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Metabolism of Drugs. LXXVI.[1] The Metabolic Fate of Prolintane in Rabbits

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The metabolism of prolintane (1-phenyl-2-pyrrolidinopentane hydrochloride) in rabbits has been performed in this study. Rabbits receiving the drug in oral dose of 270 mg/kg or 170 mg/kg excreted seven metabolites together with a trace of unchanged drug in the 24 hr urine. They were shown to be lactam, phenol, alcohol, lactam phenol, diastereoisomeric two lactam alcohols and amino acid, by infrared, nuclear magnetic resonance and mass spectra. Some of these were unequivocally identified by comparison with authentic samples.

From these results, it is concluded that the major metabolic oxidation occured at pyrrolidine and aromatic ring to form lactam and phenolic metabolites, respectively. Penultimate oxidation of alkyl chain was seemed to be the minor reaction.

Prolintane (1-phenyl-2-pyrrolidinopentane), a recently developed CNS stimulant,[3] has a simple chemical structure as well as that of amphetamine. In spite of the simplicity, this structure seems very interesting in the metabolic point of view, since it consists of three functional groups, *i.e.* phenyl, alkyl, and pyrrolidino groups, each of which has a high susceptibility to metabolic oxidation. It is, therefore, expected that the metabolic pathways of prolintane would be of complex pattern, and this seems very attractive to investigate.

By the elaborated work on the metabolism of amphetamine, Dring, *et al.*[4] have recently reported that the drug is metabolized mainly through either aromatic hydroxylation or oxidative deamination, depending on the animal species. It is further evidenced that compounds possessing a pyrrolidino group such as nicotine[5a,b] or tremorine[6a,b] are oxidized primarily into corresponding lactam derivatives.

In the present paper the metabolic pathways of prolintane in rabbits will be reported.

Material and Method

Material——Prolintane hydrochloride, mp 130—133°, was kindly supplied by Morishita Pharmaceutical Co., Ltd., Osaka, Japan. 1-(4-Hydroxyphenyl)-2-pyrrolidinopentane hydrochloride, mp 168—169°, two diastereoisomeric 1-phenyl-2-pyrrolidino-4-hydroxypentane hydrochlorides, (A) mp 126—127°, (B) mp 171—172°, and 5-phenyl-4-pyrrolidinopentanoic acid hydrochloride, mp 189—192°, were kindly gifted from C.H. Boehringer Sohn, Ingelheim, Germany. All the samples described above were of racemic forms.

Gas Liquid Chromatography (GLC)——The instrument used was a Shimadzu Model GC-1C Gas Chromatograph equipped with hydrogen flame ionization detector (dual column and differential flame type). The column used was a glass U-shaped tube, ϕ 4 mm × 2.625 m. The column packing was 1.5% OV-1 on Shimalite W (100—200 mesh). The column temperature was maintained at 180°, the sample chamber and the detector temperature at 210°. Nitrogen was used as the carrier gas with a flow rate of 40 ml/min (2.8 kg/cm^2).

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N,O-Bis(trimethylsilyl)acetamide was used as trimethylsilylating reagent.

Thin-Layer Chromatography (TLC)——Thin-layer plate of silica gel (Kiesel gel G, Merck; activated at 105° for 30 min) was used in the present experiments. Solvent systems used were (A) benzene: acetone: MeOH (7:1:2), (B) acetone: *n*-hexane (6:4), (C) ethylacetate and (D) $CHCl_3$: diethylamine (9:1).

Dragendorff reagent was used for detection of metabolites containing pyrrolidino group, and iodine vapour was applied for nonspecific detection of compounds. Phenolic compounds were visualized by 0.2% diazotized sulfanilic acid in 10% Na_2CO_3 solution.

