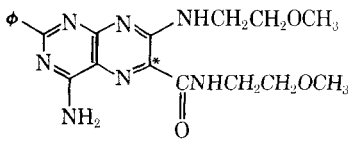
The Metabolic Fate of the Pteridine Diuretic, Wy-5256

SAMUEL F. SISENWINE and SIDNEY S. WALKENSTEIN*

Abstract □ Utilizing labeled drug, the metabolic fate of 4-amino-*N* - (2 - methoxyethyl) - 7 - (2 - methoxyethylamino) - 2 - phenyl-6-pteridinecarboxamide (Wy-5256) has been studied in the rat, dog, and squirrel monkey after a single pharmacologically active oral dose. All three investigated species were found to transform the drug by successive *O*-demethylation of both aliphatic ether side chains to two isomeric mono-demethylated metabolites and the major metabolite, a bis-demethylated product. The drug was found to be rapidly absorbed in each species, and a distribution study in male rats revealed no tendency to accumulate in any tissue. The fecal route of excretion was found to predominate in all three species and biliary excretion studies revealed that this elimination pathway was due not to poor absorption but mainly to biliary excretion.

Keyphrases □ 4-Amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide (Wy-5256)—metabolism □ Diuretic (Wy-5256), radioactive—absorption, excretion □ Biliary excretion—Wy-5256 □ Metabolites—isolated, identified □ Scintillometry, liquid—analysis □ TLC—separation, identity □ IR spectrophotometry—identity □ Mass spectroscopy—identity

The pteridine, 4-amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide (Wy-5256) has been found to be an active diuretic agent having approximately the same oral activity and potency as hydrochlorothiazide (1). The compound possesses a rapid onset of action and a short duration of activity in both the dog and rat, in which optimal effects may be noted at 2 hr. The pharmacology of this drug has been reported by Rosenthale and Osdene (1) and the chemistry by Osdene, *et al.* (2). A preliminary report (3) on the fate of the drug was given in 1967. The present report describes in detail the absorption, distribution, and excretion of the drug in rat, dog, and squirrel monkey, as well as the major metabolic transformation, which takes place by the uncommon route of *O*-demethylation of aliphatic ether side chains. The investigation was facilitated by the use of labeled drug in which the tag was placed on the carbon at the six position of the pteridine nucleus.



Wy-5256

METHODS AND MATERIALS

The labeled drug, 6-^{14}C-4-amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide (Wy-5256) (specific activity, 1.4 mc./mmole) was used.[1] The radioactive drug was prepared from 4,6-diamino-5-nitroso-2-phenylpyrimidine and 2-^{14}C-*N*,*N*′-bis-(2-methoxyethyl)malonamide (2). TLC and autoradiography on medical X-ray film (Cronex II, du Pont) revealed only one radioactive spot.

Absorption and Excretion Studies—Eighteen male rats (250–300 g., Charles River) were given a single oral 4 mg./kg. dose of labeled drug in a 1% nonionic surfactant[2] solution *via* stomach intubation. The rats were fasted overnight prior to administration of the dose and maintained in stainless steel metabolism cages with free access to food and water. Three rats each were anesthetized with ether at 0.5, 1, 3, 6, 24, and 48 hr., and their chests opened. Blood was collected by cardiac puncture, placed in heparinized tubes, and immediately centrifuged. Plasma was separated and frozen at −10° until assayed. The desired tissues and organs were removed for radioassay and urine and fecal samples were also collected and frozen at −10°.

Three pure-bred female beagels and six squirrel monkeys (3 male, 3 female) each received a single oral 4-mg./kg. dose of labeled drug in a 1% nonionic surfactant solution. The animals were fasted overnight prior to oral administration and thereafter maintained in metabolism cages with free access to food and water. Urine and fecal samples were collected up to 48 hr. and stored at −10°. Blood was sampled from each animal at various intervals and prepared for radioassay.

Biliary Excretion Studies—Rats (250–300 g.) were anesthetized with ether, and their bile ducts were cannulated (4) with a polyethylene cannula, which was connected to a tube mounted on the back of the animal. Two days later, the animals were dosed in an unanesthetized state with labeled drug (4 mg./kg. p.o.) and kept unrestrained in metabolism cages with free access to food and water. Urine, feces, and bile were collected at 1, 3, 6, 12, 24, and 48 hr. and frozen at −10° until radioassayed in order to determine the excretion values. TLC followed by autoradiography was performed in order to determine what radioactive products were present.

