Metabolism of *O,O*-Dipropyl *S*-[2-(2'-Methyl-1'-piperidiny)-2-oxo-ethyl] Phosphorodithioate (C 19 490) in the Rat

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Received April 22, 1980

The fate of *O,O*-dipropyl *S*-[2-(2'-methyl-1'-piperidiny)-2-oxo-ethyl] phosphorodithioate (C 19 490, piperophos), an active ingredient of herbicides Rilof® and Avirosan®, was investigated in rats using 2-methyl [¹⁴C]-labelled material. After single oral doses (0.5 to 30 mg/kg body weight) the radioactivity was rapidly excreted, mainly through urine. Nine days after receiving a dose of 5.4 mg/kg the residues in liver, fat, kidney, muscle, blood and brain were below 0.03 ppm C 19 490 equivalents. Thirteen urine metabolites, representing 55% of the dose applied, were isolated and their structures elucidated by spectroscopic methods. The degradation of C 19 490 in the rat proceeds *via* hydrolysis of the thio phosphate followed by methylation of the sulfhydryl group. The methylthio derivative is either oxidized at the sulfur or hydroxylated at the piperidine moiety. Hydroxylation at the α -C leads *via* ring opening to carboxylic acids. The hydroxypiperidine derivatives are conjugated with glucuronic acid. No unchanged C 19 490 was excreted with the urine and the amount of ¹⁴CO₂ expired was below 0.5% of the dose.

Piperophos (C 19 490), *i.e.* *O,O*-dipropyl-*S*-[2-(2'-methyl-1'-piperidiny)-2-oxo-ethyl] phosphorodithioate, is an active ingredient of the herbicides Rilof® and Avirosan® used for weed control in paddy rice.⁽¹⁾

Studies were initiated to assess biodegradability and metabolism to assure its safe use. The metabolic fate of piperophos in paddy rice has been reported.^(2,3)

The present report deals with the absorption, disposition, excretion and with the metabolic pathways of the compound in the rat.

MATERIALS AND METHODS

Chemicals. *O,O*-Dipropyl *S*-[2-(2'-[¹⁴C]-methyl-1'-piperidiny)-2-oxo-ethyl] phosphorodithioate ([¹⁴C]-C 19 490) with a specific radioactivity of 10.4 μ Ci/mg (5.4 mg/kg dose), 37.4 μ Ci/mg (30.3 mg/kg dose) and 37.7 μ Ci/mg (all other doses) and a purity of more than 99% was used. The labeled material and the unlabeled reference compounds (see Table I) were synthesized at CIBA-GEIGY Ltd., Basle, Switzerland.

Silicagel was purchased from E. Merck AG, Darmstadt, Germany; Sephadex LH 20 from Pharmacia Fine

Chemicals AB, Uppsala, Sweden and Amberlite XAD 4 from Serva GmbH., Heidelberg, Germany. β -Glucuronidase and arylsulfatase were supplied by Sigma Chemical Co., St. Louis, Mo., USA; ureas from Calbiochem AG, Lucerne, Switzerland. Insta-Gel® and Soluene® were obtained from Packard Instrument Co. Inc., Downers Grove, Ill., USA. HPLC columns with Lichrosorb and Spherisorb were purchased from E. Merck AG and Phase Separations Limited, Queensferry, U.K., respectively.

Treatment and maintenance of animals. Male and female rats (strain: Tif: RAI f (SPF)) bred under SPF conditions at CIBA-GEIGY, body weight 180~200 g were used. The animals were kept singly in metabolism cages at 22 $\pm$ 1°C, relative humidity 60 $\pm$ 10% and a dark/light cycle of 8/16 hours. The rats were fasted 20 hours prior to dosing and afterwards allowed free access to commercial rat food. Water was offered throughout the experiment. All individual doses were applied in 1 ml water/polyethylene glycol 200/ethanol mixtures by stomach tube. The amount of ethanol received by each rat did not exceed 0.3 ml. Urine and faeces were collected at 24 hours intervals as was the expired CO₂ by absorption into a 1:1 (v/v) ethanolamine-methylcellulose mixture. The animals were killed by decapitation and samples of tissues collected for radiometry.

