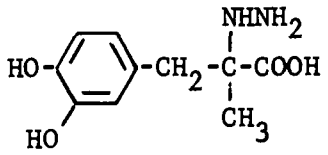

Figure 1. Carbidopa.

purification yielded compound VII. Compound VII was measured by gas–liquid chromatography. The method was similar to that described below except that solvent extraction was performed with chloroform–ethyl acetate (4:1). Analyses were performed at an oven temperature of 165°. Recovery in this analytical procedure as determined by control experiments was 50%. The method was used for the analysis of urines from man, monkey, and rat.

Determination of 2-Methyl-3′-methoxy-4′-hydroxyphenylpropionic Acid (II) in Urine. Compound II was measured by gas–liquid chromatography. Urines were hydrolyzed with Glusulase at pH 5.2 prior to analysis. Hydrolyzed urine samples (1 ml, in duplicate) were diluted with pH 9.2 buffer (4 ml) and then extracted with benzene (25 ml). After removal of the benzene the aqueous solution was adjusted to pH 2 by the use of 2 *N* HCl and reextracted with benzene (25 ml). After removal of the benzene (under reduced pressure) the extracts were dissolved in pyridine (10 μl) and BSA (40 μl). Analyses were performed using an oven temperature of 180° and an Apiezon (3%) column. A control experiment was run in which urine (1 ml) that did not contain compound II was used. A calibration curve was constructed by assay of samples containing 5–50 μg/ml of compound II. The curve was linear and passed through the origin. The recovery in this analytical procedure as determined by control experiments was 50%.

Analyses were performed on urines obtained from man, monkey, and dog.

Determination of 2-Methyl-3,4-dihydroxyphenylpropionic Acid (III). Compound III was measured by gas–liquid chromatography. Hydrolyzed urine samples (1 ml in duplicate) were diluted with pH 2 buffer (4 ml) and extracted with ether (25 ml water saturated). The ether layer was separated and extracted with pH 9 buffer (5 ml), which in turn was separated, adjusted to pH 2 (with 2 *N* HCl), and extracted with ether (25 ml water saturated). The ether layer was separated, dried ($MgSO_4$), and then evaporated (reduced pressure). The residue was dissolved in pyridine (10 μl) and BSA (40 μl). Analyses were performed over a programmed temperature range of 153–173° at 2°/min on SE-30 (1%). A control experiment was run in which urine (1 ml) that did not contain compound III was used. A calibration curve was constructed by assay of samples containing 5 to 50 μg/ml of compound III. The curve was linear and passed through the origin. Recovery in this analytical procedure as determined by control experiments was 70%. Analyses were performed on urines obtained from monkey and dog.

Determination of Hydrazine in Plasma. Hydrazine was measured by a highly sensitive fluorescence method[3] modified as follows to prevent interference by carbidopa. Plasma samples (1 ml in duplicate) were adjusted to pH 8.4 with 0.1 *N* NaOH followed by chromatography using a small column of acid-washed alumina (700 mg). The columns were washed with distilled water (2 ml), and the combined aqueous extracts were diluted with 10% trichloroacetic acid (3 ml) and then extracted with chloroform (3 ml). After centrifugation, the aqueous phase was removed and mixed with an ethanolic 0.4% solution of *p*-*N*,*N*-dimethylaminobenzaldehyde (2 ml). After heating for 45 min at 70° the tubes were extracted with $CHCl_3$ (3 ml). The latter solution was separated and dried over $MgSO_4$, and its fluorescence was determined at an excitation wavelength of 466 mμ and an emission wavelength of 546 mμ. The calibration curve was constructed by assay of samples containing 0–1 μg/ml of hydrazine hydrate and 1.0 μg/ml of carbidopa. The curve was linear and passed through the origin, showing that this amount of carbidopa does not interfere when alumina is used.

Three rhesus monkeys received carbidopa (20 mg/kg po), and blood was drawn at 1, 2, and 4 hr postdosing. The plasma obtained was then analyzed for hydrazine by the above fluorometric method.