Radioassay of Samples—Analyses of all radioactive samples were performed in a liquid scintillation spectrometer (Tri-Carb model 3000, Packard Instrument Co., Inc.) at 5° under conditions appropriate for counting ^{14}C. The external standardization technique was employed. All samples were counted in low potassium vials containing either a dioxane scintillation solution (100 g. naphthalene, 10 g. PPO, 250 mg. dimethyl POPOP, and 1 l. dioxane) or a toluene scintillation solution (5 g. PPO, 100 mg. dimethyl POPOP, 1 l. toluene, and 200 ml. absolute ethanol). Urine and bile samples were assayed directly by pipeting aliquots (100 μl.) directly into 15 ml. of the dioxane scintillation solution. Fecal samples were homogenized in distilled water, and samplings were combusted in a combustion apparatus (Thomas-Ogg) in Schoeniger flasks which contained 10 ml. of 10% aqueous ethanolamine solution. Aliquots of this solution after combustion were taken and counted in the dioxane scintillation solution. Plasma (100-μl. aliquots) and 100–250-mg. samples of each tissue and organ were placed in the proper scintillation vials. One milliliter of NCS (Nuclear-Chicago) solubilizer solution was added along with 1 drop of water to each sample after which the vials were capped. Plasma samples were digested at room temperature 18–24 hr., while tissue and organ samples were incubated in a 50° oven for a minimum of 12 hr. or until no solid material was evident upon visual scanning. Toluene scintillation solution was then added to the vials and the samples were counted.

Isolation of Metabolites—Ten dogs were given single 60-mg./kg. doses of unlabeled drug in gelatin capsules and were kept in metabolism cages for 24 hr. with free access to food and water. At the end of that period, all excreta were collected. Feces were homogenized with distilled water (1:3) in a 1-gal. blender (Oster) whereupon the aqueous homogenate was taken up in 10 volumes of acetone. The acetone–water mixture was then separated from the solids by centrifugation. The extract was concentrated at room temperature under re-

[1] Tracerlab, Inc., Waltham, Mass.

[2] Tween 80, Atlas Chemical Co., Wilmington, Del.

Table I—^{14}C Tissue Levels[a] in Male Rats Following a Single Oral 4.0-mg./kg. Dose of ^{14}C-WY-5256

	Time/hr.					
	0.5	1	3	6	24	48
Plasma[b]	0.38	0.72	0.14	0.05	*[c]	—
Liver	3.46	5.65	1.13	0.68	0.06	—
Lung	0.83	1.33	0.23	0.08	0.11	—
Spleen	0.62	1.16	0.16	0.04	*	—
Stomach[d]	76.3	28.4	22.6	5.2	0.1	*
Gastrointestinal tract[d]	20.1	46.2	72.9	94.8	23.2	1.7
Kidney	1.66	3.00	0.73	0.21	0.03	—
Brain	0.19	0.26	—	0.07	0.09	—
Heart	0.83	1.35	0.21	0.11	0.04	—
Muscle	0.47	0.81	0.13	0.05	*	—
Skin	0.51	0.99	0.19	0.23	0.11	*
Fat	0.61	1.60	0.22	0.08	*	*
Bladder	0.73	1.74	0.66	0.18	0.04	—
Testes	0.19	0.46	0.13	0.06	0.03	—
Adrenals	1.58	3.40	0.50	0.17	*	—
Feces[c,d]	*	0.04	0.10	1.84	64.5	103.0
Urine[d]	0.49	2.26	5.38	6.15	9.05	9.56

[a] Each figure represents average value for three rats expressed as equivalent amounts of drug in mcg./g. tissue. [b] mcg./ml. [c] Trace. [d] Percent of dose.

duced pressure until most of the acetone had been driven off, whereupon the remaining aqueous mixture was extracted with chloroform three times. The chloroform extracts were combined and dried over anhydrous magnesium sulfate and concentrated under reduced pressure to a small volume. The residue was chromatographed on a column of alumina (basic; M. Woelm, Eschwege, Germany) employing a chloroform–methanol (1:1) solvent mixture as an eluant. A broad yellow band was eluted from the column until the eluate became colorless. The yellow solution was then concentrated to a small volume which was streaked onto 20 × 20-cm. plates coated with 750-μ layers of alumina (DS-5, Camag) and developed in a *n*-butanol–dimethoxyethane–diethylamine (4:8:2) solvent system until the solvent front had traveled 7 cm. The plates were dried and then chromatographed in a *n*-butanol–dimethoxyethane–pyridine (4:8:3) solvent system for maximum separation. The desired areas, which fluoresced under UV light, were scraped from the plate, and each metabolite was extracted from the adsorbent with methanol. After concentration of the methanolic solutions, each metabolite was subjected to various analytical methods, such as IR spectrophotometry, mass spectrometry, and cochromatography in order to determine the structure of each metabolite.

