Use of Ion Cluster Analysis in a Metabolic Study of Pencycuron, a Phenylurea Fungicide, in Rabbits

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Two deuterium labels of pencycuron [1-(*p*-chlorobenzyl)-1-cyclopentyl-3-phenylurea], pentadeuteriolabeled at the phenyl group and tetradeuteriolabeled at the 2,2,5,5 positions of the cyclopentyl group, were synthesized. Each was mixed with equimolar nonlabeled pencycuron and administered orally to male rabbits. Excreta were purified and subjected to GC–MS, and 11 metabolites (urine, 11; feces, 3) were characterized by ion cluster analysis. Major urinary metabolites were 1-(*p*-chlorobenzyl)-1-cyclopentyl-3-(*p*-hydroxyphenyl)urea (free and glucuronide). Decyclopentylation, hydroxylation at the 3 position of the cyclopentyl moiety, diol formations, thiomethylation at the phenyl ring, and some other glucuronides were also observed in the metabolites. Three mass spectra of fully methylated glucuronides were obtained in intact form. Besides three minor metabolites, a significant amount of fecal pencycuron was found. A metabolic scheme is proposed to fit the data obtained.

Pencycuron [1-(*p*-chlorobenzyl)-1-cyclopentyl-3-phenylurea] was developed to control plant diseases caused by *Rhizoctonia solani*, e.g., rice sheath blight and potato black scurf (Yamada et al., 1978). The mammalian toxicity of this fungicide is quite low (acute oral LD_{50} for rats > 5 g/kg).

At present, information concerning the metabolic behavior of pencycuron in mammals is scanty. On the other hand, several studies on the metabolic fate of urea herbicides in mammals have been reported (Paulson, 1975). The major metabolic pathways of methyl- or methoxy-substituted ureas are demethylation and/or demethoxylation. However, as pencycuron contains no methyl group, these degradation pathways may not apply. Belasco and Reiser (1969) have demonstrated that ring hydroxylation and conjugation of siduron, which has no *N*-methyl moiety, occur in dogs. Pencycuron may be metabolized in a similar manner as siduron to produce various types of hydroxylated and conjugated compounds.

Ion cluster analysis has been used successfully in metabolic drug (Heijenoort et al., 1967; Knapp et al., 1972; Curtius et al., 1973; Baba et al., 1976, 1978) and insecticide studies (Ueyama and Takase, 1980). This method facilitates the differentiation of metabolites from other compounds native to the organisms and confirmation of the structures of the compounds by mass fragment pattern analysis. Since pencycuron contains many hydrogen atoms that are substituted by a hydroxy group or other groups, the use of regiospecifically deuterium-labeled compounds makes this method a better procedure for identifying the metabolites formed in mammals.

We now report on the metabolism of pencycuron in male rabbits.

MATERIALS AND METHODS

Chemicals. Analytical-grade pencycuron (pencycuron-d_0) was provided by the Synthesis Laboratory of our institute. The synthesizing processes for obtaining pencycuron-d_5 [1-(*p*-chlorobenzyl)-1-cyclopentyl-3-(pentadeuteriophenyl)urea] and pencycuron-d_4 [1-(*p*-chlorobenzyl)-1-(2,2,5,5-tetradeuteriocyclopentyl)-3-phenylurea] are outlined in Figure 1. Deuterioreagents were purchased from Merck Sharp & Dohme, Canada. Referential compounds (I–XXII) and their methyl-substituted derivatives were prepared. The structure, thin-layer chromatographic (TLC) and gas–liquid chromatographic (GLC) properties, and mass spectrometric fragments of pencycuron and related compounds are summarized in Table I.

Animals. Male Japanese albino rabbits weighing ca. 2.5 kg were used. They were divided into two groups of two animals each and maintained individually in stainless steel metabolism cages for 1 week prior to dosing. Each rabbit received an oral administration of 15 mL poly(ethylene glycol) 400 solution (150 mg/kg); it contained an equimolar mixture of pencycuron-d_0 and -d_5 (group 1) or pencycuron-d_0 and -d_4 (group 2). Excreta were collected for 3 consecutive days.

Analytical Procedures. *Urine.* A glass column (1.5 cm i.d.; resin height 10 cm) containing Amberlite XAD-2 resin as a methanol slurry (Junk et al., 1974) was washed with distilled water. A portion of the urine sample was added to the column, the aqueous solution was drained, and then the resin was washed with three 100-mL portions of water. Dry nitrogen gas was blown into the column, it was closed with a stopcock, diethyl ether (50 mL) was poured onto the resin, and the bubbles were sucked off, allowing the diethyl ether to equilibrate with the resin. After 30 min, the solvent was collected, and further elution, using methanol (50 mL), was performed. The organic solvents were combined and concentrated; the aqueous residue was lyophilized and treated with methyl iodide to form methyl-substituted compounds (Lawrence and Laver, 1975) or with deuteriomethyl iodide (CD_3I; Merck Sharp & Dohme) to form deuteriomethyl compounds.

Another portion of the urine sample was partitioned with dichloromethane, the aqueous solution was lyophilized, and the residue was dissolved with sodium acetate buffer (pH 4.5, 0.1 M). Then, arylsulfatase (Limpets Type V, Sigma) or β-D-glucuronidase (Bovine Liver, Sigma) was added to the solution, and the mixture was incubated for 24 h at 37 °C. After extraction with a diethyl ether–ethyl acetate (1:1) mixture, the organic phase was dried and methylated.

