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METABOLISM OF THE NON-NARCOTIC ANALGESIC, WY-535

SIDNEY S. WALKENSTEIN, ROBERT A. CORRADINO, ROBERT WISER and CATHRYN H. GUDMUNDSEN

Radiochemistry Department, Wyeth Laboratories, Radnor, Pa., U.S.A.

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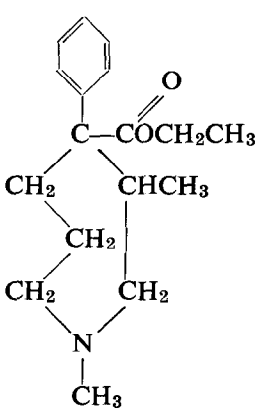
Abstract—Hexahydro-1,3-dimethyl-4-phenylazepine-4-^{14}C-carboxylic acid, ethyl ester (Wy-535), was administered to dogs and rats both orally and intramuscularly. Tissue levels were determined in the rat, and plasma levels as well as urinary and fecal excretion rates were determined in both species.

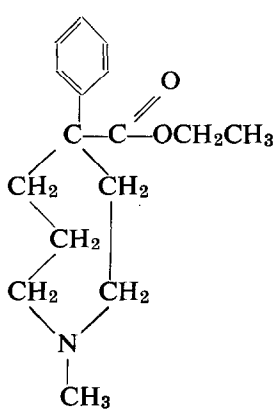
The drug appeared to be well absorbed; significant plasma levels were found at ½ hr in both species after both routes of administration. It was well distributed to all organs studied and gave no evidence of any long-term retention in tissue.

In addition to unchanged drug, three metabolites were excreted. These were, as anticipated from a previous study of the chemically related ethoheptazine, the products of N-demethylation and hydrolysis, namely, nor-Wy-535 and the de-esterified products of both Wy-535 and nor-Wy-535.

HEXAHYDRO-1,3-dimethyl-4-phenylazepine-4-carboxylic acid, ethyl ester (Wy-535), has been reported to be a potent analgesic agent free from many of the side effects of the narcotic analgesics.[1,2] A metabolic study of the chemically related ethoheptazine[3] indicated that Wy-535 would be subject to N-demethylation, de-esterification and, possibly, hydroxylation of the azepine ring. Accordingly, Wy-535 was labeled with ^{14}C in the carboxyl carbon and its biodegradation, disposition, and excretion studied in rat and dog.



Wy 535



Ethoheptazine

MATERIALS AND METHODS

Synthesis of ^{14}C*-Wy*-535. ^{14}C-Labeled phenylacetonitrile was prepared from benzyl chloride and potassium cyanide-^{14}C according to the procedure of Adams and Thal,[4] and converted to Wy-535 by a slightly modified version of the scheme of Diamond and Bruce.[5] The product, isolated as the hydrochloride, had a specific activity of 1,040 dpm/μg. Radiopurity was confirmed by paper chromatography in the two solvent systems given below.

Synthesis of non-radioactive nor-Wy-535, Hexahydro-4-cyano-1,3-dimethyl-4-phenylazepine was demethylated by means of the von Braun cyanogen bromide reaction.[6] The intermediate cyanamide was hydrolyzed and esterified to yield hexahydro-3-methyl-4-phenylazepine-4-carboxylic acid, ethyl ester (nor-Wy-535) which was isolated as the maleate, m.p. 143°–145°. Calculated for $C_{20}H_{27}NO_6$ C, 63·64%; H, 7·21%; N, 3·71%. Found: C, 63·32%; H, 7·21%; N, 3·81%. Nor-Wy-535 undergoes rapid hydrolysis in the presence of water, irrespective of pH.

Administration of drug. For the oral study in dogs, the drug was administered in gelatin capsules to each of three fasted animals at an average dose of 10 mg/kg. Three other fasted dogs were injected i.m. at a dose of 10 mg/kg with a solution containing 50 mg Wy-535-^{14}C/ml saline. Plasma, urine, and feces from all six dogs were sampled at intervals up to 96 hr.