Quantitation of the Urinary and Fecal Metabolites—Urine samples from each species were extracted with a chloroform–methanol (2:1) solvent mixture and aliquots of the organic and aqueous phases were radioassayed to determine the percentage of excreted dose in each phase. Both phases were then concentrated under reduced pressure at room temperature and the residues were chromatographed on 250-μ layers of alumina. After development of the chromatograms, the plates were dried and analyzed by autoradiography using medical X-Ray film (Cronex II, du Pont). Quantitative evaluation of each metabolite was performed by scraping each radioactive area from the plate and assaying it in dioxane scintillator solution.

The fecal samples were homogenized with distilled water (1:3) in a blender (Osterizer) until a thin, pasty consistency was attained. The radioactive products were extracted from the homogenate with ten volumes of acetone. This extract was concentrated under reduced pressure until only water remained and then shaken with chloroform. The organic extract was removed, and the aqueous phase washed twice with further portions of chloroform. The organic extracts were combined, whereupon both the organic and aqueous phases were analyzed in the same manner as the urine extracts.

Synthetic Procedures—Unlabeled drug (I) and 4-amino-*N*-(2-hydroxyethyl)-7-(2-hydroxyethylamino)-2-phenyl-6-pteridinecarboxamide (IV), the bis-demethylated metabolite, were prepared by the method reported by Osdene *et al.* (2). The mono-demethylated metabolites were prepared by the following route:

Diethyl malonate (48 g., 0.3 mole) and β-methoxyethylamine (22.5 g., 0.3 mole) were refluxed for 2 hr. The mixture was concentrated and crystallized from benzene–cyclohexane (1:4). The crude product, which consisted of ethyl-*N*-(2-methoxyethyl)malonamate and *N*,*N*′-bis-(2-methoxyethyl)malonamide, was collected by filtration, dried, and then added to 18.3 g. ethanolamine (0.3 mole). This mixture was refluxed for 1 hr. concentrated, and crystallized from diethyl ether. After recrystallization from ethanol-ether, *N*-(2-hydroxyethyl)-*N*′-(2-methoxyethyl)malonamide melted at 82–83°: yield, 33%.

Anal.—Calcd. for $C_8H_{16}N_2O_4$: C, 47.10; H, 7.84; N, 13.71. Found: C, 46.88; H, 7.88; N, 13.83.

N-(2-Hydroxyethyl)-*N*′-(2-methoxyethyl)malonamide (6.2 g., 0.03 mole) and 6.55 g. 4,6-diamino-5-nitroso-2-phenylpyrimidine (0.03 mole) were added to a solution of 0.7 g. sodium in 300 ml. absolute ethanol. The mixture was refluxed for 1 hr. and cooled to room temperature. After filtering off the yellow precipitate which contained the sodium salts of 4-amino-7-hydroxy-*N*-(2-hydroxyethyl)-2-phenyl-6-pteridinecarboxamide and 4-amino-7-hydroxy-*N*-(2-methoxyethyl)-2-phenyl-6-pteridinecarboxamide, the filtrate was concentrated under reduced pressure and the residue was chromatographed by preparative TLC on Silica Gel G (E. Merck Ag., Darm-

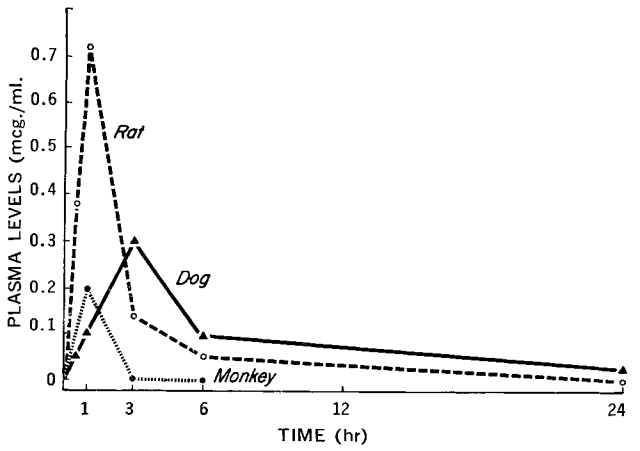


Figure 1—*Plasma levels following a single oral 4.0-mg./kg. dose of ^{14}C-Wy-5256 expressed as equivalent amounts of drug in mcg./ml.*