Administration of Drug and Extraction of Metabolites——Two male albino rabbits weighing 2.6 kg were used in this study. Prolintane hydrochloride dissolved in water was administered orally in a dose of 270 mg/kg (Rabbit A) or 170 mg/kg (Rabbit B), respectively. Each 24 hr urine collected was filtered through hyflo supercel and then treated as shown in Fig. 1. After unconjugated metabolites were extracted, conjugated metabolites in the urine were hydrolyzed with a half volume of conc. HCl and then extracted with $CHCl_3$.

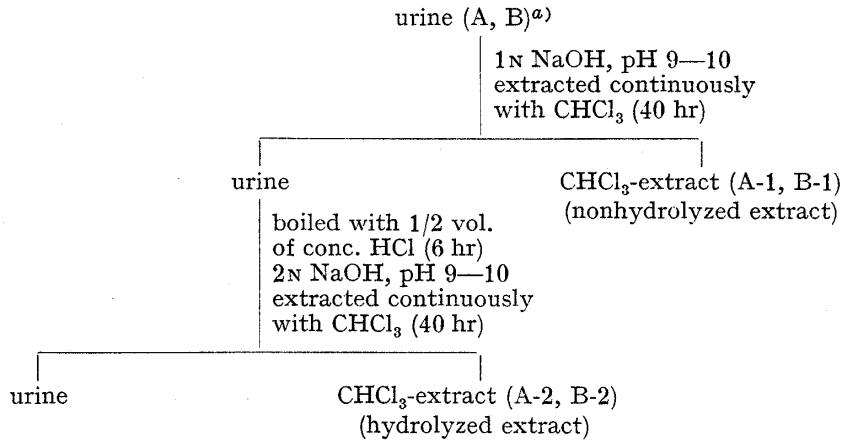

Fig. 1. Extraction Procedure of Metabolites from Urine

a) urine A: urine of rabbit A urine B: urine of rabbit B

Result

Detection of Urinary Metabolites

The 24 hr urine samples from two rabbits were separately extracted with $CHCl_3$ (see Method). Each organic phase was evaporated to dryness *in vacuo*. The residue was dissolved in 15 ml of MeOH, and 1 ml of them was reserved for quantitative analysis as described below. The remaining solution was condensed to a small volume *in vacuo* and submitted to chromatographic examinations (TLC and GLC).

As seen in Fig. 2, two spots, positive to Dragendorff reagent, were detected in nonhydrolyzed extract, whereas in hydrolyzed extract seven spots were detected. The spots of *Rf* 0.25 and 0.58 in hydrolyzed extract were also positive to diazotized sulfanilic acid reagent.

Isolation of Urinary Metabolites

About 400 mg of nonhydrolyzed extracts from two rabbits were applied to silica gel column chromatography (Silic AR CC-7, Mallinkrodt, ϕ 2.1×13.5 cm) using $CHCl_3$, $CHCl_3$: acetone (90: 10), acetone, acetone: MeOH (90: 10), acetone: MeOH (50: 50) and MeOH as effluent solvent. About 100 mg of crude Metabolite-1 (M-1) and same amounts of crude Metabolite-7 (M-7) were isolated from $CHCl_3$ and acetone: MeOH (50: 50) eluates, respectively.

By means of silica gel column chromatography using $CHCl_3$, $CHCl_3$: acetone, acetone and acetone: MeOH as effluent solvent, about 600 mg of hydrolyzed extracts was separated into three fractions; the first, $CHCl_3$ eluate contained only M-1, the second, $CHCl_3$: acetone (80: 20) eluate contained Metabolite-3 (M-3), Metabolite-4 (M-4), and Metabolite-6 (M-6), and the third, acetone: MeOH (80: 20) eluate contained Metabolite-2 (M-2), Metabolite-5 (M-5), and M-7, respectively.