Measurement of radioactivity. Radioactivity was measured on a Packard Tri-Carb Scintillation Counter,

Model 3375 or 3385. Quenching was corrected by the AES channels ratio method or by the internal standard method. Radioactivity in solution was measured using the counting medium of Bruno and Christian.⁴⁾

Tissues and Blood samples were solubilized in propan-2-ol/Soluene® (1:1 v/v) and counted in Insta-Gel®/HCl 0.5 M (9:1 v/v). All samples except brain and fat were decolorized with H₂O₂ before adding the scintillation mixture. Faeces were lyophilized and the radioactivity was determined by combustion according to Kalberer and Rutschmann⁵⁾ or with a Packard Sample Oxidizer Model 306. In the former case and for the determination of the absorbed ¹⁴CO₂ from the expired air the counting medium of Jeffay and Alvarez⁶⁾ was used.

Thin layer plates and electropherograms were scanned on a Berthold Thin Layer Scanner II (Laboratorium Prof. Berthold, Wildbad, Germany) or the radioactivity visualized with a LKB AGA 2105 Radio Chromatogram Camera (LKB Producter, Bromma, Sweden).

Quantitation of thin layer chromatography pattern was carried out by integration with the TLC Scanner.

Chromatographic methods. Thin layer chromatography (TLC) was performed on precoated Silicagel 60 F₂₅₆ plates (E. Merck) of 0.25 mm thickness. The plates were developed without chamber saturation. Reference compounds were visualized under UV-light (254 nm) with or without prior exposition to iodine vapour.

According to the character of the metabolite fractions a vast variety of solvent systems were used for analytical purposes.

For the purification of the methylated metabolite G (see Figure 1) and metabolite K, propan-2-ol / hexane (50:50 v/v) and acetone / diethylether / chloroform (15:5:80 v/v) were used, respectively.

For conventional liquid chromatography (LC) Silica gel (for column chromatography, particle size 0.04~0.063 mm), Sephadex LH 20® and Amberlite XAD-4 (particle size 0.05~0.1 mm) with the following eluents were used: (1) ethyl acetate / methanol (9:1) (2) chloroform (3) gradient chloroform → methanol (4) ethyl acetate / acetonitrile (1:1) (5) gradient hexane/chloroform (1:1) → methanol (6) acetone (7) gradient water → methanol (8) chloroform/hexane (3:1) → methanol (9) gradient acetonitrile → ethanol.

High performance liquid chromatography (HPLC) was run on a Spectra Physics Instrument, Model 3500 B (Spectra-Physics, Santa Clara, Ca., USA) equipped with a Perkin-Elmer Spectrometer, Model LC 55 (Perkin-Elmer AG, Küssnacht, Switzerland) and a Berthold Radioactivity Flow analyzer, Model BF 5026 (Laboratorium Prof. Berthold, Wildbad, Germany).

Separation was performed on commercial stainless steel columns of 3.2 or 4 mm inner diameter and 250 mm lengths filled with Silicagel 5 µm (LiChrosorb Si 60, Spherisorb Silica) or reversed phases 5µm (LiChrosorb RP 18, Spherisorb ODS) using the following eluents: (1)

acetonitrile / diisopropylether (50-60:50-40) (2) dichloromethane / diisopropylether / methanol (45:25:10) (3) gradient water → acetonitrile (4) gradient acetonitrile → methanol (5) water / acetonitrile (82:18) (6) propan-2-ol / hexane (1:9) (7) gradient water → ethanol.

High voltage electrophoresis. High voltage electrophoresis (HVE) was performed on Schleicher and Schüll paper 2043 b at 70 V/cm for 30 minutes using the HVE-system Camag (CAMAG AG, Muttenz, Switzerland). The following buffers were preferably used: (pH 1.9): acetic acid / formic acid / water (150:10:840), (pH 6.9): acetic acid / pyridine / water (3:50:947), (pH 8.8): ammonium carbonate solution (0.15 M). ¹⁴C-Labeled glucose was used for correction of electroosmotic effects and fuchsin and methylene blue to check the homogeneity of the electric field.