Table II—Excretion of Radioactive Products Following a Single Oral 4.0-mg./kg. Dose[a]

		Time, hr.				
		1	3	6	24	48
Monkey	Urine	0.03	0.06	0.07	0.3	0.75
	Feces	—	—	48.5	77.5	80.3
Dog	Urine	0.09[b]	3.58[b]	5.00[b]	5.84	6.07
	Feces	—	—	—	55.5	75.7
Rat	Urine	2.26	5.38	6.15	9.05	9.56
	Feces	—	0.1	1.84	64.5	103.0

[a] Values expressed as cumulative percent of dose. [b] Samples collected by catheterization.

Table III—Excretion of Radioactive Products in Rats Possessing Externalized Biliary Fistulae—Dose 4 mg./kg., p.o.[a]

		Time, hr. 1	3	6	12	24	Total
Rat A	Urine	0.97	2.82	1.95	0.97	0.15	7.33
	Bile	8.59	20.36	14.46	6.46	0.45	50.32
	Feces	—	—	2.3	13.2	7.5	23.0
Rat B	Urine	1.1	3.7	3.6	0.9	0.4	9.7
	Bile	6.14	22.70	16.35	4.84	1.38	57.38
	Feces	—	—	—	5.0	14.1	19.1
Rats without fistulae	Urine	2.26	3.12	0.77	[b]	2.90	9.05
	Feces	—	0–1	1.84	[b]	62.7	64.5

[a] Values expressed as percent of dose. [b] No sample taken.

stadt, Germany) using a *n*-butanol–diethylamine–dimethoxyethane (4:2:8) solvent system. The yellow band at R_f 0.77 was extracted from the adsorbent with methanol and after concentration of the methanolic solution yellow crystals appeared which were collected by suction filtration: 4-Amino-7-(2-hydroxyethylamino)-*N*-(2-methoxyethyl)-2-phenyl-6-pteridinecarboxamide (III) melted at 227–228°.

Anal.—Calcd. for $C_{18}H_{21}N_7O_3$: C, 56.39; H, 5.48; N, 25.58. Found: C, 56.26; H, 5.40; N, 25.27.

A yellow band at R_f 0.64 was also extracted from the chromatogram with methanol. After concentration of the methanolic solution, 4-amino-*N*-(2-hydroxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide (II) crystallized and was collected by suction filtration, m.p. 217–219°. Both isomers were analyzed by mass spectrometry in order to assign the proper structure for each.

RESULTS

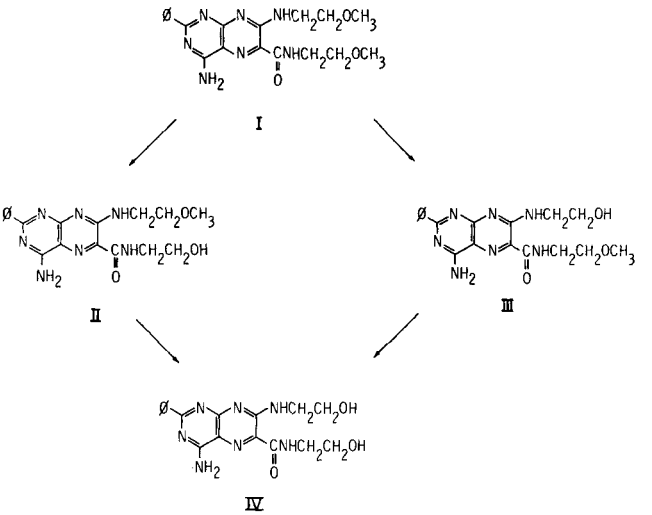
Absorption—Upon administration of a pharmacologically active dose of 6-^{14}C-4-amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide (4 mg./kg., p.o.), evidence of rapid absorption of the drug was found in each species as illustrated by the plasma levels in Fig. 1. After 0.5 hr., the rat exhibited significant radioactive levels corresponding to 0.38 mcg./ml. of Wy-5256 and within 1 hr., a peak level was obtained corresponding to 0.72 mcg/ml. The peak occurred at the same time in the squirrel monkey, but a considerably lower value corresponding to 0.20 mcg./ml. was noted. The dog achieved a peak level in plasma corresponding to 0.30 mcg./ml. after 3 hr. The half-life of the radioactive dose in the rat and monkey was estimated to be 2 hr., and 5 hr., in the dog. The variations in plasma levels and half lives observed among the species indicate differences in rates of metabolism and excretion and/or rapid uptake by tissues. Within 24 hr., negligible values were found in the plasma of the three species.