For the tissue distribution study in rats, the drug was administered either orally (stomach tube) in solution at a concentration of 1 mg/ml H_2O or i.m. at a concentration of 10 mg/ml saline. Rats were sacrificed at time intervals up to 48 hr, three rats to each interval, and organs, tissues, and excreta were taken for assay. Urine and feces were collected only at time of sacrifice; thus each collection represents the total amount excreted by the animal from the time of drug administration.

Radioassay of samples. Stomach, intestine, cecum, and feces were homogenized in water, aliquots were subjected to Schoeniger combustion, and the CO_2 formed was absorbed in aqueous ethanolamine. Aliquots of the ethanolamine solution were placed in dioxane scintillator solution for counting. Urine was pipetted directly into the dioxane counting solution. All other samples were dissolved in hydroxide of Hyamine-10x and assayed in a toluene–alcohol counting system. Assay of radioactive zones on paper chromatograms was performed with a thin-window Geiger-Müller counter.

Paper chromatography. Urine was spotted directly on Whatman 1 paper and was developed in the descending manner in methanol:ethyl acetate:1 M NH_4Ac (pH 7), 26:2:2, and ascending in butanol saturated with 1 M NH_4Ac (pH 7). For visualization of radioactive spots, chromatograms were placed in contact with Kodak No-Screen X-ray film. The chromatograms were also sprayed with Dragendorff's reagent or 0·05% bromcresol green and, for detection of secondary amines, 1% sodium nitroprusside in 10% acetaldehyde, followed by 2% sodium carbonate when the paper was still damp.[7]

RESULTS AND DISCUSSION

Plasma levels in dogs

The drug appeared to be well absorbed after oral administration, as evidenced by the appearance of a measurable level in plasma within ½ hr (Table 1). The peak occurred

at 2 hr, low levels of radioactivity persisted through the 48th hour in all three dogs, and at 72 hr activity was still measurable. By 96 hr, however, the plasma of all three animals had returned to background values.

After i.m. administration, ^{14}C was detected in plasma within the first 15 min (Table 2). A peak level of 7·6 μg/ml was reached by one dog in the first hour, the other

TABLE 1. ^{14}C LEVELS IN PLASMA AND EXCRETA OF DOGS AFTER A SINGLE ORAL 10-MG DOSE OF ^{14}C-WY-535 PER KG

Time (hr)	Dog no.	Plasma (μg/ml)	Urine	Feces
			Cumulative % of dose	
¼	A	0·00	0·00	
	B	0·00	0·00	
	C	0·00	0·00	
½	A	0·00	0·00	
	B	1·32	0·12	
	C	0·66	0·03	
1	A	2·05	0·62	
	B	5·43	2·25	
	C	4·81	1·82	
2	A	6·43	6·86	
	B	7·00	12·6	
	C	6·63	9·66	
4	A	6·22	15·2	
	B	5·71	28·9	
	C	3·44	26·1	
6	A	4·01	34·2	
	B	3·88	40·1	
	C	2·84	35·4	
24	A	0·97	67·6	0·16
	B	0·66	71·2	0·00
	C	0·34	56·4	14·2
48	A	0·54	78·0	18·00
	B	0·34	72·4	22·6
	C	0·25	68·0	19·6
72	A	0·25	80·4	20·0
	B	0·00	73·6	25·1
	C	0·00	69·1	20·3
96	A	0·00	81·9	20·3
	B	0·00	74·0	25·7
	C	0·00	69·6	21·2

two showing a maximum of just under 3 μg/ml at the fourth hour. The plasma level then decreased at about the same rate as in the orally dosed dogs, although activity was still present at 96 hr.