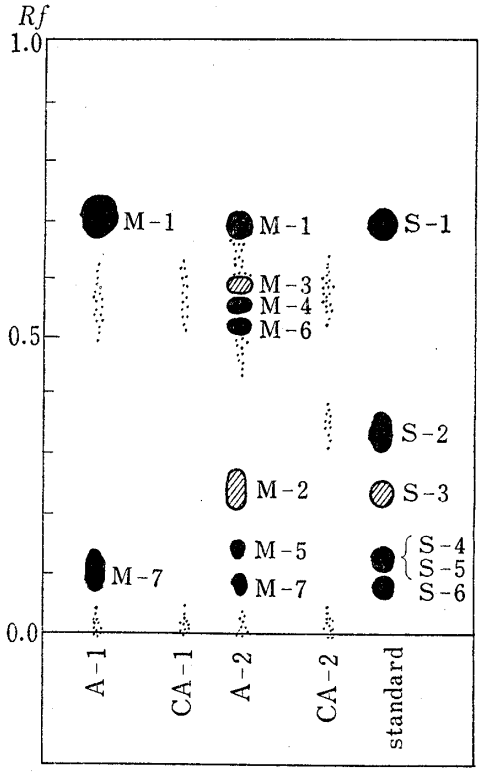

Fig. 2. Thin-Layer Chromatogram of Urinary Extracts and Authentic Samples (solvent system A)

A-1 : nonhydrolyzed extract of Urine A
CA-1: nonhydrolyzed extract of control urine
A-2 : hydrolyzed extract of Urine A
CA-2: hydrolyzed extract of control urine
S-1 : 1-phenyl-2-(2-oxopyrrolidino)pentane
S-2 : prolintane
S-3 : 1-(4-hydroxyphenyl)-2-pyrrolidinopentane
S-4 : 1-phenyl-2-pyrrolidino-4-hydroxypentane (A)
S-5 : 1-phenyl-2-pyrrolidino-4-hydroxypentane (B)
S-6 : 5-phenyl-4-pyrrolidinopentanoic acid
● : positive to Dragendorff reagent
▨ : positive to Dragendorff reagent and diazotized sulfanilic acid reagent

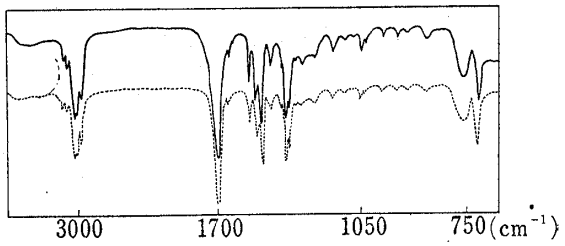

Fig. 3. IR Spectra of M-1 and Authentic Oxoprolintane (S-1)(liquid film)

——: M-1 ------: S-1

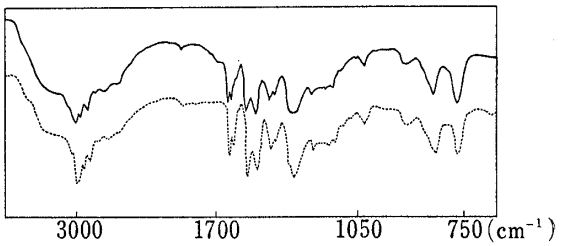

Fig. 4. IR Spectra of M-2 and Authentic Phenol (S-3) (liquid film)

——: M-2 ------: S-3

Brown oily substance obtained from the third fraction was submitted to preparative TLC using solvent system (C). The band showing *Rf* 0.50 was scrapped off, and eluted with MeOH. The eluate was filtered and evaporated to dryness. About 80 mg of pale yellow solid, M-2, was obtained. Similarly, about 2 mg of M-5 was obtained from the band having *Rf* 0.74. As to the second fraction, 20 mg of M-6 was isolated by rechromatography with silica gel column, and the remaining mixture of M-3 and M-4 was developed with solvent system (C) for preparative TLC. About 20 mg of M-3, and 30 mg of M-4 were eluted with MeOH from the bands of *Rf* 0.40 and 0.20, respectively.