Tissue Distribution—The distribution of the radioactive dose at various time intervals was investigated in male rats following a single oral dose of labeled drug (Table I). Concentrations were higher in most tissues than in plasma at all times, and remained fairly constant relative to plasma concentration throughout the experiment. Liver tissue, which exhibited the highest values, showed no tendency to accumulate any of the dose as indicated by the rapid decline after 1 hr. Peak levels were also attained in lung, kidney, spleen, and heart at 1 hr. and fairly rapid clearance occurred thereafter. At 6 hr., the major part of the dose was present in the gastrointestinal tract. By 48 hr., the drug and its metabolites were almost completely eliminated.

Excretion—The urinary and fecal recoveries of radioactivity in the rat, dog, and squirrel monkey after a single oral dose (4 mg./kg.) are detailed in Table II. The rat excreted 9.56% of the dose in the urine within 48 hr. Of this, more than half was excreted in the first 3 hr. The rest of the radioactivity was found in feces. Similarly, the dog excreted about 12 times as much of the administered dose in feces as in urine. The difference was even more marked in the squirrel monkey which excreted less than 1% of the dose in urine.

Although it was reasonable to expect a drug in this molecular weight range to be excreted chiefly in bile, the authors nevertheless investigated the possibility that the high fecal levels might have resulted from poor absorption. Rats with externalized biliary fistulae were dosed with labeled drug (4 mg./kg., p.o.) in an unanesthetized, unrestrained state, and the excretion values were compared with those of control rats (Table III). The fecal excretion rates were markedly lower in the two cannulated rats than in the controls, whereas no significant difference was noted in the urinary excretion rates. The cannulated rats excreted approximately half of the dose in bile, and most of this occurred within the first 6 hr. Reabsorption of the drug and its metabolites from the intestine did not seem to be a major factor. Therefore, the results received from this biliary excretion study indicate that the low urinary levels, at least in this species, are not the result of poor absorption of this virtually insoluble drug, but rather of physico-chemical factors favoring biliary excretion.

Nature of the Urinary and Fecal Metabolites—Examination of the excreta of each species revealed that all species qualitatively transformed the drug by the same pathway. The uncommon manner in which the drug was metabolized occurred by *O*-demethylation of the aliphatic ether side chain in either the six or seven position of the molecule. This pathway led *via* the two minor intermediate monodemethylated metabolites to the major bis-demethylated metabolite (Scheme I). These metabolites, along with unchanged drug and three



Scheme I

unidentified water-soluble metabolites, made up the radioactive products found in both urine and feces of each species. This was initially determined by autoradiography of thin-layer chromatograms containing excretory extracts.

Rat plasma extracts were found to contain unchanged drug and the demethylated metabolites but very little polar material while bile

Table IV—Distribution in Excreta of Radioactive Unchanged Drug and Metabolites[a]

	Dog Urine	Dog Feces	Rat Urine	Rat Feces	Monkey Urine	Monkey Feces
Unchanged drug I	1.1	6.1	2.1	13.1	2.2	15.2
Metabolite II	4.0	3.2	12.9	20.1	2.3	3.8
Metabolite III	4.7	7.5	4.5	2.3	3.6	15.2
Metabolite IV	67.6	45.1	33.9	22.9	45.9	33.6
Polar metabolites	22.6	38.1	46.6	41.6	46.0	32.2

[a] Within 24-hr. period percent of total ^{14}C in sample.