Enzymatic methods. For analytical purposes the samples were dissolved in 0.1 M acetate buffer (pH 5) and incubated for 24 hours at 37°C with (I) β-glucuronidase from bovine liver, Type B-3 16,000 Fishman Units/ml or (II) arylsulphatase from limpets, Type III 5 Sigma Units/ml and control (III) without enzyme.

Aliquots of the incubation mixtures were taken at the start of incubation and 0.5, 1, 5 and 24 hours thereafter for TLC control.

For preparative purposes the urine was treated with 6 Sumner Units urease B grade/ml at room temperature for 18 hours and after CHCl₃ and CH₃CN extraction with 25,000 Fishman Units β-glucuronidase from bovine liver, type B-1/ml at pH 5 and 37°C for 21 hours.

Chemical reactions. Methylation was carried out in methanol solution by adding diazomethane in ether. After 20 minutes at room temperature the solvents were removed by a gentle nitrogen stream. The procedure was repeated twice.

Oxidation was carried out in acetic acid/30% H₂O₂ (5:1 v/v) by incubation for 1/2 to 1 hour at 50°C.

Reduction was carried out according to Allenmark,⁷⁾

Spectroscopic methods. Infrared spectra (IR) were recorded on a Perkin-Elmer spectrometer, Model 221 (Perkin Elmer Corporation, Norwalk, Con. USA) equipped with a NaCl prism using the KBr preparation technique.

¹H-Nuclear magnetic resonance spectra (NMR) were recorded with a Varian spectrometer, Model HA-100 (Varian, Palo Alto, Ca., USA) or with a Bruker spectrometer, Model HX-360 (Bruker, Fällanden, Switzerland). Substances were dissolved in CDCl₃, CD₃OD and CD₃COCD₃ or mixtures thereof. TMS was used as internal standard.

Mass spectral analysis was performed using the following instrumentation. Low resolution: Varian MAT CH 7 spectrometer (ion source temperature 170°C, ionizing

energy 70 eV, direct sample insertion); high resolution: CEC 21-110B double focusing spectrometer using Ionomer plates for photographic detection; field desorption: Varian MAT CH 5-DF spectrometer equipped with a combined FI/FD/EI-ion source (10 μ m high temperature activated tungsten emitters, emitter dipping technique, best anode temperature [BAT], ion source temperature 80°C).

RESULTS

Excretion and residue pattern

The excretion pattern following an oral dose of 5.4 mg/kg and 30.3 mg/kg [14 C]-C 19 490 is presented in Table I. The radioactivity administered is very rapidly eliminated, more than 2/3 being excreted in 24 hours.

C 19 490 is readily absorbed from the intestinal tract, as about 80% of the dose is renally excreted and only 10~20% with the

faeces. Less than 0.5% of the label appeared in the expired air. More than 90% of the dose was excreted within 3 days. The excretion pattern appears to be independent of the dose.

The residual radioactivity in selected tissues of the rats nine days after dosing with 5.4 mg/kg [14 C]-C 19 490 is given in Table II. The residues in muscle, blood and brain were below 0.01 ppm and those in liver fat and kidney about 0.02 ppm C 19 490 equivalents.

Identification of metabolites

The pooled urines excreted within 48 hours after application of 30.3 mg/kg [14 C]-C 19 490, representing 83.9% of the dose (Table I) were used for the isolation of metabolites. All percent values of fractions, metabolites, *etc.* hereafter refer to this specimen as 100%.

TABLE I. EXCRETION OF RADIOACTIVITY FROM RATS AFTER SINGLE ORAL ADMINISTRATION OF 14 C-C 19 490 (in % of dose, mean values $\pm$ SEM)