Feces. Samples from individual rabbits were soaked overnight in acetonitrile and homogenized with Polytron (Kinematica GmbH). After filtration, the residue was extracted with Polytron; the filtrates were combined and washed with *n*-hexane. The lower layer was collected and the acetonitrile evaporated. Acetate buffer was added to the residues, and the mixture was partitioned 3 times with ethyl acetate. The ethyl acetate layers were combined, dried over anhydrous sodium sulfate, and concentrated. Then, a part of the residue was methylated by using

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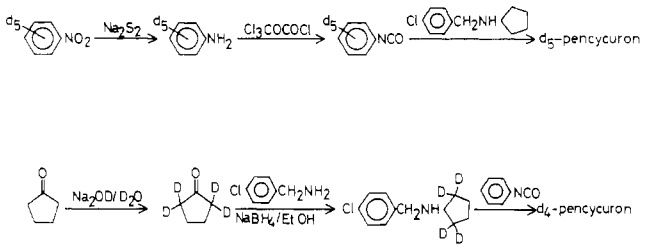


Figure 1. Procedures used in the synthesis of deuteriolabeled pencycuron.

methyl iodide. After ethyl acetate partitioning, the aqueous layer was subjected to the same enzymatic treatment as described above.

Chromatographic Method. For qualitative analyses, TLC and gas chromatography–mass spectrometry (GC–MS) were used. Prior to methylation, an aliquot of the sample solution was subjected to TLC (silica gel F_{254}, 0.25-mm thickness, Merck). The solvent systems are described in Table I; compounds were inspected under UV light (254 nm).

Methylated samples were subjected to GC–MS for ion cluster analysis. The instrument was Shimadzu-LKB 9000 connected to a computer system (GC–MS PAC 300D). The settings were as follows: a glass column (1 m, 2.6-mm i.d.) packed with 1% OV-101 on Chromosorb W (AW-DMCS, 60–80 mesh); inlet temperature, 290 °C; separator temperature, 270 °C; ion source temperature, 290 °C; helium, 30 mL/min; accelerating voltage, 3510 V; trap current, 60 μA; ionization energy, 70 eV. The column oven temperature was 200 °C; this was increased by 5 °C/min until it reached 290 °C and held at this temperature for 10 min. Mass spectrometric data were recorded every 6 s starting 1 min postinjection.

For quantitative analysis, methylated samples were subjected to a GLC (Hewlett-Packard 5730A) equipped with an N-specific FID. The glass column packing was 2% OV-101 on Chromosorb W (100–120 mesh, 4 ft, 2-mm i.d.); the column oven temperature was increased at 8 °C/min from 180 to 260 °C and held at that level. The injection and detector temperatures were 300 °C. Gas flow rates of helium (carrier), hydrogen, and air were 30, 3, and 80 mL/min, respectively.

RESULTS AND DISCUSSION

Mass Spectra of Pencycuron and of the Equimolar Mixture Samples Used for Ion Cluster Analysis. Since unsubstituted phenylurea is commonly pyrolyzed on a GLC or GC–MS column to yield the corresponding phenyl isocyanate (Büchert and Løkke, 1975), a hydrogen atom in the urea skelton was replaced by a methyl- or deuteriomethyl group (pencycuron-d_3). Figure 2 shows the proposed fragmentation pathways by four kinds of methyl-substituted pencycuron, namely, pencycuron-d_0, -d_5, -d_4, and -d_3. Decyclopentylation ($M^+ - 69$), N–CH_2 bond cleavage (m/z 125 and 217) and CO–NCH_3 bond cleavage (m/z 236) may represent first steps. The m/z 231 fragment of pencycuron-d_0 would then be formed from the m/z 273 fragment via splitting off of the NCO moiety.

For ion cluster analysis, equimolar mixtures of pencycuron-d_0/d_5 or -d_0/d_4 were administered to rabbits. Figure 3 depicts their mass spectra. The molecular ion of deuterated pencycuron shifted according to the labeled deuterium number, and peculiar doublet fragments (so-called ion clusters) appeared in the spectra of mixed specimens. Namely, seven doublet fragments differed at 5 amu; their having nearly the same ion intensity indicates that they contain the phenyl moiety of the aniline side (Figure 3, top). Similarly, the ion clusters in the spectrum of pencycuron-d_0/d_4 are indicative of these fragments containing the cyclopentyl moiety (Figure 3, bottom). Therefore, reliable fragmentation schemes of pencycuron could be obtained by examining the mass spectra of these deuterated pencycurons. The four methyl-substituted pencycurons gave the same base peak (m/z 125) due to the *p*-chlorobenzyl moiety (Figure 2).

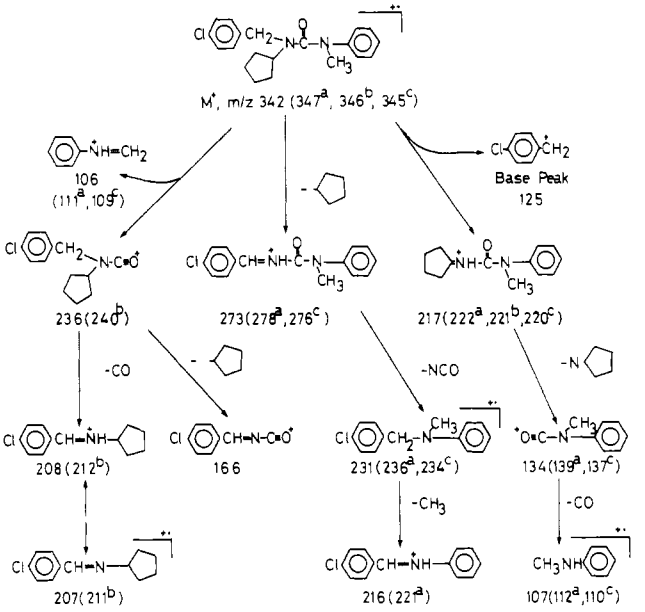


Figure 2. Proposed fragmentation process of methyl-substituted pencycuron: (a) pencycuron-d_5; (b) pencycuron-d_4; (c) pencycuron-d_3.

