Physiological and Metabolic Elimination of 1-(α,α -dimethylbenzyl)-3-(*p*-tolyl) Urea, Dimuron in Rats (1)

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Metabolic fate of a ureid herbicide, 1-(α,α -dimethylbenzyl)-3-(*p*-tolyl)urea, dimuron, was investigated in male rats. Greater than 69% of orally administered carbonyl- ^{14}C -dimuron (50 mg/kg, single) was absorbed from the gastrointestinal tract of male Wistar rats. Furthermore, the dose was entirely eliminated in the feces (66%) and urine (34%) within 48 hr after dosing. Tissue retention accounted for only 0.6% of the dose within 48 hr after administration. Radiocarbon levels in selected tissues declined to less than detectable levels (liver: 0.2 ppm, kidney: 0.03 ppm, adipose tissue: 0.15 ppm, blood: 0.025 ppm, plasma: 0.02 ppm, carcass: 0.05 ppm equivalent of dimuron) after 120 hr. The high elimination observed in the feces was mainly due to active biliary excretion (38.6% in 48 hr). A substantial part (24.3%) of the radioactive biliary excreta was reexcreted into the bile through an enterohepatic cycle. In addition to urinary and biliary excretion, the involvement of the gastrointestinal tract was observed as a minor excretion route of dimuron. *Tlc* of the urine extracts indicated that dimuron was metabolized to more than seven polar metabolites, but dimuron itself was not detected in the urine. From fecal extracts, dimuron and several of its metabolites were detected. A major metabolite found in the urine was suggested as 1-(α,α -dimethylbenzyl)-3-(*p*-carboxyphenyl) urea by *Tlc*. As minor metabolites, *p*-toluidine was detected in the urine, and the expiration of radioactive carbon dioxide was also observed (less than 0.06% of the dose). Furthermore, radioactive β -glucuronide conjugate was detected in the bile.

INTRODUCTION

Dimuron, 1-(α,α -dimethylbenzyl)-3-(*p*-tolyl)urea, is one of the new type ureid herbicides developed recently by Showa Denko Ltd, which has a strong herbicidal activity and a lower mammalian toxicity. Although there are many ureid herbicides currently used, only a limited information was obtainable on their metabolic fates in mammals in contrast to those in plants and soil. In regard to dimuron, the metabolic fate has been studied in plants and soil by Uchiyama and his coworkers,¹⁾ but in mammals it has not been reported. This paper presents the information

on absorption, elimination, and metabolism of dimuron in the male rats.

MATERIALS AND METHODS

1. Chemicals

Pure dimuron, 1-(α,α -dimethylbenzyl)-3-(*p*-tolyl)urea was supplied by Showa Denko Ltd. Dimuron used in this study was also prepared in this laboratory. Carbonyl- ^{14}C -dimuron was provided by Dr. M. Uchiyama of Tokyo University of Agriculture and Technology. The specific radioactivity was 5.38 $\mu\text{Ci}/\text{mg}$ and the radiochemical purity was greater than 96%. A potential metabolite of dimuron, 1-(α,α -dimethylbenzyl)-3-(*p*-

carboxyphenyl)urea, [carboxydimuron], was prepared by reaction of *p*-aminobenzoic acid with cumyl isocyanate.

2. Treatment and Handling of Animals

Six-weeks old male Wistar Imamichi rats weighing about 110 to 130 g were orally treated with 50 mg/kg of labeled dimuron (0.196 μ Ci/mg) dissolved in olive oil containing 0.033% dimethylsulfoxide. Immediately after administration, each rat was placed in a glass metabolic cage (Natsume KN-0070) for 120 hr. During the first 48 hr, the expiratory air was trapped with ethanolamine and the radioactive carbon dioxide was estimated. Fresh water and diet (Oriental MF) were provided *ad libitum* throughout the experiment. For the study of biliary excretion of dimuron, a polyethylene cannula (Intramedic PE-10, Clay Adams) was inserted into the common bile duct of the rat after anesthetization with 50 mg/kg of pentobarbital. During anesthesia, the animal body temperature was maintained with a 100-watt lamp. The conscious rats were administered 50 mg/kg of labeled dimuron by gastric intubation or 124 μ g/kg of the chemical through the femoral vein. In the study of enterohepatic circulation of dimuron, four of paired rats were anesthetized and cannulated. A biliary cannula was led from a rat (A) into the duodenum of a second rat (B) which had also a biliary cannula. After the intravenous injection of labeled dimuron to rat (A), the bile was collected periodically (for 2 min in every 30 min) from rat (A) and continuously from animal (B) to estimate the enterohepatic circulation.