Urinary excretion in dogs

A measurable amount of radioactivity was found in the urine as early as ½ hr, regardless of the route of administration (Tables 1 and 2). As would be expected,

those animals which showed the more rapid rise in plasma level also showed a faster urinary output of the drug in the first 4 hr. By the sixth hour, an average of approximately $\frac{1}{3}$ of the dose could be accounted for in the urine. There was no significant difference in the urinary excretion pattern between the orally and the intramuscularly dosed animals.

TABLE 2. ^{14}C LEVELS IN PLASMA AND EXCRETA OF DOGS AFTER A SINGLE INTRAMUSCULAR 10-MG DOSE OF ^{14}C-WY-535 PER KG

Time (hr)	Dog no.	Plasma (μg/ml)	Urine	Feces
			Cumulative % of dose	
$\frac{1}{4}$	A	2·96	0·00	
	B	0·95	0·00	
	C	0·31	0·00	
$\frac{1}{2}$	A	4·93	2·75	
	B	1·44	1·23	
	C	0·89	0·14	
1	A	7·61	7·08	
	B	2·14	6·98	
	C	1·76	0·55	
2	A	7·12	12·7	
	B	2·97	14·2	
	C	2·55	3·00	
4	A	5·35	31·3	
	B	2·98	32·5	
	C	2·83	10·7	
6	A	4·13	37·3	
	B	1·94	37·7	
	C	2·19	15·8	
24	A	1·23	56·5	0·00
	B	0·54	66·2	15·3
	C	0·97	38·0	0·00
48	A	0·34	68·3	4·80
	B	0·00	72·3	23·1
	C	0·67	54·8	5·69
72	A	0·00	70·3	15·5
	B	0·00	73·8	25·5
	C	0·39	65·4	9·95
96	A	0·00	70·9	15·9
	B	0·00	74·3	26·2
	C	0·25	72·8	16·8

Fecal excretion in dogs

Regardless of route of administration, an average of $\frac{1}{5}$ of the administered dose was accounted for in fecal radioactivity in 96 hr (Tables 1 and 2). Fecal excretion was initially more rapid in the orally dosed animals. In the oral dogs only a trace was excreted over the next two days, while about 9% was accounted for in the i.m. animals; the total percentage excreted in feces by the two sets of animals was approximately the same.

TABLE 3. ^{14}C-TISSUE LEVELS IN RATS AFTER A SINGLE ORAL 10-MG DOSE OF ^{14}C-WY- -535 PER KG (μg/g tissue)