Dose [mg/kg]	5.4	5.4	30.3	0.5	2.2	10.7	31.3
Sex	Males	Females	Males	Males	Males	Males	Males
Number of animals	4	4	16	2	2	2	2
<i>Urine</i>							
0~24 hr	54.4	68.9	78.7	61.1	74.8	71.5	70.7
24~48 hr	13.6	6.0	5.2	*	*	*	*
48~72 hr	3.9	1.5	1.0	*	*	*	*
72~216 hr	1.3	2.1	*	*	*	*	*
Subtotal	73.2 $\pm$ 5.7	78.5 $\pm$ 5.3	84.9	*	*	*	*
<i>Faeces</i>							
0~24 hr	13.3	9.3	9.4				
24~48 hr	7.2	4.6	1.5				
48~72 hr	2.6	0.9	0.2				
72~216 hr	0.9	1.3	*				
Subtotal	22.2 $\pm$ 6.0	16.1 $\pm$ 3.9	11.1	*	*	*	*
Expired air 0~72 hr	0.3**	0.4**	*	*	*	*	*
Tissue residues***	0.1 $\pm$ 0.0	<0.1 $\pm$ 0.0	*	*	*	*	*
Cage wash	1.1 $\pm$ 0.5	2.2 $\pm$ 1.6	*	*	*	*	*
Total recovery	96.9 $\pm$ 0.8	97.2 $\pm$ 1.2	96.0	*	*	*	*

* Not determined.

** Collected from two animals of each sex only.

*** Calculated from Table II assuming that fat, blood and muscle represent 5%, 5.9% and 42.5% of the body weight, respectively.¹⁸⁾

Preliminary tests, such as TLC, HVE as well as specific chemical and enzymatic reactions, revealed some information as to the nature of the urinary metabolites. Furthermore, the

TABLE II. RETENTION OF RADIOACTIVITY IN
MALE AND FEMALE RATS NINE DAYS
AFTER AN SINGLE ORAL DOSE OF
5.4 mg/kg ^{14}C -C 19 490
(mean values $\pm$ SEM, $n=4$)

Tissue	Residues [ppm C 19 490 equivalents]	
	Males	Females
Liver	0.022 ± 0.002	0.020 ± 0.001
Fat	0.018 ± 0.004	0.012 ± 0.004
Kidney	0.026 ± 0.002	0.025 ± 0.002
Muscle	$< L_Q^*$	$> L_Q^*$
Blood	0.008 ± 0.000	0.008 ± 0.001
Brain	$< L_Q^*$	$< L_Q^*$

* L_Q = Limit of determination according to Currie,¹⁹
i.e. 0.009 ppm for all tissues except blood (0.003 ppm).

presence of a number of theoretically possible metabolites, which were available as authentic reference compounds could be ruled out, e.g. 2-(2'-methyl-1'-piperidiny)-2-oxo-ethane sulfonic acid and 2-(2'-methyl-1'-piperidiny)-2-oxo-ethanoic acid being major metabolites in paddy rice.

About 60% of the total radioactivity moved toward the anode upon HVE at pH 8.8, indicating this portion to be acidic in character. None of the components however move towards the cathode, either at pH 8.8 or 6.9 or 1.9, thus ruling out the presence of any basic functional groups.

Some of the metabolites were susceptible towards β -glucuronidase, but not towards arylsulfatase.

Conclusive information on the metabolite structures, however, was obtained from their IR, NMR and mass spectra after their isolation according to Fig. 1. The structure relevant data are summarized in Table III.

FIG. 1. The Isolation and Purification of Urinary Metabolites from Male Rats after a Single Oral Administration of 30.3 mg/kg ^{14}C -C 19 490 (number in parenthesis refer to the mobile phases given in section chromatographic methods).