3. Analyses of tissues

Animals were sacrificed by intercardial perfusion with physiological saline at 3, 6, 24, 48, 72 and 120 hr after the oral administration of labeled dimuron, and the following tissues were collected: liver, kidney, adipose tissue, and remaining carcass. Total radioactivity in the tissues was determined by oxygen combustion (Packard Tricarb Sample Oxidizer 306) of 300-

mg homogenized tissue samples in triplicate.

4. Urine

Urine samples were collected at 6, 12, 24, 36, 48, 72, 96 and 120 hr after dosing. Total radioactivity in each of the urine sample was determined by radioassay of triplicate 100- μ l aliquotes in a liquid scintillation counter (Beckman LC-355). Remaining urine samples (0 to 48-hr urine) were combined and fractionated by extracting the radioactivity with ether under acidic (0.1 N HCl, acidic fraction), basic (0.1 N NaOH, basic fraction) and neutral (neutral fraction) conditions, successively. The aqueous phase was then divided into a chloroform soluble fraction and an aqueous fraction. Each fraction was radioassayed, dried over anhydrous sodium sulfate and concentrated under a stream of nitrogen. The concentrates were spotted on precoated silica gel thin-layer plates (Merck, silica gel GF-254, 0.25 mm thickness). Generally, the plates were developed in benzene-acetone (2 : 1). Spots were located on the plates by visualization with UV (254 nm) light, Ehrlich's reagent, and by autoradiography.

5. Feces

Fecal samples were collected from each animal at identical time intervals as adopted for the collection of urine. Samples were homogenized in a Polytron homogenizer with five volumes of methanol and total radioactivity was determined by oxygen combustion of 300-mg samples of the homogenate in triplicate. Remaining fecal samples (0 to 48-hr feces) were combined and successively extracted by shaking with five volumes of the following solvents: ether, acetone, methanol, and water. Extracts were then combined and analyzed as described for the fractionated urine samples.

6. Bile

Bile was bleached with hydrogen peroxide prior to radioassay. In some cases, approximately 0.05 to 0.2 μ Ci of the radioactive bile was lyophilized, extracted with ether, acetone, and methanol, and then evaporated to dryness. The residue was dissolved in 100 mM acetate buffer (pH 5.4), and incubated with 4000 Fish-

man units of calf-liver β -glucuronidase (Sigma) for 12 hr at 37°C in a shaking water bath incubator. For the inhibition of β -glucuronidase, 50 mM of D-saccharo- γ -lactone (Sigma) was added to the incubation mixture. After incubation, the sample was again lyophilized and extracted with acetone and methanol. This sample was then analyzed by *tlc*.

7. Gas Chromatographic Identification of Dimuron Hydrolysate

Five rats were treated intraperitoneally with 200 mg/kg/day of dimuron for 2 days. Urine was collected for 4 days and fractionated as described before. The basic fraction was evaporated and treated with monochloroacetic acid anhydride. Monochloroacetylated samples were then introduced into ECD gas chromatographs (Yanako G-1800 equipped with 50 cm glass column containing 5% GE-XE 60 on 60-80 mesh chromosorb-W and Shimadzu GC-4BM equipped with 2 m glass column containing 3% GE-XE 60 on 60-80 mesh chromosorb-W) and compared with authentic monochloroacetyl-*p*-toluidine. Operating conditions: temperatures—injector and detector 325°C, column 200°C; flow rate of

nitrogen—60 ml/min. Retention times of monochloroacetyl-*p*-toluidine were 3.1 min (Shimadzu GC-4BM) and 3.4 min (Yanako G-1800).