Hydrazine was also measured by a spectrophotometric assay.[4] Plasma samples (1 ml in duplicate) were mixed with 10% trichloroacetic acid (3 ml) and centrifuged. The supernatant was removed and reacted with a 0.4% ethanolic solution of dimethylaminobenzaldehyde (2 ml) for 45 min at 70°. After the chromophore was extracted into water-saturated butanol (3 ml) in the presence

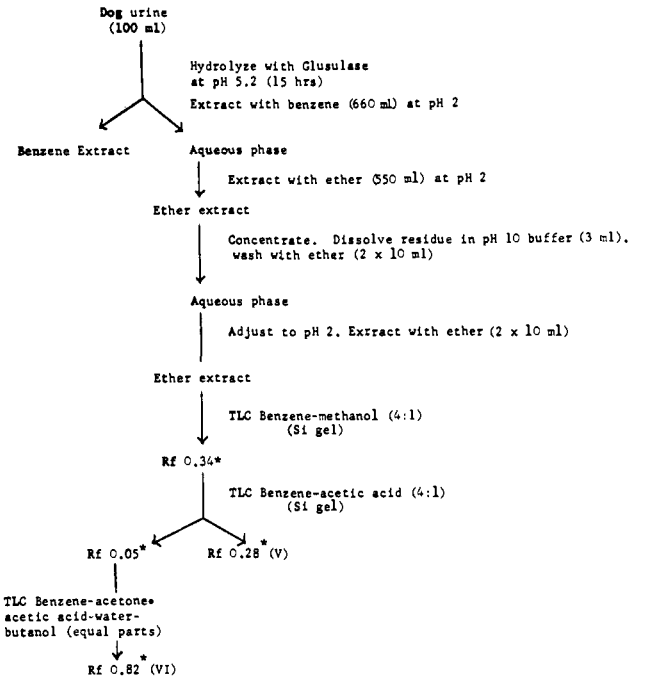

Figure 2. Isolation of compounds V and VI from dog urine. An asterisk indicates radioactive.

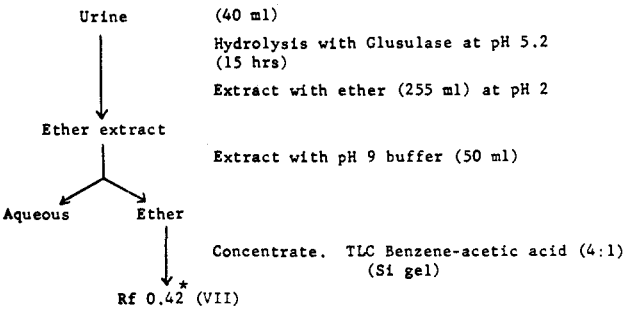

Figure 3. Isolation of VII from urine. An asterisk indicates radioactive.

of excess sodium chloride its absorbance was determined at 465 nm. An experiment was run in which control plasma (1 ml) was treated in the way described above. A calibration curve was constructed by assay of samples containing 1–6 μg of hydrazine hydrate. The curve was linear and passed through the origin.

Three monkeys were dosed with hydrazine hydrate§ (1.4 mg/kg ip). At measured time intervals (*viz.* 1, 2, 3, 4, 5, and 6 hr) following dosings, blood was drawn from the animals. The plasma samples were analyzed for hydrazine by the spectrophotometric method described above.

Detection of Hydrazine in Urine. Urines (0–24 hr) (diluted 1:1 or 1:2 with distilled water) from three monkeys that had received carbidopa (20 gm/kg po) were individually analyzed for hydrazine. Aliquots (1 ml) were adjusted to pH 8.4 with 0.1 *N* NaOH and then applied to a small column of alumina (700 mg). The column was then washed with pH 7 buffer (3 ml), and the combined aqueous eluates were mixed with a 0.4% ethanolic solution of benzaldehyde (2 ml). The mixture was heated at 70° for 30 min, cooled, and extracted with benzene (3 ml). The benzene solution (2 ml) was evaporated and analyzed by gas–liquid chromatography–mass spectrometry using repetitive scanning (*m*/*e* values 10–280) 0.5 min before and 0.5 min after the retention time for benzalazine.

A control experiment using OV-17 was performed in which urine (1 ml) containing 0.1 μg of hydrazine hydrate was subjected to the above assay. Hydrazine (as benzalazine) was detected, and this was the limit of detection. Experiments similar to the above

§ This equivalent dose was determined by multiplying the dose of carbidopa (20 mg/kg calculated as the anhydrous compound) by 0.07 in order to correct for the difference in molecular weight, and assuming that of the drug absorbed (40%), 20% would be excreted as unchanged drug ($50/226 \times 2/5 \times 4/5 = 0.07$).