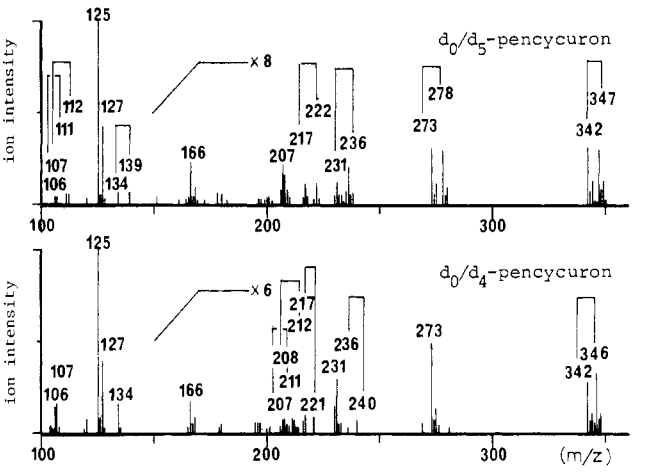


Figure 3. Mass spectra of the equimolar mixtures of pencycuron-d_0/d_5 and -d_0/d_4 used for ion cluster analysis.

Characterization of Urinary Metabolites. Urine samples, obtained from rabbits treated with pencycuron-d_0/d_5 or -d_0/d_4, were purified by XAD-2 resin, methylated, and subjected to GC–MS. Figure 4 shows a typical mass chromatogram of total ion and 10 mass numbers when pencycuron-d_0/d_5 was administered. Most metabolites with a *p*-chlorobenzyl group could be detected by identifying the mass spectra whose ion intensity ratio of m/z 125/127 was about 3 ($^{35}Cl/^{37}Cl$). However, there were no peaks corresponding to the compounds having no *p*-chlorobenzyl moiety [I, VI, and X, m/z 106, 111; II, m/z 208, 212; III, m/z 232, 237; XIV, m/z 155, 157 (in Figure 4)]. Therefore, we could hypothesize with respect to the fecal sample that cleavage of the CH_2–NCO bond (I–III), reductive dechlorination (VI), hydrolytic dechlorination

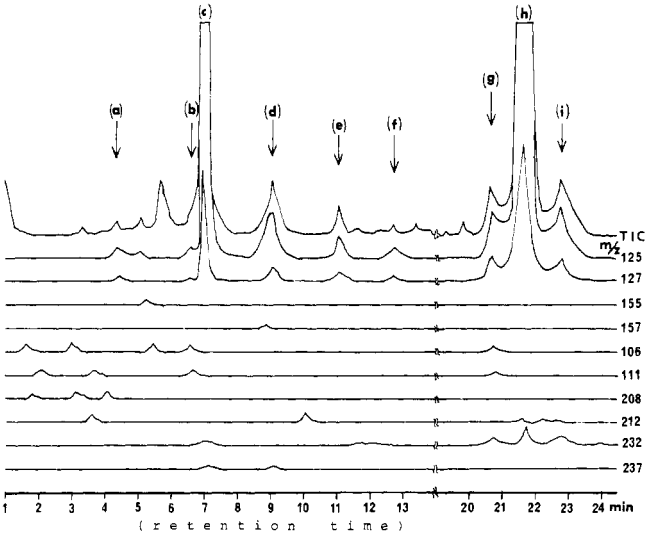


Figure 4. Mass chromatogram of urinary sample from rabbits treated with pencycuron-d_0/d_5.

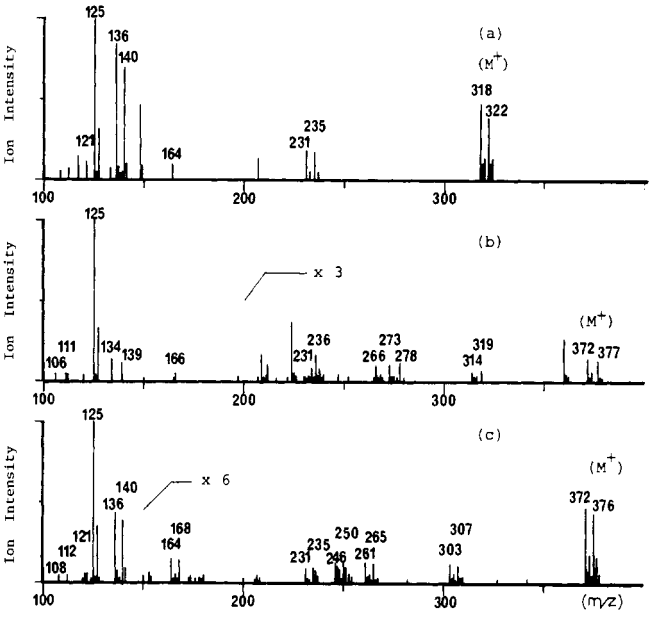


Figure 5. Mass spectra of peaks a–c.