Time (hr)	Rat no.	Plasma	Liver	Lung	Spleen	Stomach*	Small Intestine*	Cecum*	Kidney	Brain	Heart	Muscle	Skin	Fat	Bladder	Feces*	Urine*
½	1	2·59	27·6	22·1	4·84	21·0	36·1	0·7	14·2	2·14	2·64	1·91	2·03	3·78	10·2	0·00	1·76
	2	2·65	30·8	28·3	9·52	25·2	26·2	1·0	19·5	2·86	5·58	1·95	1·30	6·03	7·33	0·00	1·43
	3	2·21	29·1	19·4	7·34	32·7	34·5	0·6	12·2	1·65	2·70	1·19	1·10	2·45	9·33	0·00	0·12
1	4	2·38	21·7	17·8	8·43	15·7	41·2	0·5	13·9	3·15	4·41	3·02	2·81	5·26	13·8	0·00	6·39
	5	2·34	28·2	13·5	7·18	7·15	49·0	0·9	13·3	2·37	3·93	2·64	2·60	2·76	9·10	0·00	5·13
	6	2·31	21·5	13·5	6·57	15·4	48·3	0·3	11·4	2·66	3·36	2·58	3·10	3·66	7·17	0·00	6·18
2	7	1·47	10·8	6·61	3·24	1·01	58·8	0·4	4·82	1·00	1·58	1·07	1·01	1·22	9·77	0·00	8·60
	8	1·30	11·3	11·3	4·49	1·07	66·4	0·5	7·82	1·32	2·94	1·75	2·12	2·10	4·77	0·00	8·21
	9	1·04	10·8	7·15	3·00	0·64	72·6	0·5	6·71	1·14	1·70	1·20	1·25	2·20	17·1	0·00	15·1
4	10	0·80	9·72	3·76	1·09	0·27	49·0	19·1	2·91	0·42	0·75	0·43	0·00	0·79	23·7	0·00	14·1
	11	0·48	7·42	0·56	0·17	0·00	72·5	5·2	1·57	0·00	0·00	0·00	0·00	0·00	4·02	0·00	13·1
	12	0·38	7·92	1·48	0·28	0·30	72·3	4·3	2·14	0·17	0·00	0·21	0·00	0·16	13·8	0·00	11·5
6	13	0·33	6·77	0·00	0·00	0·00	26·2	47·4	1·27	0·00	0·00	0·00	0·00	0·00	0·40	0·00	23·3
	14	1·28	8·55	1·74	0·00	0·42	65·4	0·7	3·19	0·22	0·36	0·00	0·24	0·19	12·7	0·00	26·3
	15	0·64	9·59	0·47	0·00	0·36	24·9	44·5	2·06	0·12	0·00	0·00	0·00	0·16	23·8	0·00	22·8
24	16	0·00	0·54	0·00	0·00	0·00	2·47	13·5	0·00	0·00	0·00	0·00	0·00	0·00	0·00	34·2	45·9
	17	0·21	0·55	0·00	0·00	0·00	1·99	10·4	0·00	0·00	0·00	0·00	0·00	0·00	0·00	38·2	33·5
	18	0·13	0·65	0·00	0·00	0·00	3·30	12·6	0·00	0·00	0·00	0·00	0·00	0·00	0·07	43·1	37·9
48	19	0·00	0·00	0·00	0·00	0·00	0·00	1·3	0·00	0·00	0·00	0·00	0·00	0·00	0·00	65·6	38·5
	20	0·00	0·00	0·00	0·00	0·00	0·61	2·4	0·00	0·00	0·00	0·00	0·00	0·00	0·00	53·0	35·3
	21	0·00	0·34	0·00	0·00	0·00	0·53	3·4	0·00	0·00	0·00	0·00	0·00	0·00	0·00	50·5	48·6

* Total content in per cent of dose.

TABLE 4. ^{14}C TISSUE LEVELS IN RATS AFTER A SINGLE INTRAMUSCULAR 10-MG DOSE OF ^{14}C-WY-535 PER KG ($\mu g/g$ tissue)