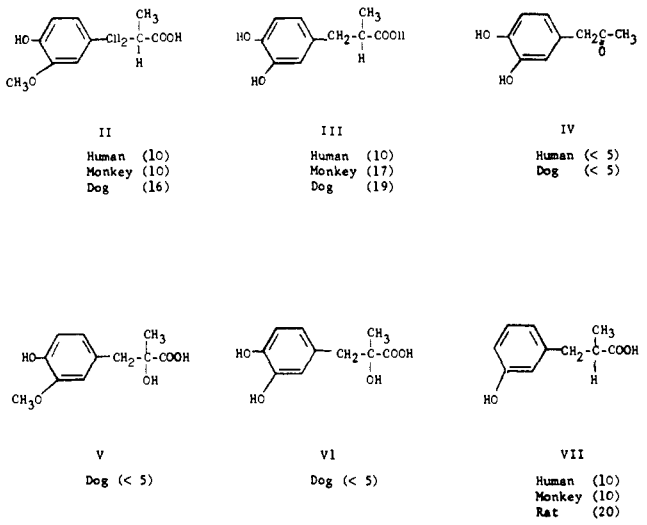

Figure 4. Urinary metabolites of carbidopa. Per cent of dose, where determined, is given in parentheses; that for III (human) was based on the recovery of radioactivity. Unchanged carbidopa was present to the extent of 30, 20, 65, and 38% in human (50 mg po), monkey, dog, and rat (all 20 mg/kg po) urine, respectively.[4]

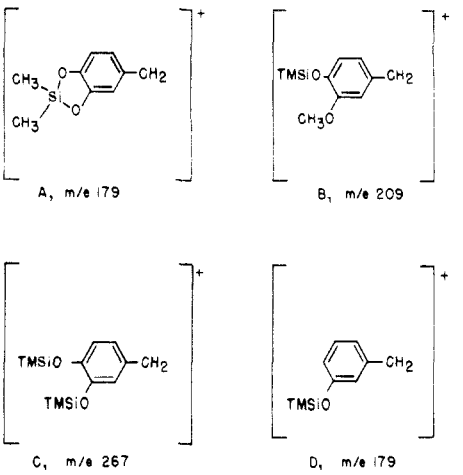

Figure 5. Major fragment ions of carbidopa metabolites.

were also run using control urine containing 60 μg/ml of carbidopa as controls; hydrazine (as benzalazine) was not detected.

Computer Techniques. The Varian spectrosystem 100 with the statos 21 plotter was used.

Reaction between Carbidopa, Dimethylaminobenzaldehyde (DMAB), and Pyridoxal. The methodology employed was essentially that reported earlier[5] except that radioactive carbidopa was employed. When DMAB was used, the reaction mixture was extracted with benzene, $CHCl_3$, and ether. After evaporation, the organic extracts were analyzed by thin-layer chromatography (tlc) and glc-MS as the methylated or BSA derivative. When pyridoxal was used, a yellow oil was formed by evaporation of the reaction mixture. The oil was analyzed by mass spectrometry either directly or by conversion to a BSA derivative.

Results

The urinary metabolites of carbidopa are shown in Figure 4. The previously reported compounds II and III were present along with unchanged carbidopa& (I) in human, monkey, dog, and rat urine, while compound IV was found in the human and dog urine (its presence or absence in monkey urine appears to be erratic).[2] It is now reported that compounds V and VI are present in dog urine (they were not detected in the urine of other species) while compound VII is present in human, monkey, and rat urine following dosing with carbidopa (its presence of absence in dog urine was not established).

Compounds V and VI were identified in dog urine by glc-MS techniques after preliminary solvent extraction and thin-layer chromatography. The compounds possessed molecular ions, and confirmation of structure was obtained by the use of BSA-d_{18} (to obtain the predicted shifts in m/e values) as well as by the use of the authentic compounds. It was possible to examine large amounts of glc-MS data with the aid of a computer and this approach revealed the presence of an additional carbidopa metabolite VII. All known silylated metabolites of carbidopa were found to possess one of two ring systems which were characterized by ions at m/e 179 and 209 or m/e 179 and 267 (Figure 5, structures A, B, and C). Several metabolite fractions (urinary extracts from monkeys and also tlc zones from dog urine) were derivatized and then subjected to combined glc-MS with repetitive MS scanning (m/e 10–550 every 10 sec) for 15 min. All the resulting data were taken into the computer. The "D plot" routine was then employed to plot out *vs.* time the production of the ions 179, 209, and 267 (*i.e.*, to provide mass chromatograms[6]). The appropriate pairs of ions (*e.g.*, 179 and 209, 179 and 267) were found to match at the retention times of the known metabolites. A complete spectrum was obtained from the stored computer data for those components which exhibited the characteristic ions; in each case the component was proven to be a carbidopa metabolite present in dog or monkey urine (Figures 6 and 7).