RESULTS

1. Tissue Retention and Overall Elimination

Orally administered dimuron was rapidly cleared from the gastrointestinal tract with a biological half life of 6 hr and the radioactive content in the gut was reduced to less than 5% of the original dose in 24 hr (Fig. 1). Tissue

Fig. 1 Clearance of radiocarbon from the gastrointestinal tracts.

Single oral dose of 50 mg/kg of ^{14}C -dimuron was administered and residual radioactivity was determined in the gut including contents. Each point represents the average value ($\pm$ S.D.) obtained with more than 4 rats.

Fig. 2 Distribution of radiocarbon following single oral administration of ^{14}C -dimuron (50 mg/kg).

The detectable limits of radiocarbon expressed as ppm equivalents of carbonyl- ^{14}C -dimuron were as follows: the liver: 0.2 ppm, kidney: 0.03 ppm, adipose tissue: 0.15 ppm, blood: 0.025 ppm, plasma: 0.02 ppm, remaining carcass: 0.05 ppm.

Each bar represents the average value ($\pm$ S.D.) obtained with more than 4 rats.

Table 1 Elimination of radiocarbon after intraperitoneal administration of carbonyl- ^{14}C -dimuron.

hr	Feces	Urine	Urine plus feces
0-24	$37.0 \pm 5.3\%$	$46.9 \pm 7.2\%$	$83.9 \pm 17.2\%$
24-48	9.5 ± 2.3	5.5 ± 1.4	15.1 ± 5.2
48-72	0.8 ± 0.2	0.4 ± 0.2	1.3 ± 0.7
0-72	47.4	52.9	100.3

Values are means $\pm$ S.D. of 4 rats and expressed as percentages of dose (50 mg/kg).

retention (except the gut) accounted for 0.6% of the radioactivity after 48 hr. Figure 2 shows the residual radiocarbon levels in selected tissues expressed as ppm equivalents of carbonyl- ^{14}C -dimuron at various time intervals. All tissues reached a maximum level within 6 hr. After 120 hr, radiocarbon levels in either of the tissues declined to less than detectable levels (liver: 0.2 ppm, kidney: 0.03 ppm, adipose tissues: 0.15 ppm, blood: 0.025 ppm, plasma: 0.02 ppm, remaining carcass: 0.05 ppm). Residual radiocarbon level was higher in the liver, kidney, adipose tissue, and remaining carcass than in the total blood or plasma. The liver contained the highest level of radioactivity at all time intervals. Figure 3 shows the cumulative elimination of radiocarbon into the urine and feces. The radiocarbon from orally administered dimuron was quantitatively eliminated in 48 hr with 66% recovery from the feces and 34% from the urine. On the other hand, when dimuron was administered intraperitoneally, 47.4% of the dose was excreted in the feces in 72 hr (Table 1) indicating the involvement of biliary excretion. Table 2 shows the radiocarbon eliminated from biliary cannulated rats which were orally treated with 50 mg/kg of ^{14}C -dimuron. Within 48 hr, 38.6% of the dose was excreted into the bile. In the same period, 27.4% and 30.2% of the dose were recovered in the feces and urine, respectively.

2. Enterohepatic Circulation

After intravenous injection of 124 $\mu\text{g/kg}$ of labeled dimuron, 41.8% of the dose was recovered in the 24-hr bile (Fig. 4). This dose was determined on the basis of the maximal blood level estimated after a single oral dosage of 50 mg/kg of the chemical. When rat (A) was given ^{14}C -dimuron (124 $\mu\text{g/kg}$)

Fig. 3 Cumulative elimination of radiocarbon to urine and feces after single oral administration of ^{14}C -dimuron (50 mg/kg).

●: Urine, ○: feces.

Each point represents the average value ($\pm$ S.D.) obtained with more than 4 rats.

