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Studies on Absorption, Biotransformation and Excretion of Drug. V.[1] Metabolism of Pentazocine in the Rat

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The metabolism of pentazocine was studied in the rat following oral administration. After the enzymatic hydrolysis of the urine, eight metabolites including unchanged pentazocine were extracted with organic solvents and XAD-2 column, and isolated by preparative thin-layer chromatography. These metabolites were characterized by gas-liquid chromatography, and infrared, nuclear magnetic resonance and mass spectrometries.

Unchanged pentazocine and three metabolites, 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*trans*-2-buten-1-ol (*trans*-OH), 1,2,-3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-crotonic acid (*trans*-COOH) and norpentazocine were identified by comparison with corresponding authentic samples, and four other metabolites were characterized to be 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*cis*-2-buten-1-ol (*cis*-OH), 9-methoxy pentazocine (or its 8-methoxy-9-hydroxy isomer) and 8,9(or 9,8)-methoxy hydroxy metabolites of *cis*-OH and *trans*-OH by spectral and chromatographic data.

During 24 hr after oral administration, unchanged pentazocine was mainly excreted in the urine as the nonconjugated and conjugated forms. The amount of the other metabolites was found to be small.

Keywords——pentazocine; metabolism (*in vivo*); metabolic pathway (rat); methoxy hydroxy metabolites; mass spectra; NMR spectra; TLC; GLC

Pentazocine is a potent analgesic agent and very useful for clinical application. Pittman, *et al.*[3] studied the metabolism of pentazocine in the 10000 ***g*** supernatant of the monkey, mouse and rat liver homogenates and in the monkey *in vivo* and reported that pentazocine was mainly metabolized through oxidation of the terminal methyl groups of the dimethylallyl side chain to yield two isomeric alcohols (*cis*-OH and *trans*-OH) and one of the corresponding carboxylic acid (*trans*-COOH). Norpentazocine was also identified in the bile of mouse,[4] but El-Mazati and Way[5] could not detect the metabolite in the urine of the rat.

Recently, Lynn, *et al.*[6] investigated the metabolism of benzomorphans in the isolated perfused rat liver and reported that pentazocine was hydroxylated on the aromatic ring and further metabolized by methylation of one hydroxy group, in addition to the formation of norpentazocine and of hydroxy and carboxylic acid metabolites oxidized on the dimethylallyl substituent.

We previously[7] carried out the evaluation of the biological fate of pentazocine after oral administration in the rat and also compared it with that after subcutaneous administration in order to understand the pharmacological effects in both administered routes. These

1) Part IV: K. Fukawa, O. Irino, and Y. Ohsawa, *Pharmacometrics*, **16**, 483, (1978).
2) Location: *2-12-3 Sakurashin-machi, Setagaya-ku, Tokyo.*
3) K.A. Pittman, D. Rosi, R. Cherniak, A.J. Merola, and W.D. Conway, *Biochem. Pharmacol.*, **18**, 1673 (1969).
4) S.D. James, G.C. Paugh, and R.H. Waring, *Xenobiotica*, **4**, 521 (1974).
5) A.M. El-Mazati and E.L. Way, *J. Pharmacol. Exptl. Therap.*, **177**, 332 (1971).
6) R.K. Lynn, R.G. Smith, R.M. Leger, M.L. Deinzer, D. Griffin, and N. Gerber, *Drug Meta. Dipos.*, **5**, 47 (1977).
7) K. Fukawa, O. Irino, K. Maruyama, Y. Ohsawa, and K. Saitoh, *Pharmacometrics*, in press.

studies revealed that the excretion of pentazocine in the urine and feces during 24 hr was 21% of the oral dose. This corresponded with the result described by El-Mazati and Way[5] that the recovery from the urine and feces was found to be low after oral administration in the rat. In contrast, a large amount was excreted in human[8] and monkey[3] urine after oral administration.

The authors attempted to study the metabolism of pentazocine in the rat because it is considered that the metabolism of pentazocine in the rat differs from that in the primates.