Characterization and Identification of Urinary Metabolites

M-1——Crude M-1 was further purified by preparative TLC using solvent system (A). The position of M-1 was determined by spraying of Dragendorff reagent on one end of plate. The remaining band corresponding to M-1 was scrapped off and eluted with MeOH. The eluate was evaporated to dryness *in vacuo*. The residue was dissolved with 20 ml of ether and filtered. After evaporation of ether, about 60 mg of brown oily residue (pure M-1) was obtained. This purified metabolite gave a single peak on GLC and a single spot on TLC developed with all of the solvent systems used.

As seen in Fig. 3, the infrared (IR) spectrum of M-1 showed a strong peak at 1680 cm^{-1}, suggesting that this metabolite should be a lactam derivative. In addition, the mass and nuclear magnetic resonance (NMR) spectra also supported a lactam structure (see Table I and II). It was finally evidenced by IR, NMR and mass spectra, and GLC and TLC that M-1 was identical with authentic lactam which was synthesized chemically as described below.

M-2——Pure M-2 gave an orange-violet spot on TLC with diazotized sulfanilic acid reagent, suggesting that it should be phenolic compound. This was further confirmed by the

ultraviolet (UV) spectra showing a maximum absorption at 279 mμ in EtOH and 293 mμ in 0.1N NaOH. In addition to this UV spectra, the IR (Fig. 4), NMR (Table I) and mass spectra of M-2 were identical with those of authentic phenol, 1-(4-hydroxyphenyl)-2-pyrrolidinopentane.

M-3——M-3, recrystallized from acetone to colorless needles, mp 137°, showed UV spectra similar to those of M-2, in neutral and alkaline media, which suggested that M-3 was also phenolic metabolite. The IR spectrum indicated the presence of phenolic OH at 3160 cm^{-1} and lactam carbonyl at 1650 cm^{-1}. The NMR (Table I) and mass spectra also suggested that M-3 was a lactam phenol, 1-(4-hydroxyphenyl)-2-(2-oxopyrrolidino)pentane.

M-4——The IR spectrum of M-4 gave characteristic bands due to lactam carbonyl group at 1660 cm^{-1} and hydroxyl group at 3450 cm^{-1}, but the bands in finger print region were apparently different from those of M-3. The UV spectrum showed no bathochromic shift. The NMR spectrum showed a similar pattern as M-1, however the signal of primary methyl group of M-1 (0.89 ppm) changed to doublet at 1.18 ppm, indicating that a hydroxyl group must be introduced at (ω-1)-position of alkyl chain of M-1 (Table I).

From these findings it was concluded that M-4 should be a lactam alcohol, 1-phenyl-2-(2-oxopyrrolidino)-4-hydroxypentane.

M-5——This was obtained only in a trace amount, but enough to carry out TLC, GLC, and mass spectral analysis. The mass spectrum of M-5 showed a molecular ion peak at *m/e* 233 and characteristic fragment ion peaks at *m/e* 218[M—CH_3]$^+$, 172[M—CH_2-CH(OH)-CH_3]$^+$, 142 [M—C_6H_5-CH_2]$^+$, 45[CH_3-CH=OH]$^+$, identical with those of authentic two diastereoisomeric (ω-1)-alcohols. Furthermore this metabolite behaved as same as authentic (ω-1)-alcohol