Table 2 Elimination of radioactivity after oral administration of ^{14}C -dimuron (50 mg/kg) in the biliary cannulated rats.

Excreta	% of dose in 48 hr after administration
Bile	38.6 ± 5.4
Urine	30.2 ± 3.7
Feces	27.4 ± 7.2
Total	96.2

Each value represents the average $\pm$ S.D. obtained with 5 rats.

and the bile was transferred for 12 hr into the duodenum of animal (B) by biliary cannula, 24.3% of the radiocarbon which was estimated to be received by rat (B) was subsequently recovered in the bile as a result of entero-

Fig. 4 Biliary excretion of radiocarbon following single intravenous injection of $124 \mu\text{g/kg}$ of ^{14}C -dimuron.

Excretion rate (○: % of dose/hr and cumulative elimination (●: % of dose) were determined. Each point represents the average value ($\pm$ S.D.) obtained with 4 rats.

Table 3 Elimination of radiocarbon after intravenous injection of carbonyl- ^{14}C -dimuron.

Excreta	Rat(A) ^{a)}	Rat(B) ^{b)}
Feces	$7.2 \pm 1.1\%$	$16.7 \pm 5.4\%$
Urine	30.8 ± 1.6	22.5 ± 4.2
Bile	$53.0 \pm 2.6^*$	24.3 ± 2.4
Summ.	91.0 ± 1.8	63.5 ± 4.4

^{14}C -Dimuron ($124 \mu\text{g/kg}$) was injected into the femoral vein of rat(A), and the bile passed continuously into the duodenum of animal (B) for 12 hr (biliary excretion of ^{14}C from rat (A) reduced to trace level in 12 hr). Bile was collected periodically from rat (A) [for 2 min in 30 min intervals] for 12 hr and continuously from animal (B) for 24 hr to calculate the total radioactivity received by animal (B) and to estimate the enterohepatic circulation in 24 hr.

^{a)}: % of injected dose in 24 hr.

^{b)}: % of received radioactivity from rat (A) in 24 hr.

*: % of injected dose in 12 hr.

hepatic circulation in 24 hr (Table 3). In spite of the approximately constant flow rate of bile throughout the experiment ($41\text{--}59 \mu\text{l/kg/min}$), two peaks were observed in the biliary excretion of radiocarbon from rat (B) as shown in Fig. 5. Maximal excretion of radioactivity

Fig. 5 Biliary excretion rate of radioactivity in double cannulated rat.

Radioactive bile which was excreted by rat (A) injected intravenously with $124 \mu\text{g/kg}$ of ^{14}C -dimuron was recycled into the duodenum of rat (B). The radioactive bile of rat (A) was passed continuously into the duodenum of rat (B) for 12 hr except the sample was withdrawn (for 2 min/30 min), and the biliary excretion rates of radioactivity were measured (●: from rat (A); dpm/2 min; Bars: from rat (B), dpm/hr).

into the bile of animal (B) appeared 8 to 11 hr after that of rat (A). In addition to the main peak, a small peak was observed almost simultaneously with the occurrence of maximal biliary excretion in animal (A). In this experiment from rat (A), 91% of the intravenous dose was recovered: 7.2% in the feces, 30.8% in the urine, and 53% in the bile. On the other hand, from rat (B) 63.5% of the received radioactivity from rat (A) was recovered: 16.7% in the feces, 22.5% in the urine, and 24.3% in the bile.

3. Metabolites

Fractionation of 48-hr urine specimens indicated that 59, 1.2, and 1.4% of the radioactivity from the initial dosage were recovered in the acidic, basic, and neutral fractions, respectively. Although the urine was repeatedly extracted with ether, 13% of the radioactivity remained in the aqueous phase. Only 36% of the administered radioactivity was extracted from the 48-hr feces samples. Similar to urine, most of the extracted radioactivity was recovered in the acid fraction (52%) and in the aqueous fraction (32%).

Tlc radioautograms of the ether fraction

Fig. 6 Tlc of extracts from urine and feces.

D: dimuron, U: Urine, F: feces, t: total, a: acid fraction, b: basic fraction, n: neutral fraction, c: chloroform fraction.