Materials and Methods

Materials——Pentazocine[1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-8-ol] was synthesized by Kametani, *et al.*[9] Norpentazocine, 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*trans*-2-buten-1-ol (*trans*-OH) and 1,2,3,4,5,6-hexa-

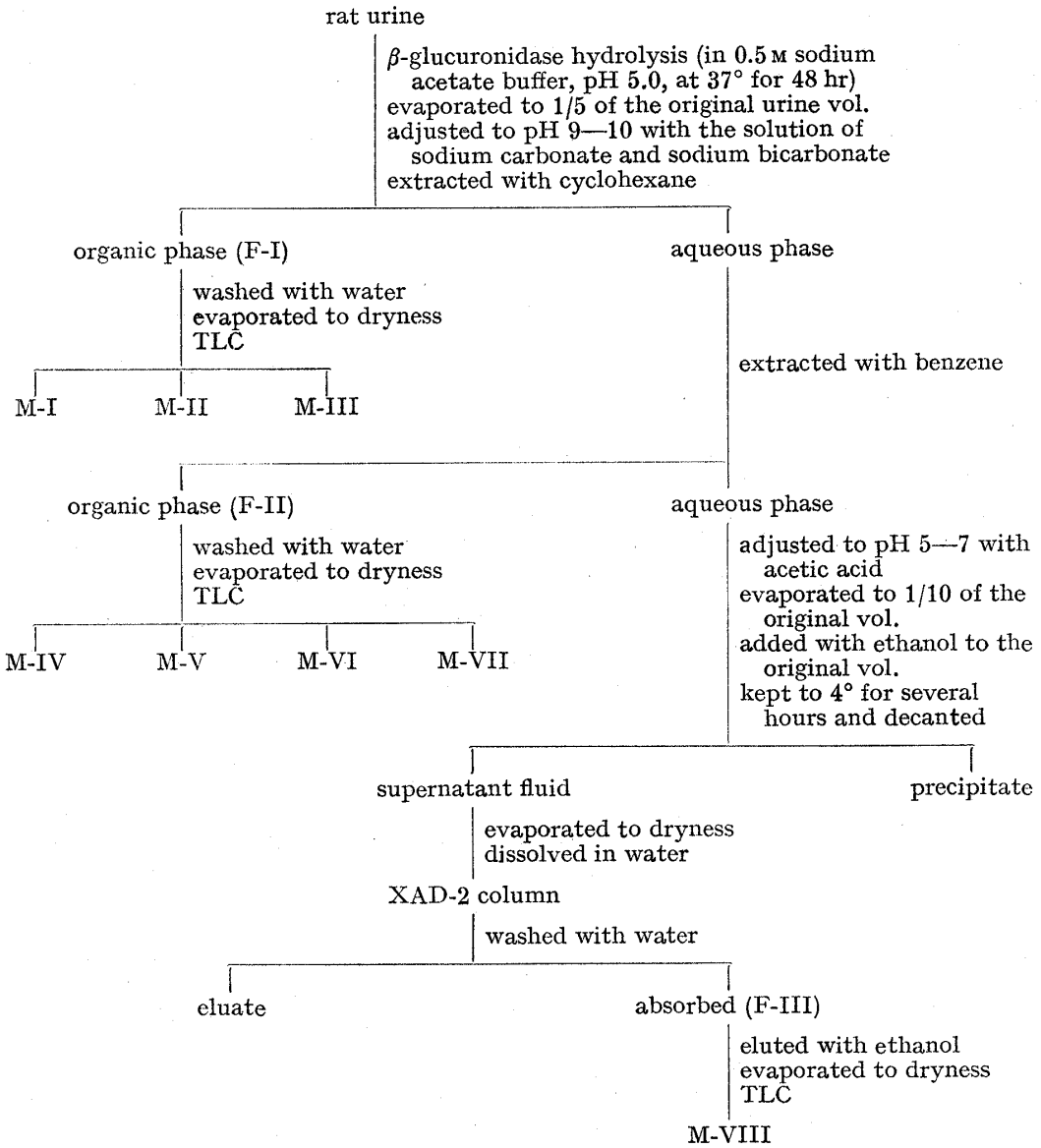

Chart 1. Scheme for Fractionation and Isolation of the Urinary Metabolites in Rat Urine