Table I. NMR[a] Signals of Metabolites and Authentic Samples

Compound	NMR Signal (ppm in $CDCl_3$)
M-1 (lactam)	0.89 (3H, -CH_3); 1.10—1.60 (4H, m, -CH_2-); 1.60—2.50 (4H, m, -CH_2-) 2.77 (2H, d, C_6H_5-CH_2-); 3.18 (2H, t, N-CH_2-); 4.37 (1H, m, -CH-); 7.19 (5H, s, aromatic)
M-2 (phenol)	0.79 (3H, -CH_3); 1.15—1.58 (4H, m, -CH_2-); 1.60—2.10 (4H, m, -CH_2-); 2.33—3.20 (4H, N-CH_2-;2H, C_6H_4-CH_2-; 1H, -CH-); 5.20 (1H, broad, C_6H_4-OH); 6.70 (2H, d, aromatic); 7.03 (2H, d, aromatic)
M-3 (lactam phenol)	0.91 (3H, -CH_3); 1.07—1.70 (4H, m, -CH_2-); 1.70—2.50 (4H, m, -CH_2-); 2.73 (2H,C_6H_4-CH_2-); 3.23 (2H, t, N-CH_2-); 4.10—4.80 (1H, -CH-; 1H, C_6H_4-OH); 6.65 (2H, d, aromatic); 6.98 (2H, d, aromatic)
M-4 (lactam alcohol)	1.18 (3H, d, -CH_3); 1.58 (2H, t, -CH_2-); 1.75—2.60 (4H, m, -CH_2-); 2.86 (2H, d, C_6H_5-CH_2-); 3.24 (2H, t, N-CH_2-); 3.30—3.90 (1H, -CH-; 1H,-OH); 4.72 (1H, m, -CH-); 7.18 (5H, s, aromatic)
M-6 (lactam alcohol)	1.18 (3H, d, -CH_3); 1.50—1.70 (2H, -CH_2-); 1.70—2.50 (4H, m, -CH_2-); 2.83 (2H, d, C_6H_5-CH_2-); 3.10 (1H, -OH); 3.27 (2H, t, N-CH_2-); 3.80(1H, m, -CH-); 4.48 (1H, m, -CH-); 7.20 (5H, s, aromatic)
M-7 (amino acid)	0.81 (3H, -CH_3); 1.10—1.72 (4H, m, -CH_2-); 1.72—2.20 (2H, m, -CH_2-); 2.20—2.63 (2H, m, -CH_2-COO^-); 2.70—3.40 (2H, N-CH_2-; 2H, C_6H_5-CH_2-; 1H, -CH-); 7.20 (5H, s, aromatic); 9.97 (2H, broad, -N^+H_2-)
S-1[b] (lactam)	0.89 (3H, -CH_3); 1.10—1.60 (4H, m, -CH_2-); 1.60—2.50 (4H, m, -CH_2-); 2.77 (2H, d, C_6H_5-CH_2-); 3.18 (2H, t, N-CH_2-); 4.37 (1H, m, -CH-); 7.20 (5H, s, aromatic)
S-2[b] (prolintane)	0.82 (3H, -CH_3); 1.10—1.63 (4H, m, -CH_2-); 1.63—2.18 (4H, m, -CH_2-); 2.30—2.90 (4H, N-CH_2-; 2H, C_6H_5-CH_2-); 2.96 (1H, m, -CH-); 7.23 (5H, s, aromatic)
S-3[b] (phenol)	0.79 (3H, -CH_3); 1.10—1.57 (4H, m, -CH_2-); 1.57—2.10 (4H, m,-CH_2-); 2.30—3.20 (4H, N-CH_2-; 2H, C_6H_4-CH_2-; 1H, -CH-); 5.20 (1H, broad, C_6H_4-OH); 6.70 (2H, d, aromatic); 7.03 (2H, d, aromatic)
S-4[b] (alcohol)	1.05 (3H, d,-CH_3); 1.47 (2H, t, -CH_2-); 1.60—2.10 (4H, m, -CH_2-); 2.50—3.30 (1H, -CH-; 4H, N-CH_2-; 2H, C_6H_5-CH_2-); 4.13 (1H, m, -CH-); 5.02 (1H, broad, -OH); 7.19 (5H, s, aromatic)

a) NMR spectra were measured by JEOL-JNM-C-60H spectrometer (60 Mc) with tetramethylsilane as internal reference.
b) See legend of Fig. 2.