Solvent system: benzene: acetone (2 : 1)

Radioactivity of Ut applied was less than 5% of that of Ua and other fractions.

concentrates derived from urine and feces are shown in Fig. 6. Tlc of the total urine revealed major metabolite at R_f values of 0 and 0.35 in the benzene-acetone (2 : 1) system. The acid fraction from urine contained at least seven metabolites, and a major metabolite (R_f : 0.35) was recovered from this fraction. Dimuron was not detected in any of the urine fractions. On the other hand, dimuron was detected in the basic and neutral fecal extract.

The major metabolite in the urine-acid fraction (R_f : 0.35) was extracted from the tlc plates with methanol and cochromatographed with authentic carboxydimuron. R_f values were identical by cochromatography [R_f : 0.36 in benzene-acetone (2 : 1), R_f : 0.52 in benzene-acetone-chloroform-methanol (15 : 5 : 2 : 1)]. Furthermore, upon treatment with diazomethane the methylated products also demonstrated identical mobilities [R_f : 0.72 in benzene-acetone (2 : 1), R_f : 0.73 in benzene-acetone-chloroform-methanol (15 : 5 : 2 : 1)].

Dimuron was not detected in the bile. Radioautograms of the bile sample showed a single spot at the origin. Pretreatment of the bile with β -glucuronidase produced a new radioactive spot on the tlc plates, which was not observed when D-saccharo- γ -lactone, a specific inhibitor of β -glucuronidase, was used. R_f value of the new spot was approximately the same as that observed with carboxydimuron.

Monochloroacetyl-*p*-toluidine was detected in trace amounts (less than 0.075% of the intraperitoneal dose) by ECD-gas chromato-

graphy. With the occurrence of *p*-toluidine, the expiration of radioactive carbon dioxide was estimated, but the level accounted for less than 0.06% of the oral dose.

DISCUSSION

Orally administered dimuron was absorbed rapidly from the gastrointestinal tract and distributed to all body tissues with liver having the highest level. Tissue retention was low with complete chemical elimination in the feces (66%) and urine (34%) within 48 hr after oral dosing. All tissues showed less than detectable levels of radioactivity after 120 hr. In spite of the lipophilic nature of dimuron, accumulation of dimuron in the adipose tissue was not indicated. These findings suggest that rats are capable of rapidly transforming dimuron into polar metabolites *in vivo*.

The major excretion was found in the bile (40.3% of the oral dose) resulting in high discharge of radiocarbon in the feces and significant involvement of enterohepatic circulation (24.3% of radioactive biliary excreta). The only example of studies involving enterohepatic circulation with pesticides was demonstrated with DDT. Gingel²³ recently reported that 97% of intravenously injected DDA was excreted into the bile and 67% of the DDA metabolite (β -glucuronide) in the bile was reexcreted into the bile via the enterohepatic cycle.

In addition to urinary and biliary excretion system, dimuron was also excreted through the gut. When the bile, urine, and feces were collected separately, 7.2% of the intravenously injected ¹⁴C-dimuron was eliminated in the feces. With regard to this particular excretion route of foreign chemicals through the gut, several neutral chemicals [diphenylhydantoin,³ dieldrin,⁴ and PCB²³] have been suggested to be excreted by this route. In the case of PCB, the route was demonstrated to play an important role in its overall elimination because PCB is highly lipophilic and it is inactive in urinary and biliary excretion. In the case of dimuron, however, this route did not appear to be important in the overall elimination of dimuron from rats because of the active metabolic transformation of dimuron into polar metabolites which were rapidly excreted into the

urine and bile. At this time, the mechanism of excretion and the chemical form of dimuron involved in this excretion process remain unknown.

Biliary cannulated rats excreted 69% of the oral dose in the bile and urine. Part of the absorbed radioactivity was excreted by the gastrointestinal tract, therefore the net absorption ratio of dimuron (single oral dosage of 50 mg/kg) was 69% plus that contributed by the gastrointestinal tract (7.2% of the intravenous dose).