Time (hr)	Rat no.	Plasma	Liver	Lung	Spleen	Stomach	Small Intestine	Cecum	Kidney	Brain	Heart	Muscle	Skin	Fat	Bladder	Feces*	Urine	Inject site*
½	1	0·42	5·22	33·6	9·51	0·00	12·5	1·87	10·9	5·73	3·35	1·52	1·59	2·46	4·93	0·00	0·00	70·6
	2	0·48	5·94	44·2	9·74	0·00	11·0	0·00	12·5	6·66	5·12	1·39	1·82	3·41	2·81	0·000	0·36	68·8
	3	1·15	11·7	60·6	17·5	13·6	26·1	4·67	20·4	11·1	7·92	3·96	2·01	8·68	11·5	0·00	0·00	61·6
1	4	1·13	8·51	49·4	9·47	0·78	23·2	3·43	16·2	7·45	5·76	1·66	1·57	5·03	19·4	0·00	0·00	66·9
	5	1·35	9·02	45·1	13·4	2·88	31·2	3·95	15·6	7·10	4·67	1·87	1·64	5·38	42·7	0·00	0·00	73·0
	6	1·05	8·20	42·4	8·74	0·00	18·0	2·25	10·9	6·24	4·47	1·81	1·27	3·82	26·4	0·00	0·00	72·6
2	7	1·72	16·1	49·8	15·0	9·53	153	5·69	13·9	7·75	7·09	2·59	3·12	8·54	49·2	0·00	8·02	18·9
	8	1·89	18·8	48·2	17·4	21·5	140	8·63	18·6	8·78	6·23	3·61	2·62	10·4	27·8	0·00	0·87	21·5
	9	1·78	16·2	37·7	11·7	11·2	133	5·76	13·6	6·89	5·06	2·49	3·29	6·81	74·0	0·00	12·6	13·1
4	10	1·18	13·1	28·2	8·52	3·82	127	6·72	14·5	5·24	3·77	1·32	1·83	6·25	14·7	0·00	12·3	25·3
	11	1·31	17·6	16·0	6·48	3·29	187	36·9	11·1	3·47	1·62	1·02	1·45	5·57	84·4	0·00	21·5	4·56
	12	1·07	13·7	31·7	7·97	9·15	184	13·8	12·4	4·21	3·99	1·34	2·16	5·38	26·2	0·00	8·65	17·2
6	13	0·76	10·8	3·09	1·91	7·88	197	99·0	2·98	0·66	0·87	0·63	0·66	1·32	1·20	0·06	27·6	1·03
	14	1·51	15·3	9·73	3·47	2·38	139	75·6	8·10	1·24	0·99	0·72	0·88	2·52	—	0·09	17·0	2·13
	15	0·65	13·4	2·04	0·92	8·86	136	151	3·43	0·47	0·76	0·49	0·58	0·66	2·13	0·00	22·2	12·9
24	16	0·00	3·15	0·00	0·27	0·00	9·67	25·7	0·94	0·00	0·00	0·00	0·00	0·00	0·40	35·6	52·6	0·32
	17	0·00	2·49	0·00	0·00	0·00	16·3	17·9	0·71	0·00	0·00	0·20	0·00	0·00	0·47	38·7	48·1	0·28
	18	0·00	4·53	0·00	0·38	0·00	10·0	38·7	0·59	0·00	0·00	0·00	0·00	0·00	0·44	26·9	48·1	0·00
48	19	0·00	1·03	0·00	0·00	0.00	1·61	6·85	0·00	0·00	0·00	0·00	0·00	0·00	0·18	36·4	53·7	0·00
	20	0·00	0·88	0·00	0·00	0·00	0·00	4·43	0·00	0·00	0·00	0·00	0·00	0·00	0·00	40·7	46·3	0·00
	21	0·00	1·00	0·00	0·00	0·00	0·00	3·41	0·00	0·00	0·00	0·00	0·00	0·00	0·00	44·7	57·8	0·00

* Total content in per cent of dose.