In one case (a monkey urine ether extract) a strong response for m/e 179 was observed but no corresponding 209 or 267 (Figure 5, structure D). Further work using a radioactivity monitor on the glc effluent showed that this unknown was radioactive. Structure VII was proposed on the basis of the mass spectral analysis and predicted shifts of m/e values occurred when BSA-d_{18} was used. Synthetic samples of VII and its 4-hydroxy isomer were obtained, and the mass spectral properties were examined. The base peak of the 3 (meta) isomer and of the metabolite was the ion at m/e 206 with a molecular ion at m/e 324 (Figures 7 and 8), whereas the 4 (para) isomer had a base peak at m/e 179 with a molecular ion of m/e 324 (Figure 9). It was concluded that the carbidopa metabolite present in monkey urine was the meta isomer VII, which was subsequently identified in human and rat urine.

The effect of positional isomerism on the mass spectrum of VII may be explained by a resonance effect since the para-substituted group increased the stability and the abundance of the m/e 179 ion. This was, of course, not true for the meta isomer.

Estimates were made, based on the recovery of radioactivity or by glc analysis of pooled urines, of the amounts of the metabolites II, III, IV, V, VI, and VII present in urine. Metabolite II represented ~10% of the urinary radioactivity in both man and monkey and 16% in the dog. Metabolite III represented 10, 17, and 19% of the urinary radioactivity in man, monkey, and dog. Metabolite IV represented <5% of the urinary radioactivity in man and dog. Metabolite VII represented ~10% of the urinary radioactivity in man and monkey. The corresponding figure for the rat was ~20%. In the case of the dog, compounds V and VI represented <5% of the urinary radioactivity.

Assays for hydrazine both in plasma and urine were performed using alumina to remove carbidopa which would interfere[5,7] (see Experimental Section). Hydrazine was not detected (limit of detection 80 ng of hydrazine hydrate/ml) in the plasma of monkeys that received a 20 mg/kg dose of carbidopa. Monkeys that received hydrazine intraperitoneally at a dose level (1.4 mg/kg of

& Values for the amounts of I excreted have been reported previously.[2]

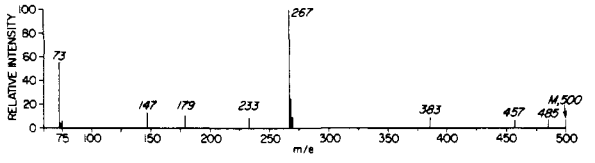

Figure 6. Mass spectrum of the metabolite VI (TMSi derivative).

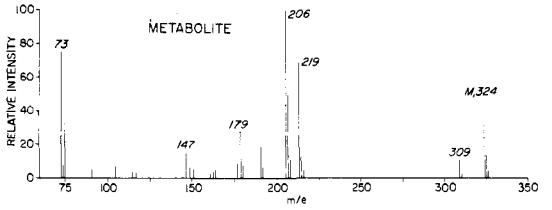

Figure 7. Mass spectrum of the metabolite VII (TMSi derivative).

$NH_2NH_2 \cdot H_2O$) comparable to that which could be released from an oral dose (20 mg/kg) of carbidopa had levels of hydrazine hydrate which ranged from 600 to 1000 ng/ml within the first hour and which were readily detected even by an insensitive colorimetric procedure.

A sensitive method of detecting hydrazine in the presence of carbidopa in urine was devised (see Experimental Section). Control urines containing 60 μg/ml of carbidopa were extracted with alumina in order to remove the carbidopa and then they were treated with benzaldehyde. The product was dissolved in ethyl acetate and aliquots were injected into a glc–MS system (OV-17 column). Repetitive scanning from m/e 10 to 280 at the retention time of benzalazine did not produce the response characteristic of this compound, and thus there was no interference by carbidopa. This approach was employed for the analysis of urine from monkeys dosed with carbidopa. There was no evidence for the presence of hydrazine.