As suggested from the excretion rates in the bile and urine, dimuron was actively metabolized into polar metabolites in rats. The radioactive biliary excreta contained a β -glucuronide conjugate. But, whether the aglycone was the same as the major metabolite in the urine was not determined. In addition to this β -glucuronide, at least one additional metabolite was suggested to be involved in the biliary excreta. The biliary excretion of radiocarbon via enterohepatic cycle showed a biphasic pattern which might indicate that at least two different metabolites were involved in the biliary excretion of dimuron. One showed no time lag while the other required 8 to 11 hr for absorption (probably through the hydrolysis of the conjugate by the intestinal flora and mucosa).

In rats, dimuron appeared to be hydrolyzed at trace levels (less than 0.06% of the oral dose) as estimated from expiration of $^{14}\text{CO}_2$ and measurement of *p*-toluidine level in the urine. Results of the *tlc* analyses on the acid fraction of urine suggested that the major metabolite in the urine was carboxydimuron [$1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-carboxyphenyl})\text{urea}$]. Isolation of this major metabolite in the urine was attempted using column chromatography and *tlc*. The IR spectra of the isolated metabolite indicated the existence of a carbonyl group in addition to the ureid moiety of dimuron at 1695 cm^{-1} . From these results the major metabolite of dimuron in rat urine was strongly suggested to be $1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-carboxyphenyl})\text{urea}$.

Previously Uchiyama and coworkers¹⁾ reported the glucoside of $1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-hydroxymethylphenyl})\text{urea}$ as the main metabolite of dimuron in plants and

soil. Furthermore, in soil amide linkage of dimuron was hydrolyzed and active decarboxylation was observed. In this study, attempts were made to detect the intermediate of carboxydimuron, $1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-hydroxymethylphenyl})\text{urea}$ in the urine, however, the results were inconclusive. Perhaps $1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-hydroxymethylphenyl})\text{urea}$ is easily converted to the carboxylic acid derivative in rats.

According to preliminary work, dimuron was not metabolized or degraded in the gastrointestinal tract. Therefore, the metabolites found in the feces may be attributed to the biliary excreta which were not reabsorbed from the intestine or the excreted metabolites of dimuron by the gut.

Further investigations to identify the metabolites of dimuron in animals are needed and are now in progress.

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要 約

尿素系除草剤ダイムロン [$1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-tolyl})\text{urea}$] の吸収、排泄を Wistar 系雄ラットを用い carbonyl- ^{14}C -ダイムロン (50 mg/kg) の 1 回経口投与で調べた。 ^{14}C の排泄はすみやかで 48 時間以内に尿および糞中に投与量のおおの 34, 66% が見いだされ、120 時間後には調査した全組織ともに検出限界以下となった。糞中への高い排泄率は活発な胆汁排泄に起因するものであり 48 時間で経口投与量の 40% が胆汁中へ排泄された。静脈内投与 (124 $\mu\text{g/kg}$) では投与量の 42% が胆汁中に排泄されまた胆汁排泄量の 24% が腸肝循環により再び胆汁中に排泄された。これら尿、胆汁排泄系の外にダイムロンは腸管からも分泌排泄された。経口投与におけるダイムロンの吸収率は胆管カニュレートしたラットの尿および胆汁中への排泄率の和に消化管からの分泌排泄率を加えたものであり 69% 以上と推定される。ダイムロンはラット体内で容易に代謝され尿中に 7 種以上、また胆汁中にグルクロン酸抱合体を含む代謝物の形で排泄される。糞中からは未変化物のほか数種類の代謝物が検出された。TLC 分析の結果から尿中の主代謝物は $1-(\alpha,\alpha\text{-dimethylbenzyl})-3-(p\text{-$

carboxyphenyl)urea と推定された。また微量代謝物として *p*-toluidine が ECD ガスクロマトグラフィーにより尿中から検出されるとともに呼気中に投与量の 0.06% 以下の微量の $^{14}\text{CO}_2$ の排泄が観察された。

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