TABLE III. SPECTROSCOPIC DATA OF C 19 490 METABOLITES

Structure	Relevant spectroscopic data
<p>Met. A</p>	<p>MS. (m/e (%)): 188 (1) M - CH₃; 140 (100) M - SOCH₃; 126 (17) M - CH₂SOCH₃; 97 (47) C₆H₁₁N; 63 (7) CH₃SO.</p> <p>IR. (KBr): μm = 6.15 (C=O, amide); 6.95 (C-N + CH); 9.65 (S - O).</p> <p>NMR. (CDCl₃ and CD₃OD) δ: 1.2 - 1.3 ppm (2 d): >CH-CH₃; 1.5 - 1.9 ppm (m): 6 ring protons; 2.78 ppm (s): SO-CH₃; ~ 3.8 ppm (m): CO-CH₂-SO</p>
<p>Met. H₁ and H₂</p>	<p>MS. m/e^a): 220 M - CH₃; 156 M - CH₃SO₂; 114 M - CH₃SO₂CH₂CO; 100 156 - C₃H₄O; 79 CH₃SO₂⁺.</p> <p>FD. m/e : 235 M.</p> <p>NMR. (CD₃OD) δ: 1.1 - 1.3 ppm (4d): CH-CH₃; 3.1 ppm (2s): SO₂-CH₃</p>
<p>Met. B - methylester Met. F - methylester</p>	<p>MS. (m/e (%)): 234 (8) M - CH₃; 202 (4) M - CH₃OH/CH₃; 187 (100) M - CH₂SO; 186 (40) M - CH₃SO; 154 (72) M - CH₃SO/CH₃OH; 148 (98); M - CH₃OOC-CH₂CH₂CH₂; 105 (78) CH₃SOCH₂CO; 63 (44) CH₃SO.</p> <p>FD. m/e 250 M + H; 272 M + Na.</p> <p>IR. (KBr): μm = 5.77 (C=O, ester); 6.04 (C=O, amide); 9.65 (S + O).</p> <p>NMR. (CD₃OD) δ: 3.95 ppm (m): >CH-CH₃; 3.65 ppm (s): COOCH₃; 2.75 ppm (s): SO-CH₃; 2.35 ppm (t) -CH₂-COO; 1.4 - 1.7 ppm (2 m) CH-CH₂-CH₂-; 1.15 ppm (d) >CH-CH₃.</p>
<p>Met. I₂</p>	<p>MS. (m/e (%)): 203 (18) M; 157 (100) M - CH₂S; 142 (64) M - CH₂SCH₃; 124 (10); 114 (16); 100 (42) 142 - C₃H₆; 61 (50) CH₂=S-CH₃.</p> <p>NMR. (CD₃OD) δ: 4.75 and 4.23 ppm (2 m): H-2, 4.5 and 3.8 ppm (2 d,d): H-5; 3.6 and 3.4 ppm (2 m): H-6 eq; 3.4 ppm (AB,s): CO-CH₂; 2.95 and 2.5 ppm (2d,d): H-6 ax; 2.6 - 2.9 ppm (m): H-4, H-3; 1.3 and 1.16 ppm (2d): >CH-CH₃</p>
<p>Met. I₁, I₃, C, D</p>	<p>MS. m/e^b): 203 M; 157 M - CH₂=S; 142 M - CH₂SCH₃; 139 157 - H₂O; 114 157 - CH₃CO; 100 142 - C₃H₆; 61 CH₂=S-CH₃.</p>
<p>Met. E₁ and E₂</p>	<p>MS. m/e^c): 152 M - SOCH₃; 138 156 - H₂O; 114 M - CH₃SOCH₂CO; 63 CH₃SO⁺.</p> <p>FD. m/e: 220 M + H.</p>

The following now is a somewhat more detailed discussion of the individual preparations.

CHCl₃-Extract 1

The CHCl₃-extract 1, which contained 33%

of the urinary radioactivity could be further separated into a main portion, metabolite A, representing 28% and into a minor, 5% portion, which on TLC showed to consist of several small fractions, each of them certainly contributing less than 0.5% of the total

Met. G - methylester

MS. (m/e (%)): 250 (1) M - CH₃; 234 (7) M - OCH₃; 186 (39) M - SO₂CH₃; 164 (100) CH₃CH=NH-COCH₂SO₂CH₃; 154 (11) M - SO₂CH₃/CH₃OH; 144 (22) M - CH₃SO₂CH₂CO; 121 (10) CH₃SO₂CH₂CO⁺; 114 (30) H₂NCH(CH₃)CH₂CH₂CH₂CO⁺; 98 (41); 79 (31) CH₃SO₂⁺.

FD. m/e : 266 M + H.