(X), and hydroxylation at the chlorobenzyl moiety (XIV) scarcely occurred in the body of our experimental animals. Nine peaks with *p*-chlorobenzyl moiety were tentatively separated from the mass chromatogram (Figure 4).

Peak a. Figure 5 (a) illustrates the mass spectrum of this peak. The molecular ion was m/z 318; three characteristic doublet fragments were shifted to 4 amu (m/z 136/140, 231/235, 318/322). This is ascribable to the occurrence of hydroxylation at the phenyl moiety of the aniline side. Since, in addition, the retention times on GLC and GC–MS and the R_f value of TLC coincided with authentic IX, the metabolite was identified as decyclopentyl *p*-hydroxyphenyl pencycuron (IX).

Peak b. On the basis of the data shown in Figure 5 (b), the molecular ion of this metabolite was determined to be m/z 372; six doublet fragments (m/z 106/111, 134/139, 231/236, 273/278, 314/319, 372/377) were shifted to 5 amu, suggesting that no substitution at the phenyl group occurred. Inspection of the mass spectrum obtained when the rabbits were given pencycuron-d_0/d_4 made it clear that all hydrogen atoms at the 2 and 5 position of the cyclopentyl group were retained (M^+; m/z 372/376). Therefore, the remaining hydroxylated position were probably the 1

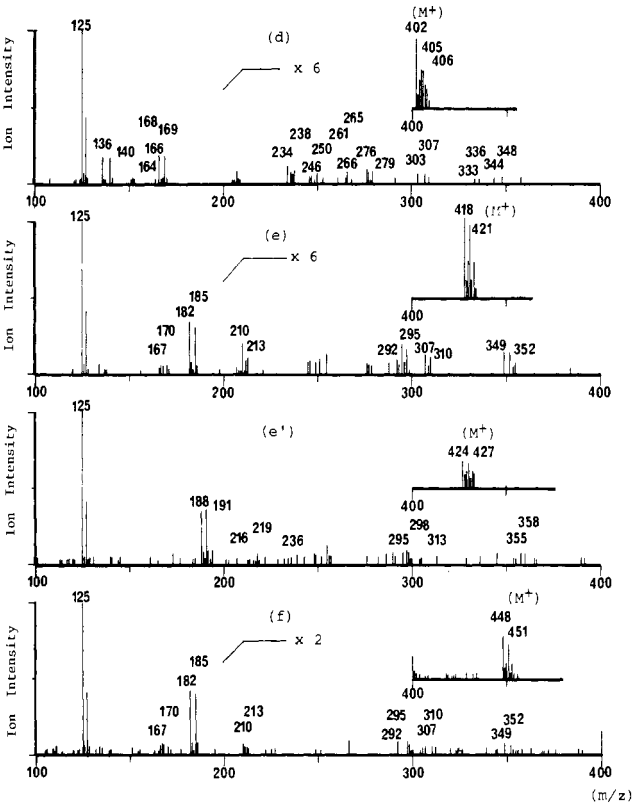


Figure 6. Mass spectra of peaks d and e, its deuteriomethyl substitution (peak e'), and peak f.

or 3 (=4) position on the cyclopentyl group. However, preferential attack on the carbon atom may occur, thus this metabolite was estimated as 3-hydroxycyclopentyl pencycuron (XII). Upon TLC analysis, this metabolite could be divided into two spots, namely, trans and cis isomers (Table I); their ratio was calculated to be about 2:1.

Peak c. Figure 5 (c) shows the mass spectrum of this peak. The molecular ion of peak c (m/z 372), corresponding to pencycuron-d_0 was the same as that of peak b. However, as there were many doublet fragments with a 4-amu shift, hydroxylation at the phenyl group may be proposed as the most reasonable reaction to interpret this metabolite. On the basis of the retention time and the R_f value, this was determined to be *p*-hydroxyphenyl pencycuron (XV), and *o*- (XI) or *m*-hydroxyphenyl (XIII) pencycuron was not observed.

Peak d. The mass spectrum of peak d is presented in Figure 6 (d). Since M^+ (m/z 402) was 30 amu greater than that of peaks b and *c*, we estimated that two hydroxy groups were introduced into the pencycuron molecule. In fact, several fragments of the authentic compound XVIII coincided with those in peak d (m/z 136/140, 164/168, 246/250, 261/265, 303/307). thus, this could be assumed to contain 3-(hydroxycyclopentyl)-*p*-(hydroxyphenyl)-pencycuron. However, this does not account for the shift at 3 amu of four doublet ions (m/z 166/169, 276/279, 333/336, 402/405). Therefore, we posit that a slight amount of pyrocatechol-type pencycuron (XVII) was formed and intermingled in peak d. Since the retention times of the three authentic compounds, XVII, *trans*-XVIII, and *cis*-XVIII, were very close (Table I), it was difficult to completely separate these peaks by GLC or GC–MS. However, TLC analysis revealed that the ratio of trans and cis isomers was about equal.