Table V—R_f Values of WY-5256 and Metabolites

	I	II	III	IV
BuOH–DME[a]–DEA[b](4:8:2)	0.84	0.39	0.75	0.18
BuOH–DME–pyridine (4:8:3)	0.75	0.34	0.63	0.15
BuOH–Bu_2O[c]–AcOH(4:8:2)	0.70	0.51	0.61	0.43

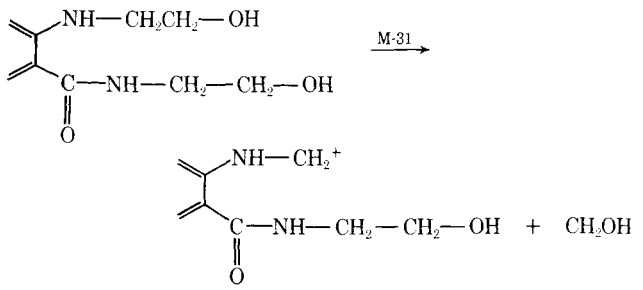
[a] DME = dimethoxyethane. [b] DEA = diethylamine. [c] Bu_2O = dibutyl ether.

extracts contained mainly the bis-demethylated metabolite, IV, and the polar fraction. Negative results were obtained upon reaction of the polar fraction with β-glucuronidase, and no further attempts have been made to identify this fraction.

Table IV details the distribution pattern of the metabolites in the excreta of each species. The parent drug I, was extensively transformed, and recovered in feces only to the extent of 15.2% in the monkey, 13.1% in the rat, and 6.1% in the dog. The bis-demethylated metabolite, IV, was found to be the major metabolite excreted by each species, and both mono-demethylated metabolites were present in minor amounts.

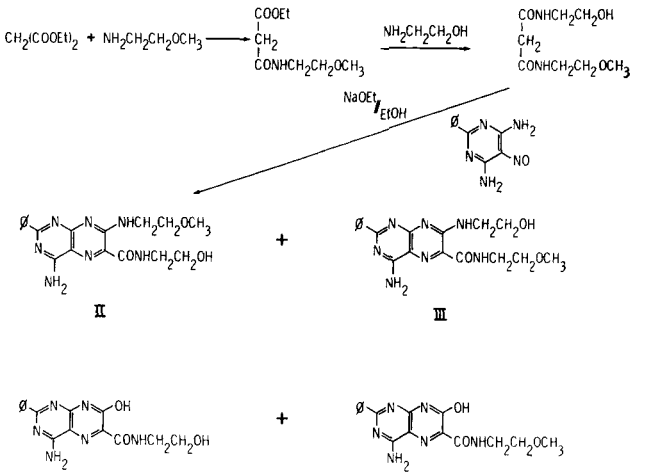
Identification of the Metabolites and Parent Drug—After each product had been isolated by preparative TLC as described in *Methods and Materials*, the product with the highest R_f value (see Table V) was compared with synthetic 4-amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide. The identity of the isolated material (I) was established by cochromatography with synthetic Wy-5256, mass spectrometry, and IR spectroscopy (Table VI).

The bis-demethylated metabolite, 4-amino-*N*-(2-hydroxyethyl)-7-(2-hydroxyethylamino)-2-phenyl-6-pteridinecarboxamide, IV, was characterized by comparison of its IR spectrum with that of authentic material, by mixed melting point, and cochromatography and the mass spectrum, which, although showing some impurities, gave mass peaks at *m/e* 369, 338, and 325. The mass peak at *m/e* 338 arose from the rupture of the bond β to the nitrogen atom of the amino group.



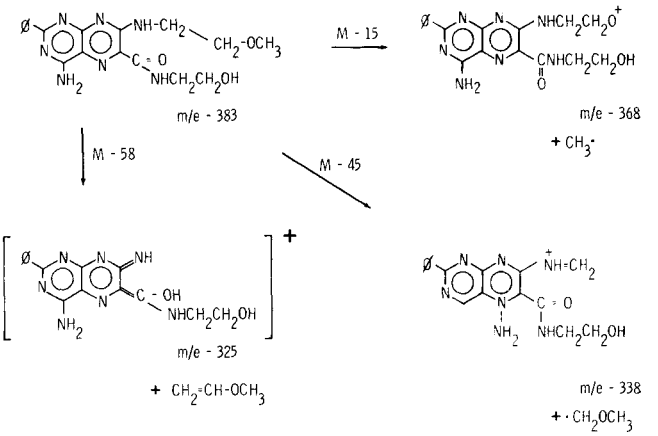
This cleavage would predominate over any cleavage of the amide group in the six position.