(A) with TLC and GLC (Table II). By these, M-5 was concluded tentatively to be one of the diastereoisomeric (ω-1)-alcohols of prolintane, 1-phenyl-2-pyrrolidino-4-hydroxypentane (A).

M-6——The IR spectrum of M-6 was very similar to that of M-4, showing absorption bands of lactam carbonyl at 1650 cm^{-1} and alcoholic hydroxyl group at 3460 cm^{-1}. In addition, the NMR and mass spectra were similar to that of M-4. It, however, showed a different retention time with GLC and *Rf* values with TLC from those of M-4 (Table II).

These results suggested that M-6 should be a diastereoisomer of M-4.

M-7——Crude M-7 was recrystallized from ethylacetate to give 50 mg of colorless needles, mp 143—144°. It gave a single peak on GLC with the same retention time as M-1, whereas on trimethylsilylation with BSA prior to injection, the sample gave a different retention time from M-1 (Table II). The IR spectrum showed strong peaks at 1550 and 1400 cm^{-1}, and broad band between 2000 and 2600 cm^{-1}, suggesting that M-7 was amino acid with zwitter-ion structure. Furthermore the NMR spectrum showed the signals indicating the opening of pyrrolidine ring (Table I). The mass spectrum gave a molecular ion peak at *m/e* 249 and the main fragment ion peaks at *m/e* 140, 98, and 91, were identical with those of M-1.

These data indicated that the structure of M-7 should be N-(1-phenyl-2-pentyl)-γ-amino-butyric acid, which afforded a ring-closed compound, M-1, by heating on GLC.

Synthesis of 1-Phenyl-2-(2-oxopyrrolidino)pentane (Oxoprolintane)

A mixture of 2 g of 1-phenyl-2-aminopentane[7] and 1 g of γ-butyrolactone was heated at 110—130° for 3 hr under occasional agitation and then at 250—270° for another 3 hr on

Table II. Summary of Analytical Data of Metabolites and Authentic Samples

Compound	IR (cm^{-1})	UV λ_{max} (mμ)	Mass M^+ (*m/e*)	GLC (Relative t_R)		TLC (*Rf*)	
				untreated	TMS	A[a]	D[a]
M-1 (lactam)	ν_{CO} 1680	—	231	2.05	2.05	0.70	0.83
M-2 (phenol)	ν_{OH} 3300	EtOH 279 0.1N NaOH 293	233	2.47	2.84	0.25	0.66
M-3 (lactam phenol)	ν_{CO} 1650 ν_{OH} 3160	EtOH 279 0.1N NaOH 293	247	6.05	5.84	0.55	0.45
M-4 (lactam alcohol-1)	ν_{CO} 1660 ν_{OH} 3450	—	247	3.26	3.79	0.51	0.69
M-5 (alcohol)	—	—	233	1.79	1.95	0.21	0.84
M-6 (lactam alcohol-2)	ν_{CO} 1650 ν_{OH} 3460	—	247	3.42	4.11	0.49	0.58
M-7 (amino acid)	ν_{CO} 1550 1400	—	249	2.05	3.12	0.10	0.07
S-1[b] (lactam)	ν_{CO} 1680	—	231	2.05	2.05	0.70	0.83
S-2[b] (prolintane)	—	—	217	1.00[c]	1.00[c]	0.33	0.85
S-3[b] (phenol)	ν_{OH} 3300	EtOH 279 0.1N NaOH 293	233	2.47	2.84	0.25	0.67
S-4[b] (alcohol-A)	ν_{OH} 3400	—	233	1.79	1.95	0.21	0.83
S-5[b] (alcohol-B)	ν_{OH} 3300	—	233	1.79	2.05	0.22	0.83
S-6[b] (pentanoic acid)	ν_{CO} 1740	—	247	0.00	3.53	0.08	0.05

a) A: solvent system A; D: solvent system D
b) authentic samples (see legend of Fig. 2.)
c) Prolintane showed t_R 2.0 min