Peaks e and e'. Mass spectra of peak e and its deuteriomethyl substitution (peak e') are shown in parts e and

Table I. Structure, Chromatographic Behavior, and Mass Spectrometric Fragments of Pencycuron and Related Compounds

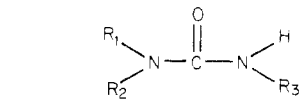
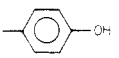
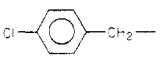
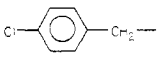
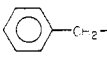
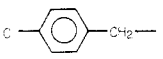
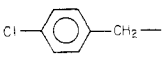
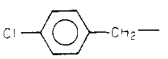
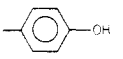
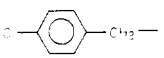
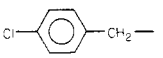
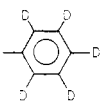
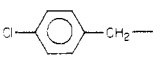
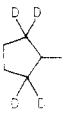
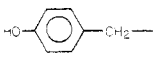
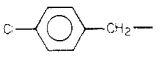
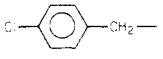
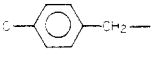
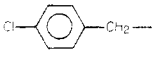
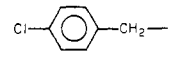
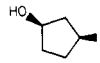
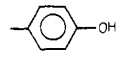
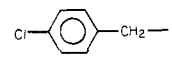
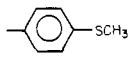
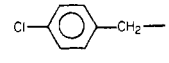
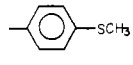
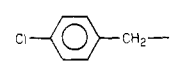
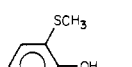
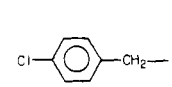
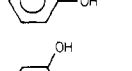
compound	 R_1	R_2	R_3	R_f values of TLC in solvent system[a] A	B	C	methyl-substituted compound GLC,[b] min	GC-MS[c] (M^+); fragment ions, m/z
I	H	H		0.29	0.24	0.14	0.80	(178), 72, 77, 106, 134, 120, 147, 163, 92, 108, 163
II[d]	H	H		–	–	–	1.59	(208), 72, 136, 85, 121, 120, 149, 92, 108, 163
III	H			0.73	0.74	0.27	2.13	(232), 106, 107, 126, 134, 69, 77, 89, 146, 163
IV			H	0.58	0.50	0.26	3.86	(280), 125, 155, 211, 208, 166, 207, 140, 178
V		H		0.69	0.78	0.24	4.71	(288), 125, 107, 106, 120, 230, 201, 134, 216, 163
VI				0.97	0.92	0.68	5.85	(308), 91, 106, 77, 107, 134, 239, 202, 174, 217
VII		H		0.57	0.68	0.16	6.09	(318), 125, 136, 260, 137, 121, 237, 149, 164, 122, 106
VIII		H		0.46	0.37	0.12	6.45	(318), 125, 137, 150, 121, 136, 260, 193, 207, 108
IX		H		0.40	0.28	0.09	6.65	(318), 125, 136, 137, 121, 231, 111, 134, 207, 246, 260
pencycuron-d_0				0.95	0.92	0.62	7.45	(342), 125, 134, 106, 107, 273, 231, 217, 166, 207
pencycuron-d_5				0.95	0.92	0.62	7.45	(347), 125, 139, 111, 112, 278, 236, 222, 207, 166
pencycuron-d_4				0.95	0.92	0.62	7.45	(346), 125, 134, 106, 107, 273, 231, 221, 166, 211
X[d]				–	–	–	7.92	(338), 121, 106, 107, 134, 269, 162, 203, 204, 231, 227
XI[d]				–	–	–	8.56	(372), 125, 136, 137, 121, 149, 164, 120, 122, 303, 314, 138
trans-XII				0.46	0.37	0.15	8.64	(372), 125, 134, 106, 107, 273, 238, 231, 266, 166
cis-XII				0.53	0.62	0.18	8.71	(372), 125, 134, 106, 107, 238, 266, 273, 207, 314
XIII				0.62	0.64	0.22	9.08	(372), 125, 137, 184, 136, 303, 150, 261, 108, 107, 247
XIV[d]				–	–	–	9.18	(372), 155, 106, 107, 134, 303, 217, 196, 237, 261
XV				0.48	0.46	0.15	9.29	(372), 125, 136, 121, 122, 108, 164, 303, 261, 120, 246
trans-XVI				0.38	0.20	0.09	9.90	(402), 125, 136, 137, 266, 164, 108, 121, 122, 149, 304, 247, 238, 231, 344
XVII[d]				–	–	–	10.40	(402), 125, 166, 122, 152, 150, 151, 138, 276, 333, 291, 194
trans-XVIII				0.28	0.09	0.05	10.44	(402), 125, 136, 137, 121, 122, 164, 303, 261, 207, 246, 237, 234

Table I (*Continued*)

compound	R_1	R_2	R_3	R_f values of TLC in solvent system[a] A	B	C	methyl-substituted compound GLC,[b] min	GC-MS[c] (M^+); fragment ions, *m/z*
cis-XVIII				0.35	0.16	0.07	10.49	(402), 125, 136, 137, 122, 207, 106, 121, 142, 108, 205, 140, 288, 266, 303
XIX				0.97	0.93	0.62	11.12	(388), 125, 152, 137, 138, 153, 273, 319, 105
XX				0.82	0.80	0.43	11.53	(418), 125, 152, 137, 266, 180, 165, 320, 273, 360
XXI[d]				–	–	–	12.25	(418), 125, 182, 183, 167, 168, 134, 166, 292, 210, 307, 138, 349, 120
XXII[d]				–	–	–	12.36	(418), 125, 182, 183, 134, 167, 168, 166, 138, 292, 307, 210, 195, 349

[a] The following solvent systems were used in an unsaturated chamber: A = chloroform-ethanol (9:1); B = chloroform-nitromethane-methanol (16:4:1); C = cyclohexane-chloroform-methanol (7:3:1). [b] min = retention time. For GLC conditions, see Materials and Methods. [c] (M^+) = molecular ion. The fragments without M^+ are arranged according to ion intensity. [d] Only methyl-substituted compounds were prepared.