With the identification of the bis-demethylated compound as Metabolite IV, the two mono-demethylated precursors were then postulated as primary metabolites and the authentic materials were synthesized as illustrated in Scheme II.



Scheme II

The two isomers were assigned proper structures by employing mass spectrometric analysis. Both compounds had the expected parent ion at *m/e* 383. Synthetic Compound II, however, gave a fragmentation pattern with mass peaks at *m/e* 368, 338, and 325 and the pattern may be postulated as illustrated in Scheme III.



Scheme III

This pattern was similar to that of the parent drug, and was quite different from that of the bis-demethylated metabolite. The cleavage which gave rise to the M-58 ion has been reported frequently and has been discussed in detail (5, 6). Compound III had significant mass fragments at *m/e* 353 and 352 while peaks at *m/e* 338 and 325 were

Table VI—Physical Constants of Isolated Metabolites and Standards

	M.p., °C.	R_f[a]	IR (μ)	Mass Spectra, *m/e*
Unchanged Drug (I)	231–233	0.83	6.02(s),6.19(s) 6.3(m),6.5(vs)	397(M^+·),382(M-15) 352(M-45),339(M-58)
4-Amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide[b]	234	0.84	6.02(s),6.19(s) 6.30(m),6.50(vs)	397(M^+·),382(M-15) 352(M-45),339(M-58)
Metabolite II	—	0.39	—	383(M^+·),368(M-15) 338(M-45),325(M-58)
4-Amino-*N*-(2-hydroxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide(II)[b]	217–219	0.39	6.02(s),6.18(s) 6.3(m),6.52(vs)	383(M^+·),368(M-15) 338(M-45),325(M-58)
Metabolite III	—	0.75	—	—
4-Amino-7-(2-hydroxyethylamino)-*N*-(2-methoxyethyl)-2-phenyl-6-pteridinecarboxamide(III)[b]	227–228	0.75	6.01(s),6.10(s) 6.30(m),6.48(vs)	383(M^+·),353,352(M-31)
Metabolite IV	249–252	0.18	6.01(m),6.12(s) 6.28(m),6.55(vs)	369(M^+·),338(M-31),325(M-44), 103
4-Amino-*N*-(2-hydroxyethyl)-7-(2-hydroxyethylamino)-2-phenyl-6-pteridinecarboxamide(IV)[b]	252	0.18	6.01(m),6.12(s) 6.28(m),6.55(vs)	369(M^+·),338(M-31),325(M-44) 103

[a] Al-250 μ BuOH–DME–DEA (4:8:2). [b] Synthetic metabolites.

Table VII—Diuretic Testing of WY-5256 and its Metabolites in Rats

	Dose, mg/kg.	Route	T/U[a] Vol.	T/U[a] Na
Wy-5256	6	p.o.	2.43	2.25
Metabolite II	6	p.o.	1.27	1.23
Metabolite III	3	p.o.	1.21	1.29
	6	p.o.	1.89	1.80
Metabolite IV	12	p.o.	0.89	0.91
	25	p.o.	0.82	0.75
	25	i.p.	1.51	1.57

[a] Ratios of urine volumes and urinary sodium levels in animals administered test drug or metabolite (T) to those administered corresponding doses of urea (U). Ratios > 1.00 represent statistically significant differences in diuretic activity.

negligible. This splitting out of 31 mass units could be expected for a β-hydroxyethylamino group in the seven position, as evidenced by the mass spectrum of the bis-demethylated metabolite. This *m/e* 352 fragment was negligible in the mass spectrum of Compound II.

With the establishment of structure for the two synthetic isomers, a comparison was made between the mass spectra of the synthetic Compounds II and III, and the isolated Metabolite II. The fragmentation pattern of the metabolite was similar to synthetic Compound II and cochromatography confirmed the structure of that metabolite as 4-amino-*N*-(2-hydroxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide. Finally, drug Metabolite III, although too small in quantity and highly impure, cochromatographed with authentic 4-amino-7-(2-hydroxyethylamino)-*N*-(2-methoxyethyl)-2-phenyl-6-pteridinecarboxamide, III.

Diuretic Activity of the Metabolites—The pharmacological activity of the metabolites was investigated and the structure-activity relationship is illustrated in Table VII. It appears that demethylation of either chain reduces the diuretic activity. Demethylation of both side chains appears to interfere with absorption of the drug, as indicated by the diuresis following intraperitoneal administration when compared with the lack of activity when the dose is given orally.