8) K.A. Pittman, *Biochem. Pharmacol.*, **19**, 1833 (1970).
9) K. Kametani, K. Kigasawa, M. Hiiragi, T. Hayasaka, N. Wagatsuma, and K. Wakisaka, *J. Heterocyclic Chem.*, **6**, 43 (1969).

hydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-crotonic acid (*trans*-COOH) were synthesized according to the method described by Albertson, *et al.*[10] (See Chart 2 for the structures).

Animals and Sampling——Aqueous solution of pentazocine hydrochloride (0.6%) was administered orally at a dose of 60 mg/kg/day to 10 male Sprague-Dawley rats weighing about 200 g (obtained from Shizuoka Agriculture Cooperative Association for Laboratory Animals). Urine sample was collected at 24 hr intervals after daily administration in a metabolic cage for 10 days in a flask containing toluene to prevent decomposition and frozen for subsequent experiment. Urine of untreated rats was also collected as control.

Enzymatic Hydrolysis——The urine (500 ml) adjusted to the pH-range between 5 and 6 with acetic acid was incubated with 2 g of β-glucuronidase (70000 to 100000 Fishman units/g, Tokyo Kasei Kogyo Co. Ltd.) in 1000 ml of 0.5 M acetate buffer (pH 5.0) at 37° for 48 hr.

Trimethylsilylation——The isolated metabolites were treated with N,O-bis(trimethylsilyl)-acetamide (BSA kit, Tokyo Kasei Kogyo Co. Ltd.) at room temperature.

Chromatography——Thin-layer Chromatography (TLC): The chromatography was carried out on Kieselgel 60/Kieselgur F_{254} plate (Merck), with the following solvent systems:

System I: benzene–methanol–isopropylamine (95: 5: 3)
System II: dioxane–water–ammonia (100: 15: 5)
System III: dioxane–methanol–formic acid (100: 5: 5)
System IV: ethyl acetate–triethylamine (90: 10)
System V: benzene–methanol–ammonia (79: 20: 1)

The chromatograms were visualized under ultraviolet light of 254 nm wavelength and by spraying with iodoplatinate, Dragendorff and Folin-Ciocalteu reagents.

Gas–liquid Chromatography (GLC): A Hitachi model 063 gas chromatograph equipped with a hydrogen flame ionization detector was employed. The glass column, which was 1.5 m×3 mm (i.d.), was packed with 3% OV-1 on Gas-Chrom Q or 5% SE-30 on Chromsorb W, and the temperatures set at 230° and 260°, respectively.

Determination of Spectra——Mass Spectrometry: A Shimadzu-LKB model 9000 gas chromatograph–mass spectrometer was used. The separation was made on a 1.5 m×3 mm(i.d.) glass column packed with 3% OV-1 on Gas-Chrom Q at 230°. The flow rate of helium carrier gas was 30 ml/min. The ionizing potential and the trap current were 70 eV and 60 μA, respectively.

Nuclear Magnetic Resonance (NMR) Spectrometry: A JEOL model JNM-PMX 60 NMR spectrometer was employed in measuring the spectra of the metabolites dissolved in CD_3OD or CD_3Cl, with tetramethylsilane as an external reference.

Infrared (IR) Spectrometry: A Hitachi model 215 spectrometer was used to determine the spectra of the metabolites in KBr or chloroform.

Isolation of Urinary Metabolites——After enzymatic hydrolysis of the urine, the metabolites were extracted with cyclohexane (Fraction I) and then benzene (Fraction II). The remaining aqueous layer was submitted to Amberlite XAD-2 (Rohn and Hass Co.) column chromatography to obtain Fraction III as described in Chart 1. The metabolites in each fraction were isolated individually by preparative TLC (Wakogel B-5F, Wako Pure Chemical Industries, Ltd.) in mainly solvent system V.