7) S. Ishiwatari and T. Suzuki, *Yakugaku Zasshi*, **71**, 1265 (1951).

silicon oil bath. After it was allowed to stand overnight at room temperature, the excess reactants were distilled off under reduced pressure. The brown oily residue was dissolved in ether, and the solution was washed by shaking with dil. HCl and then with water. After the solvent was evaporated, about 2.4 g of reddish brown gum was obtained. It was further purified by silica gel column chromatography using benzene, benzene: acetone (95: 5) and benzene: acetone (90: 10) as the effluent solvent. About 0.6 g of brown oily substance was obtained from benzene: acetone (95: 5) eluate.

The IR spectrum of this synthetic oxoprolintane is shown in Fig. 3. Characteristics of the NMR and mass spectra are summarized in Table I and II.

Quantitative Analysis of Urinary Metabolites

Reserved extracts from the urine samples of two rabbits, were applied to GLC for quantitative analysis in acetone solution (see Method). Pure metabolites described above were used as standards for making calibration curves. Results were summarized in Table III.

Table III. Excretion Percentage of Urinary Metabolites in Rabbits Given Prolintane

Metabolite	Animal			
	Rabbit A (% of dose)		Rabbit B (% of dose)	
	A-1 (nonhydrolyzed)	A-2 (hydrolyzed)	B-1 (nonhydrolyzed)	B-2 (hydrolyzed)
M-1 (lactam)	12.4	2.8	14.4	1.3
M-2 (phenol)	0.0	11.9	0.0	5.1
M-3 (lactam phenol)	0.0	5.1	0.0	3.1
M-4 (lactam alcohol-1)	0.0	7.9	0.0	5.1
M-5 (alcohol)	0.0	trace	0.0	trace
M-6 (lactam alcohol-2)	0.0	4.2	0.0	2.7
M-7 (amino acid)	5.7	trace	trace	trace
prolintane (unchanged)	trace	0.0	trace	0.0
Total	18.1	31.9	14.4	17.3

Discussion

The present paper has shown that prolintane is metabolized to seven or more metabolites in rabbits (Fig. 5). The quantitative aspects of the urinary metabolites which are summarized in Table III indicate that in rabbits, the major metabolic route of prolintane is oxidation of pyrrolidine ring to lactam ring as well as nicotine[5a,b] and tremorine.[6a,b] It is also shown that aromatic hydroxylation occurs in lesser extent and penultimate oxidation of alkyl chain does in the least. These reactions also take place with combination to produce lactam phenol and diastereoisomeric two lactam alcohols. Almost of these metabolites are excreted as conjugate forms, most of which are shown to be hydrolyzable with β-glucuronidase by our preliminary experiment and therefore considered as glucuronides. Optical rotations of metabolites were measured in MeOH solution. However, amount of isolated metabolites is too little to obtain precise value of $[\alpha]_D$, although it is supposed that some of metabolites are optically active.

Dring, *et al.*[4] have recently investigated the metabolism of amphetamine and reported that the major metabolic pathway in rabbits is oxidative deamination to yield benzyl methyl ketone which is, in turn, conjugated perhaps with sulfuric acid. In the case of prolintane,