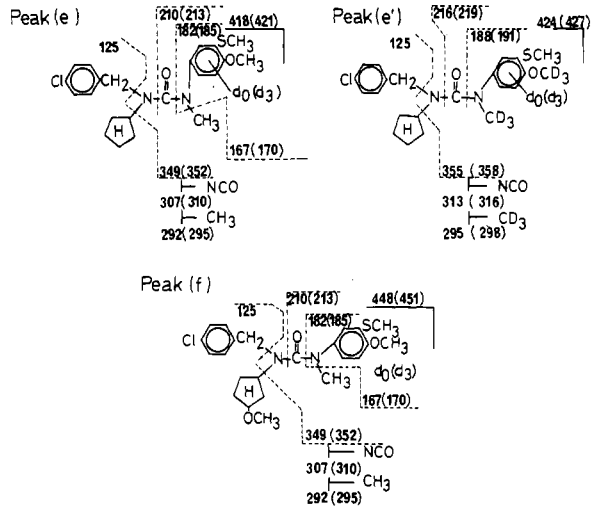


Figure 7. Proposed structure and fragmentation of two methyl-substituted *S*-methyl-containing metabolites in peaks e, e', and f.

e', respectively, of Figure 6. Seven doublet fragments in peak e were shifted at 3 amu, indicating that two hydrogens at the phenyl group were replaced by some other groups. The molecular ion (*m/z* 418) suggested that one was a methoxy and another an *S*-methyl group. The retention time of this peak was greater than of authentic XX, shorter than of XXII, and coincided with that of authentic XXI. Proposed fragmentation schemes are presented in Figure 7. Since, upon deuteriomethylation, the molecular ion(s) moved to 6 amu (*m/z* 418/421 → 424/427) [Figure 6 (e')], the methyl moiety in the phenyl ring originates in the rabbit body rather than from methyl iodide. Although it could not be decided from the mass spectra whether this biotical methyl was located at the methoxy or *S*-methyl group, we estimated the methyl connected with an SH group, because methylation of phenol may not be metabolically feasible (Bakke, 1970). Thus, the structure was postulated as 1-(*p*-chlorobenzyl)-1-cyclopentyl-3-[3-(methylthio)-4-hydroxyphenyl]urea.

Peak f. The mass spectrum of this peak is presented

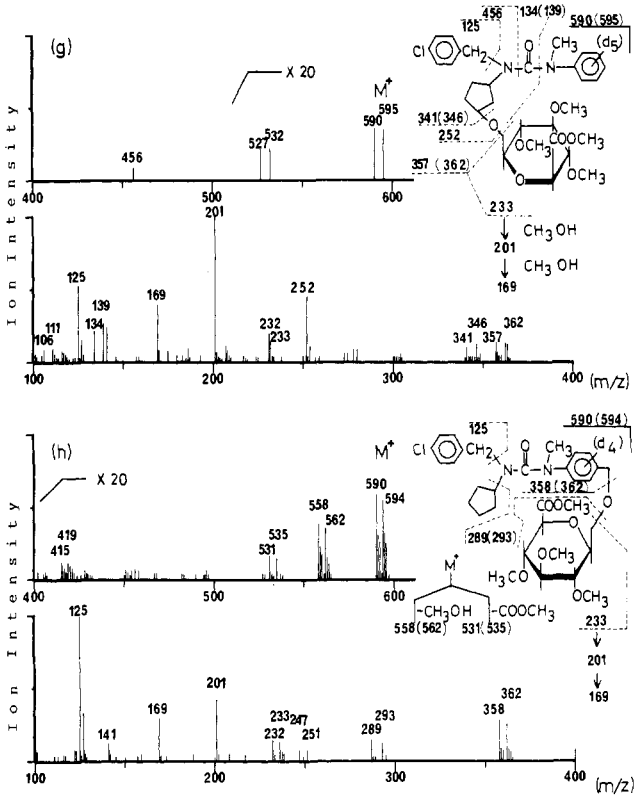


Figure 8. Mass spectra of peaks g and h.

in Figure 6 (f). Due to the similarity of the fragments, peak f was estimated as the analogue of peak e; on the other hand, the former peak had a molecular weight 30 amu greater than that of peak e. The increase in M^+ may be ascribable to the occurrence of further hydroxylation to the metabolite of peak e. Since M^+ in the case of pencycuron-d_0/d_4 dosing was *m/z* 448/452, the position of the additional hydroxy group was not at the 2 or 5 position of the cyclopentyl moiety. Moreover, the M^+ of peak f upon pencycuron-d_0/d_5 dosing increased 6 amu by deuteriomethylation, as was the case with respect to peak e. Therefore, we propose a structure of 1-(*p*-chlorobenzyl)-