Discussion

It has been demonstrated that loss of the hydrazine functional group of carbidopa is a metabolic event common to all of the species studied. The carbidopa metabolites, 2-methyl-3-(3′-methoxy-4′-hydroxyphenyl)lactic acid (V) and 2-methyl-3-(3′,4′-dihydroxyphenyl)lactic acid (VI), were found in dog urine. Man, monkey, and the rat excrete 3-hydroxy-α-(methylphenyl)propionic acid (VII) in the urine. These metabolites are excreted primarily as conjugates, as are the previously reported metabolites II–IV.[2] Together with unchanged carbidopa, they account for ~60% of the excreted urinary radioactivity in man, monkey, and rat while they account for ~90% of the same in the dog.

Experiments were performed to establish that hydrazine was not a metabolic product of carbidopa. Hydrazine was not detected in the plasma of monkeys that received an oral dose of carbidopa. Since the levels of hydrazine in the plasma of monkeys that received an equivalent dose of hydrazine (after correction for absorption and metabolism of carbidopa) were easily detectable, then the absence of hydrazine in the plasma following dosing with carbidopa could be ascribed to a lack of its formation rather than hydrazine sequestering by tissue. Nor was hydrazine detectable in the urine of monkeys given 20 mg/kg of carbidopa, using a glc–MS procedure modified to avoid interference by carbidopa. Thus, it appears that hydrazine is not a metabolic by-product of carbidopa.

It is known that the oxidation of hydrazine and its derivatives may result in the evolution of molecular nitrogen. In 1927, it was reported that the theoretical amount

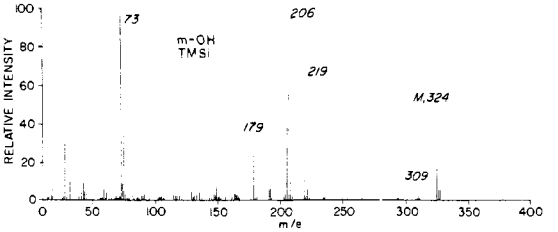

Figure 8. Mass spectrum of the authentic compound VII (TMSi derivative).

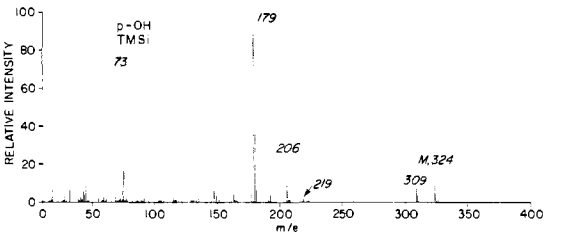

Figure 9. Mass spectrum of the para isomer of VII (TMSi derivative).

of nitrogen gas was liberated from a hydrazine solution when it was mixed with a solution of potassium iodate in a manometric apparatus.[8] An instantaneous and quantitative reaction occurred according to the stoichiometric equation

$$2NaIO_3 + 3N_2H_4 = 2NaI + 3N_2 + 6H_2O$$

It was later demonstrated that methylhydrazine, isonicotinylhydrazine, benzoylhydrazine, and asymmetric dimethylhydrazine yield nitrogen quantitatively upon treatment with acidic iodate solution.[4] This method was used to determine the excretion of hydrazine by dogs after injection of hydrazine (50 mg/kg). It was discovered that the gaseous products resulting from the reaction of monomethylhydrazine with dilute sodium hypochlorite were nitrogen, methane, carbon monoxide, and traces of alkyl chlorides.[9] Identifications were made by both gas chromatography and mass spectrometry. Radioactive methane was produced by rate receiving monomethylhydrazine at dose levels of 5.5, 11, and 22 mg/kg.[10] At a later date the presence of a microsomal enzyme system in rat liver, which is capable of enzymatically converting various simple monoalkylhydrazines to the corresponding hydrocarbons, was reported.[11,12] From these studies, both chemical and biological, it can be expected that monoalkylhydrazines will be metabolized to hydrocarbons and nitrogen gas by xenobiotic enzymes (eq 1).

$$RNHNH_2 \xrightarrow[\text{system}]{\text{oxidizing}} RH + N_2 + H_2O \qquad (1)$$