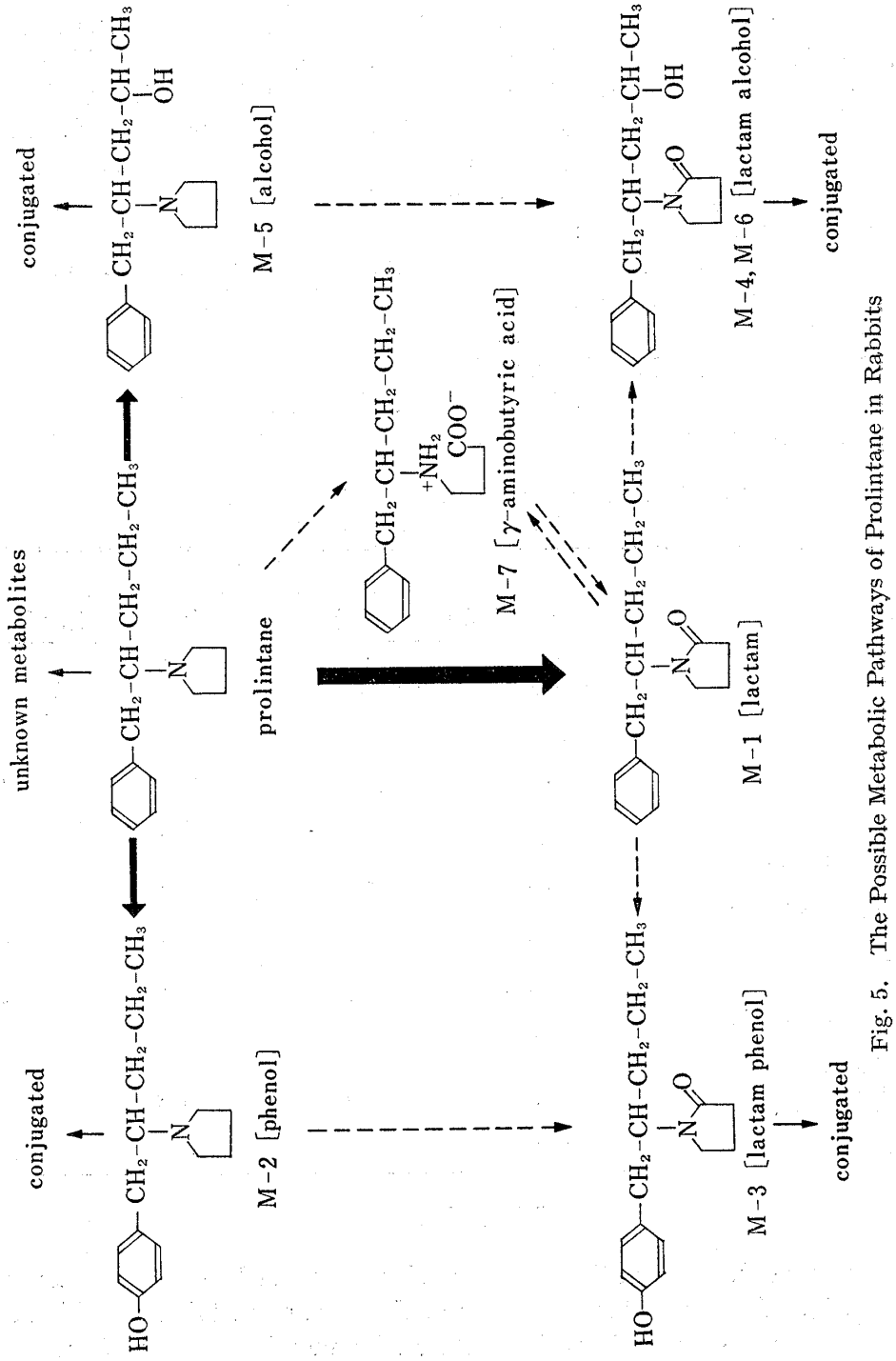

Fig. 5. The Possible Metabolic Pathways of Prolintane in Rabbits

although it has an analogous structure to amphetamine, such a deamination could not be observed.

As described above, a lactam formation is the most important pathway in the metabolism of prolintane, however, it has not yet been determined whether direct dehydrogenation of a possible primary metabolite, hydroxyprolintane, concerns with this lactam formation or M-7 (amino acid derivative produced by opening of pyrrolidine ring) is an obligatory intermediate.

In the preliminary investigation on pharmacological action in mouse, oxoprolintane (M-1) showed a weak stimulant activity on ambulation and rearing.

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