NMR. (CD₃OD) δ : 3.9 ppm (m): $\geq$ CH-CH₃; 3.65 ppm (s): -COOCH₃; 3.1 ppm (s): -SO₂-CH₃; 2.35 (t): -CH₂-COO-; 1.4 - 1.7 ppm (2 m): CH-CH₂-CH₂-; 1.15 ppm (d) $\geq$ CH-CH₃

Met. K

MS. (m/e (%)): 187 (25) M; 141 (60) M - CH₂S; 126 (100) M - CH₂SCH₃; 98 (19) 126 - CO.

a) H₁, rel. Int. (%): m/e 220 (46); 156 (100); 114 (40); 100 (86); 79 (32). H₂, rel. Int. (%): m/e 220 (24); 156 (100); 114 (32); 100 (70); 79 (28).

b) C, rel. Int. (%): 203 (60); 157 (100); 142 (84); 124 (20); 114 (13); 100 (76); 81 (24); 61 (42).
D, rel. Int. (%): 203 (50); 157 (100); 142 (88); 124 (34); 114 (16); 100 (76); 81 (26); 61 (44).
I₁, rel. Int. (%): 203 (20); 157 (100); 142 (33); 139 (30); 114 (40); 99 (66); 98 (29); 97 (50); 61 (70). I₃, rel. Int. (%): 203 (12); 157 (100); 142 (43); 139 (32); 114 (28); 100 (38); 99 (29); 98 (36); 97 (45); 61 (62).

c) E₁, rel. Int. (%): 156 (100); 138 (17); 114 (68); 63 (21). E₂, rel. Int. (%): 156 (100); 138 (21); 114 (81); 63 (26).

activity.

Metabolite A is characterized by the absence of the phosphate group, which after all is true for all metabolites identified during this work. The interpretation of the mass-, IR- and NMR spectra was straightforward and they agreed well with those of an authentic reference sample. In addition, the TLC behaviour after oxidation, reduction and in the genuine state was identical to the latter.

Some metabolites, metabolite A included, are present in the syn as well as in the anti form. Because the exchange rate between these two forms is slow with regard to the NMR time scale, they show different chemical shifts, but they were only identifiable more precisely when the sample purity was great enough.

CH₃CN-extract

Upon fine separation the acetonitrile extract yielded metabolites H₁ and H₂ and an acidic metabolite B which was methylated with diazomethane prior to further investigation. Metabolite H₁ as well as H₂ had a molecular

weight 32 mass units (amu) higher than A, corresponding to two extra oxygen functions in the molecule. In addition, the mass spectra of H₁ and H₂ were qualitatively identical. The appropriate loss of 79 amu (CH₃SO₂) and the fragment ion at m/z 79 (CH₃SO₂⁺) revealed readily the presence of a methyl sulfone group, and the fragment ion at m/z 114 (M-CH₃SO₂CH₂CO) was indicative of a hydroxyl group in the piperidine ring. The NMR spectra confirmed H₁ and H₂ to be isomers, but the position of the hydroxyl group could not be determined.

The IR spectrum of metabolite B exhibited two carbonyl absorption bands, one characteristics of an ester, the second of a secondary amide (Table III). The latter leads to an open chain structure which is evidenced by the lack of the CH₂N protons in the NMR spectrum and the loss of the (CH₂)₃COOCH₃ fragment due to α -cleavage in the mass spectrum. The presented data confirm the genuine metabolite to be the corresponding free acid.

CHCl₃-Extract II

This extract contained the aglycones of conjugates liberated by treatment with β -glucuronidase, prior to the chloroform extraction. In the HVE test the aglycones remained at the start at pH 9.0. Further separation of this extract resulted finally in 7 preparations, metabolites C, D, E₁, E₂, I₁, I₂ and I₃.

Metabolites C, D, I₁, I₂ and I₃ gave very similar mass spectra whose interpretation led to the proposed sulfide structure with one hydroxyl group in the piperidine ring. Signals, from sample impurities in the anyhow rather complicated NMR spectra (*syn-anti*-isomerism), however, made the location of the hydroxyl group in C, D, I₁ and I₃ impossible. On the contrary, the well recognizable coupling constants in the NMR spectrum of I₂ as well as the availability of an authentic reference compound allowed to establish the conformation of this metabolite.

From their electron impact and field desorption mass spectra, metabolites E₁ and E₂ are again isomers. The small sample amounts available did not allow further spectroscopic investigations, however, the formation of less polar compounds, both upon oxidation with CH₃COOH / H₂O₂ as well as reduction with KI gave additional evidence for the proposed structures.