Because 3,4-dihydroxyphenylacetone was present in human and dog urine, as well as being a product of the reaction *in vitro* between carbidopa and dimethylaminobenzaldehyde (which is also known to decarboxylate amino acids[13,14]), it was considered that its presence in human and dog urine might result from a reaction between carbidopa and a biologically available aldehyde, *e.g.*, pyridoxal. Such a reaction might be expected to produce pyridoxalazine and 3,4-dihydroxyphenylacetone. However, under chemical conditions, neither of the above compounds was isolated from the reaction between pyridoxal and carbidopa. Furthermore, recoveries of the label were the same (100%) when either carbidopa-*1*-^{14}C or carbidopa-2-^{14}C was treated with pyridoxal (this was in contrast to the finding that 98% of the label in carbidopa-*1*-^{14}C was lost upon treatment with dimethylaminobenzaldehyde). Also, when dogs were predosed with pyridoxine

(1 mg/kg) before receiving carbidopa-^{14}C (20 mg/kg), the solvent extraction properties of the radioactive urinary metabolites remained unchanged. It has been found that acidic solutions of carbidopa may decompose in air to yield 3,4-dihydroxyphenylacetone. Thus, the presence of the latter in urine may well result during manipulation of the urine samples.

It would be expected that the flora of the lower intestine assume significance in the metabolism of carbidopa since incomplete absorption of the compound occurs.[15] Evidence was obtained for bacterial decarboxylation of the racemate of carbidopa in rat feces.[16] Dehydroxylation of catecholic acids following their ingestion is well known and *m*-hydroxyphenylacetic acids have been identified in urine. Although it is reported[17] that mammalian tissues do have the capacity to dehydroxylate catecholamino acids (in the meta position), it is suggested that metabolite VII, having a *m*-hydroxyl group, is a product of bacterial dehydroxylation. The amino acid Dopa or one of its metabolites is subject to bacterial dehydroxylation.[18,19] It seems the same conclusion applies to carbidopa, the hydrazino analog of Dopa.

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Norepinephrine Uptake Sites in Cardiac Tissue. Lack of Affinity of 6-Hydroxynorepinephrine and Related Compounds

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The effects of a series of phenethylamines and the corresponding phenethanolamines on (i) rate of uptake of radioactive norepinephrine into cardiac tissue *in vivo* and (ii) the rate of efflux of radioactive norepinephrine from prelabeled cardiac storage sites have been determined. The results indicate that *m*- and *p*-hydroxyphenethylamines and the corresponding phenethanolamines have high affinities for uptake into cytoplasm and storage vesicles of noradrenergic terminals in the heart. *o*-Hydroxyphenethylamines such as 2-hydroxyphenethylamine and 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine) also have moderate to high activity as inhibitors of norepinephrine uptake and as releasing agents for norepinephrine, but *o*-hydroxyphenethanolamines such as 2-hydroxyphenethanolamine, 2,5-dihydroxyphenethanolamine, and 2,4,5-trihydroxyphenethanolamine (6-hydroxynorepinephrine) have little or no activity as inhibitors of uptake or as releasing agents. 2,6-Dihydroxyphenethylamines have little or no activity as inhibitors of uptake or as releasing agents. The results are consonant with significant binding of the *gauche* conformers of 2-hydroxyphenethylamines to uptake sites. Such conformers would be preferred because of stabilization by hydrogen bonding between nitrogen and phenolic oxygen. Apparently a hydrophobic region of the site prevents binding of such stabilized gauche conformers of 2-hydroxyphenethanolamines and 2,6-dihydroxyphenethylamines.

Extensive investigations on the effects of various compounds on the uptake and release of radioactive norepinephrine from cardiac tissues *in vivo* have been reported.[1-20] Such studies provide the following information relevant to the design of drugs and pharmacologically active research tools: (i) structure–activity correlations with respect to inhibition of norepinephrine uptake at plasma membrane, a process important to the termination of the action of this neurotransmitter; (ii) structure–activity correlations with respect to displacement of norepinephrine from storage sites, a phenomenon which results in a lowered availability of norepinephrine as a neurotransmitter and/or replacement of the norepinephrine with "false neurotransmitters" of greater or lesser physiological efficacy. With suitable modifications the basic *in vivo* test system has provided data on (i) long-term effects on uptake and storage of norepinephrine by neurotoxic agents such as 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine) and 5,7-dihydroxytryptamine;[19,20] (ii) efficacy of compounds as *in vivo* inhibitors of the decarboxylation of Dopa;[11] and (iii) the relative importance of monoamine oxidase to the *in vivo* metabolism of phenethylamines and tryptamines.[20]

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