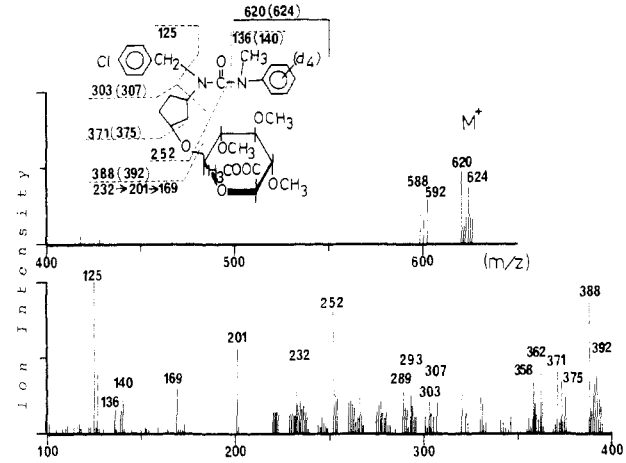


Figure 9. Mass spectrum of peak i.

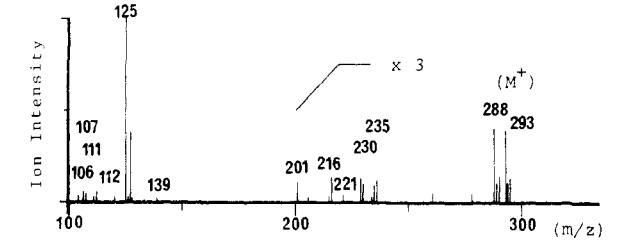


Figure 10. Mass spectrum of decyclopentylpencycuron (V) found in the urinary aqueous-soluble fraction after β-D-glucuronidase treatment.

1-(3-hydroxycyclopentyl)-3-[3-(methylthio)-4-hydroxyphenyl]urea (Figure 7).

Peak g. Figure 8 (g) shows the mass spectrum of peak g. The distance of the ion cluster was 5 amu (m/z 590/595), suggesting that there was no metabolic attack on the hydrogen in the phenyl group of the aniline side. Some single fragments containing no chlorine atom were observed (m/z 169, 201, 232). There were the characteristic fragmentation patterns for fully methylated glucuronic acid (Kováčik et al., 1968a,b). Therefore, the metabolite was estimated as glucuronic acid conjugated hydroxycyclopentyl pencycuron. β-Glucuronidase hydrolysis afforded a compound identical with the authentic XII, proving that the structure of this metabolite was 1-(*p*-chlorobenzyl)-1-[3-(β-glucuronyl)cyclopentyl]-3-phenylurea.

Peak h. The mass pattern of peak h [Figure 8 (h)] was similar to that of peak g; however, the distance of the ion cluster was 4 amu (M^+; m/z 590/594), suggesting that the metabolite was glucuronic acid conjugated hydroxyphenyl pencycuron. In fact, β-glucuronidase treatment gave the same peak as authentic XV. Thus, the structure of this compound was characterized as 1-(*p*-chlorobenzyl)-1-cyclopentyl-3-[*p*-(β-glucuronyl)phenyl]urea.

Peak i. The molecular ion of peak i (Figure 9) was 30 amu greater than of peak g or h, indicating that an additional atom of the methoxy group was introduced into peak g or h. The ion cluster was observed at m/z 136/140; m/z 252 containing one chlorine atoms was as in peak g, suggesting that a glucuronic acid was conjugated to the cyclopentyl site and that the hydroxy group was substituted at the phenyl site of pencycuron. Ion cluster analysis upon pencycuron-d_0/d_4 dosing revealed the M^+ of this metabolite to be m/z 620/624, indicating that the site of glucuronide conjugation was not at the 2 or 5 position of the cyclopentyl moiety. By enzymatic analysis, the structure of this compound was characterized as 1-(*p*-chlorobenzyl)-1-[3-(β-glucuronyl)cyclopentyl]-3-(*p*-hydroxyphenyl)urea.

Table II. Metabolism of Pencycuron in Male Rabbits after the Oral Administration of 150 mg/kg[a]

	% of dose at indicated time interval				
	urine		feces		
metabolite found	0-1 day	1-3 days	0-1 day	1-3 days	total
pencycuron	–[b]	–	34.5	8.4	42.9
V: glucuronide	0.1	–	–	–	0.1
IX: free	0.1	–	–	–	0.1
glucuronide	0.9	–	–	–	0.9
XII: free	0.2	–	0.1	–	0.3
glucuronide	4.1	0.1	–	–	4.2
XV: free	2.4	0.1	0.3	0.6	3.4
glucuronide	13.7	0.3	–	–	14.0
XVII: free	0.2	–	–	–	0.2
XVIII: free	0.3	–	0.3	0.9	1.5
glucuronide	5.8	0.1	–	–	5.9
XXI: free	0.3	–	–	–	0.3
total	28.1	0.6	35.2	9.9	73.8

[a] Values represent the percent equivalent of the initial dose and the average of four rabbits. [b] Below the detectable limit (< 0.1%).

After dichloromethane partition, an aqueous fraction of the urine sample was subjected to enzymatic hydrolysis; five mass spectra containing m/z 125/127 (ion intensity, 3/1) were obtained by treatment with only β-glucuronidase. Among these, four mass spectra coincided with the referential compounds IX, XV, XII, and XVIII. The other mass spectrum is depicted in Figure 10. The distance of the ion cluster was 5 amu; the mass pattern was very similar to that of authentic V, suggesting the metabolite to be *N*-glucuronide of decyclopentylpencycuron.