Aqueous phase

Two metabolites, F and G were isolated from the aqueous phase, both being acidic and hence methylatable with diazomethane. F was shown to be identical to B, while metabolite G was found to be the corresponding sulfone. Upon chemical oxidation and reduction both metabolites behaved accordingly.

For the completion of this study rats were dosed with 0.5, 2.2, 10.7 and 31.3 mg/kg ¹⁴C-C 19 490 in order to establish the metabolic profile in dependence on the applied dose and to assure the genuine character of the metabolites identified. As a result a new metabolite, K, not present in earlier experiments, was observed. Independent of the dose, K account-

ed for 20~25% of the urinary radioactivity and it was less polar than metabolite A. At the same time the contribution of the latter was reduced. The sum of A and K, however, remained constant at about 28% in all cases as it was found for A alone in the first experiment. Metabolite K could be easily isolated and purified by extraction with chloroform, preparative TLC and finally by HPLC on reversed phase using system.³⁾ Its structure, being a sulfide with an unaltered piperidine ring, was readily deduced from its mass spectrum. For comparison, an authentic reference sample was available in addition.

In order to confirm that K and A are genuine metabolites, and that A is not an artifact due to air oxidation, K was intraperitoneally injected into male rats (2 animals, body weight 206, 207 g, dose 0.3/0.2 mg/kg dissolved in 0.25/0.15 ml glycerol formal). Within 48 hours 81% of the dose was excreted with the urine. Thereof 71% was found to be oxidized to metabolite A, 16% was a rather complex mixture, presumably of ring oxidized products, but still 13% was eliminated as unchanged metabolite K. The conditions and the extent to which the sulfide or the sulfoxide is formed, as not known at present.

DISCUSSION

C 19 490 orally applied to rats is readily absorbed from the intestinal tract, as about 80% of the applied material is renally eliminated. This appears to be independent of the dose (0.5 mg/kg to 30 mg/kg).

The low residual radioactivity found in liver, fat, kidney, muscle, blood and brain nine days after a single oral dose of 5.4 mg/kg [¹⁴C]-C 19 490 indicates, that no retention of the herbicide and/or its metabolites had occurred.

C 19 490 is readily degraded by rats as shown by the large variety of metabolites and the absence of unchanged C 19 490 in the urine.

From the structures of the metabolites identified, covering 65% of the urinary

perhydroazepine¹⁶⁾ and perhydroazocine.¹⁷⁾

Microsomal sulfoxidation of thioethers to sulfoxides and sulfones is a quite common reaction. Traficante *et al.*¹²⁾ reported recently, that the same microsomal preparation from bovine liver was either capable to oxidize mesoridazine to sulforidazine or as well, to reduce sulforidazine to mesoridazine. The unknown factors governing the corresponding steady state condition may be the same ones, responsible for the excretion ratio of Metabolite A and Metabolite K in this study.

The biotransformation of C 19 490 in paddy rice has also been studied recently,^{2,3)} and a brief comparison of the degradation pathways seems to be appropriate. Cleavage of the thioester bond, methylation of the sulfhydryl derivative followed by sulfoxidation leading to Metabolite A is common in both systems. Whereas in the rat the piperidine ring is involved in further oxidation processes, all the rice metabolites contain an unchanged piperidine moiety and the site of oxidation concentrates on the 1-methyl sulfinyl-2-oxo-ethane side chain. Thus, the major metabolites, were found to be 2-(2'-methyl-1'-piperidinyl)-2-oxo-ethane sulfonic acid and 2-(2'-methyl-1'-piperidinyl)-2-oxo-ethanoic acid. These metabolites, not being formed in the rat, represent terminal products of detoxifying pathways, as there oral LD₅₀ in rats is much lower than that of C 19 490 (C 19 490: 324 mg/kg, both metabolites: >4000 mg/kg).

Acknowledgments. The authors are indebted to Mrs. M. Imhof for her excellent technical assistance during the isolation of the metabolites.

The ¹⁴C-labeled C 19 490 was synthesized by H. Baumann, H. Mory and W. Küng, the unlabeled reference compounds by A. Lukaszczyk, all from CIBA-GEIGY Ltd., Basle, Switzerland.

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