DISCUSSION

The results reported herein demonstrate that the metabolite fate of the parent pteridine is quite similar in all three investigated species, although there is some difference in the absorption and excretion of the drug among the monkey, rat, and dog. The drug, which was labeled, thereby greatly facilitating the study, was given orally at the pharmacologically active dose in order to provide some insight into the activity of the drug. These studies find that the rat absorbs the drug into plasma rapidly as does the monkey, but there is a 3.5-fold difference in the plasma levels between the two. The dog absorbs the drug slightly slower, and not to as great an extent as the rat. Both peak levels and half-life values, however, are found to correlate well with the length of diuretic activity in all three species.

The short acting properties of the drug may be due to the rapid absorption and release from tissues, as observed in a tissue distribution study in male rats. No accumulation was seen in any tissue, and negligible values were found in tissues, as had been found in plasma after 6 hr. As our results show a rapid absorption, they also show a rapid excretion of the dose. A biliary excretion study demonstrates that a major part of the dose is excreted rapidly through the bile into feces as deactivated metabolites. This was anticipated because of the drug's high molecular weight. The biliary excretion is found to occur to a major extent within 6 hr., and the urinary excretion rate is also greatest in the first 6 hr. All of these data point to the fact that the drug does not remain in the body of any animal to any great extent after 6 hr., thereby probably creating the short duration of activity. But this rapid excretion is not the only property that has to be taken into account as the cause of the drug's short activity. Metabolic transformation of the parent drug by *O*-demethylation of both aliphatic ether side chains also reduces the diuretic activity of the drug as shown by the structure-activity relationship, in which if only one methyl group is removed a slight lessening of activity occurs, but removal of both methyl groups to the major bisdemethylated metabolite prevents activity of the drug. This metabolic transformation, though rare, occurs in all three species and does provide a method of deactivation. Therefore, although the data give no direct information on the diuretic properties of Wy-5256, they do provide some understanding of how the metabolic fate of the drug relates to the drug's activity.

SUMMARY

The absorption, distribution, metabolism, and excretion of an orally active pteridine diuretic, 4-amino-*N*-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide, was studied with the aid of the ^{14}C-labeled drug, in the rat, dog, and squirrel monkey. All three species metabolized the drug similarly by successive removal of the methyl ether groups of the two aliphatic side chains. Identification of three metabolites, two monodemethylated isomers, and the bis-demethylated compound, was made by cochromatography, mass spectrometry, and IR spectrophotometry.

The fecal route of excretion predominated in all three species with only 10% or less of an oral dose appearing in urine. That this was not due to poor absorption of drug was demonstrated by studies in bile fistula rats who excreted about half the dose in bile within 6 hr. There was rapid uptake in tissue, but no accumulation in any organ. The rapid metabolism and excretion of the drug are believed to contribute to its relatively short duration of activity.

REFERENCES

(1) M. E. Rosenthale and T. S. Osdene, *Arch. Intern. Pharmacodyn.*, **164,** 11(1966).

(2) T. S. Osdene, A. A. Santille, L. E. McCardle, and M. E. Rosenthale, *J. Med. Chem.*, **9,** 697(1966).

(3) S. F. Sisenwine, and S. S. Walkenstein, *Pharmacologist*, **9** (2), 215(1967).

(4) G. T. Stewart and P. M. Harrison, *Brit. J. Pharmacol.*, **17,** 414(1961).

(5) K. Biemann, "Mass Spectrometry," McGraw-Hill, New York, N. Y., 1962, Chap. 3.

(6) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, Chap. 4.

ACKNOWLEDGMENTS AND ADDRESSES

Received January 27, 1969, from the *Metabolic Chemistry Department, Wyeth Laboratories, Inc., Philadelphia, PA 19101*

Accepted for publication March 31, 1969.

Presented in part at the Fall meeting of the American Society of Pharmacology and Experimental Therapeutics, Washington, DC, August, 1967.

* Present address: Smith, Kline & French Laboratories, Philadelphia, PA

The authors thank Mrs. E. Tober and Miss H. Kimmel for their technical assistance; also Dr. G. Owen for his cooperation in the animal studies, Dr. S. Shrader for his aid in interpreting the mass spectra, and Dr. M. Rosenthale for the pharmacological studies on the activity of the drug and its metabolites.