Characterization of Fecal Metabolites. The ethyl acetate soluble fraction of the fecal sample yielded a large peak. Determination of the retention times on GLC, the mass spectrum upon GC–MS, and the R_f values on TLC confirmed this to be pencycuron per se. Furthermore, compounds XII, XV, and XVIII were found in the same fraction. However, even after enzymatic treatment, the aqueous-soluble fraction of the fecal sample yielded no peaks that could be regarded as the metabolite.

Quantitative Determination of the Metabolites. Several previously prepared metabolites were quantitatively analyzed by using GLC. Approximately 74% of the initial dose were tentatively confirmed (Table II).

While it was below the limits of detection in the urine, 43% of the administered pencycuron was present in feces collected during 3 days after dosing. In our preliminary study, in which male rabbits received 5, 50, or 150 mg/kg, 26.1%, 42.2%, and 42.9% of the parent compound was excreted in the feces. These findings suggested that the absorption of pencycuron from the gastrointestinal tract was quite low and that to this may be ascribable its remarkably low toxicity upon ingestion by mammals.

The main metabolites were *p*-hydroxyphenyl pencycuron (XV, 3.4%) and its β-glucuronide (14.0%). Next, two metabolites hydroxylated at the cyclopentyl moiety were determined as both free and conjugated forms (XII, 4.5%; XVIII, 7.4%). Compounds XVII and XXI were detected as minor urinary metabolites (XVII, 0.2%; XXI, 0.3%), and small amounts of decyclopentylated metabolites (V, 0.1%; IX, 1.0%) were also found. Since authentic compounds could not be prepared, quantitative analysis for peaks f in Figure 6 were not performed. Arylsulfatase hydrolysis made it clear that the excreta did not contain

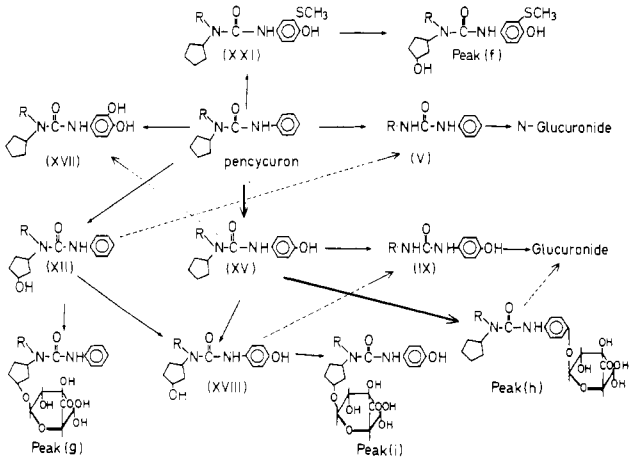


Figure 11. Proposed metabolic pathways of pencycuron in male rabbits (R = *p*-chlorobenzyl).

any sulfate-conjugated metabolites.

Metabolic Pathways of Pencycuron in Rabbits. The metabolic pathways of pencycuron in male rabbits outlined in Figure 11 can be deduced from the data acquired in this study. The main pathways were para-hydroxylation at the phenyl moiety and its β-glucuronic acid conjugation. Hydroxylation at other positions in this ring, namely, ortho- or meta-hydroxylated metabolites, was not observed. However, further hydroxylation occurred at the 3 position of the cyclopentyl moiety. We also recognized that both of these oxidations can occur in one molecule, as is the case for siduron (Belasco and Reiser, 1969). On the other hand, the benzyl group was hardly oxidized. Five β-glucuronide-conjugated metabolites were found; three of these were detected directly by ion cluster analysis. This may be a novel finding in the field of pesticide metabolism study.

Because compound IV, a dephenylated metabolite, was scarcely detected in any excreta, and because none of the three de-*p*-chlorobenzyl metabolites (I–III) were found, it is reasonable to posit that the cyclopentyl moiety may be the most easily released among the three substituents in pencycuron. Thereafter, glucuronic acid may connect at any side of the nitrogen atoms in the urea skelton. On the basis of the results of our ion cluster analysis, we tentatively propose three unique metabolites. Two of these contain the methylthio group; the other was of a pyrocatechol type at the phenyl ring. It should be noted that the metabolite(s) whose phenyl ring was substituted by only the *S*-methyl group, such as authentic XIX or XX, was not detected; both *S*-methyl and hydroxy groups were always posed simultaneously on the phenyl ring. Jerina et al. (1968, 1970) demonstrated that benzene and naphthalene were enzymatically converted to dihydrodiol, pyrocatechol, and dihydrohydroxy-*S*-gluthathion metabolites through the corresponding arene oxides. In the case of pencycuron, therefore, the occurrence of epoxide formation as an intermediate metabolite was suggested. In the present study, however, no gluthathion-conjugated compounds were found. Then, it would be postulated that meta-hydroxylation or meta-S-methylation occurred after para-hydroxylation was performed. Tateishi et al. (1978) demonstrated that the *S*-methyl group was formed through three reactions: mercapturic acid conjugation, S–C cleavage, and methylation by *S*-methyltransferase of liver microsome. Although Muecke et al. (1976) confirmed the methylthiolation of chlorotoluron in the rat, the mechanisms of those pathways are not understood at present.

As indicated in this report, estimating the structure of an unknown metabolite can be facilitated by the use of ion cluster analysis, even if the metabolite was not completely separated and its amount was very small. Therefore, we plan to use this technique in further studies on the metabolism of pesticides in conjunction with radioactively labeled compounds.

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