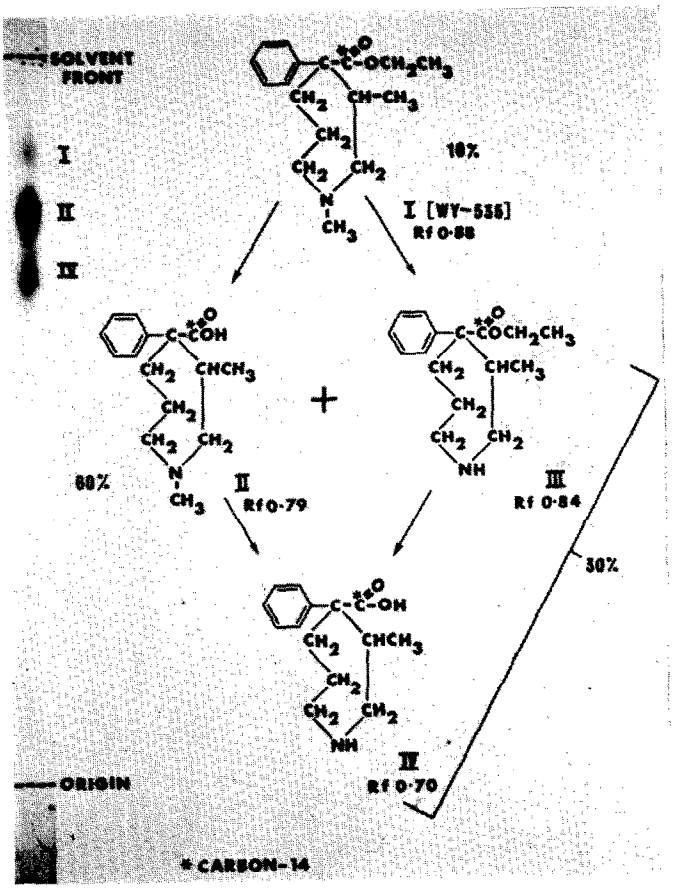


FIG. 1. Biodegradation of Wy-535. Radioautograph of dog urine. Descending solvent system, MeOH:EtOAc:1 M NH_4Ac (pH 7), 26:2:2.

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Tissue distribution in the rat

Regardless of the dosage form used, ^{14}C-Wy-535 activity was rapidly distributed in the tissues of the rat, although not in an identical pattern (Tables 3 and 4). Oral administration gave rise to the earlier and higher peak plasma level. A similar picture was obtained in liver. On the other hand, certain organs (lung, spleen, brain, heart, fat) showed a significantly higher peak activity level after intramuscular administration. This was especially noticeable in brain where the i.m. rats showed a peak level of 7·81 μg/g tissue (av.) as against a peak of only 2·73 μg/g (av.) in the oral rats.

There was no evidence of accumulation of drug in any of the organs studied. By the sixth hour, the levels in most tissues had decreased to a fraction of the peak value. This was true of all tissues except liver and gastrointestinal tract in both sets of animals, and bladder in the orally dosed rats. The activity continued to decrease until, by the 48th hour, only liver of the oral rats, and small intestine and cecum of both groups, showed significant levels. Even in these organs the activity was of a low order.

Excretion in the rat

After oral administration, activity appeared in the urine within the first ½ hr (Table 3). An average of 40% of the dose was accounted for by this route of excretion in 48 hr. Except for the few per cent remaining in the gastrointestinal tract, the remainder of the dose was accounted for in the feces.

After intramuscular administration, radioactivity appeared less quickly in the urine (Table 4). With the exception of one animal, which showed a trace at ½ hr, no activity was detectable until the second hour. By the fourth and sixth hours, however, 14% and 22%, respectively, of the dose appeared in urine—approximately the same amounts as in the oral rats. By the 24th hour, 50% of the administered drug was accounted for by this route; another 5% was excreted in the next 24 hr. Here again, the remainder of the dose other than the small amount still in the intestine and cecum was excreted in the feces in 48 hr.

Metabolism

As anticipated, Wy-535 was found to undergo N-demethylation and de-esterification in dogs (Fig. 1) and rats. No $^{14}CO_2$ was detected in respired air. Owing to the rapidity with which nor-Wy-535 (compound III) undergoes hydrolysis, we were unable to demonstrate this compound in urine until we took the precaution of collecting samples in containers kept immersed in dry ice. No such precautions were considered necessary for preservation of Wy-535, which hydrolyzed at a rate of 10%/24 hr at a neutral pH. Although the mobility of nor-Wy-535 in the solvent system used in Fig. 1 would place it overlapping compounds I and II, the nitroprusside spray made differentiation possible. For purposes of clarity we chose a chromatogram of urine for which no precautions were taken to avoid hydrolysis of nor-Wy-535.

We found no evidence of a hydroxy-metabolite analogous to hydroxyethoheptazine.[3] This could indicate that the hydroxyl group in the ethoheptazine metabolite is located on either carbon 2 or 3 of the azepine ring, positions which are blocked directly or sterically by the 3-methyl group of Wy-535.

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