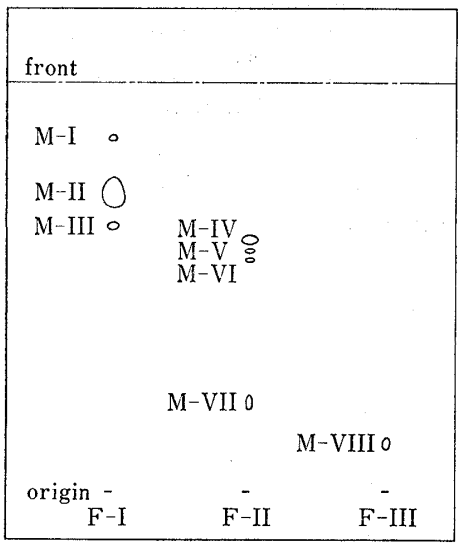

Fig. 1. Thin–Layer Chromatogram of F-I, F-II and F-III in Solvent System V

Results

Chromatographic Analysis of Urinary Metabolites

Thin–layer chromatography of F-I, F-II and F-III (Fig. 1) indicated the presence of eight iodoplatinate-positive spots, M-I through M-VIII. These spots were also visualized by Dragendorff and Folin-Ciocalteu reagents. In all solvent systems described in Table I, M-II had a *Rf* value identical with that of the authentic pentazocine, M-VI with that of *trans*-OH, M-VII with that of norpentazocine and M-VIII with that of *trans*-COOH (Table I).

The result of GLC of the metabolites is shown in Table II. M-VIII was determined only when trimethyl-

10) N.F. Albertson, D. Rosi, and A.J. Merola, U.S. Patent 3639410 (1972) [C. A., 76, 113101 *w* (1972)].

Table I. *Rf* Values of Pentazocine and Its Metabolites in Thin-Layer Chromatography

Metabolites[c]	*Rf* value[a]			
	Solvent system[b]			
	I	III	IV	V
M-I	1.29	1.01	1.09	1.20
M-II	1.00	1.00	1.00	1.00
M-III	0.89	0.86	0.75	0.90
M-IV	0.80	0.86	0.66	0.86
M-V	0.69	0.76	0.43	0.82
M-VI	0.64	0.76	0.40	0.79
M-VII	0.49	0.37	0.05	0.30
M-VIII	0.05	0.90	0.00	0.17

a) *Rf* values are relative to the *Rf* of authentic pentazocine. The absolute *Rf* values of authentic pentazocine in the solvent systems were as follows: 0.73(I), 0.79(III), 0.65(IV), 0.72(V).
b) The solvent systems (v/v) were described in Methods.
c) For the structures of these metabolites, refer to Chart 2.

Table II. Relative Retention Times[a] of Pentazocine and Its Metabolites by Gas-Liquid Chromatography

Metabolites[d]	Analysis conditions			
	I[b]		II[c]	
	Free	Trimethylsilyl derivative	Free	Trimethylsilyl derivative
M-I	1.35	1.35	1.44	1.33
M-II	1.00	1.00	1.00	1.00
M-III	3.00	3.02	2.45	2.48
M-IV	2.17	2.26	2.00	1.91
M-V	3.08	3.82	2.64	2.96
M-VI	2.38	2.38	2.11	2.22
M-VII	0.41	0.41	0.44	0.44
M-VIII		3.26		2.83

a) Retention times are relative to that of authentic pentazocine. The retention times of authentic pentazocine on 5% SE-30 and 3% OV-1 columns were 1.9 and 2.6 min, respectively.
b) 5% SE-30 column on Chromosorb W at 260°.
c) 3% OV-1 column on Gas-Chrom Q at 230°.
d) For the structures of these metabolites, refer to Chart 2.

silylated, and showed t_R value identical with that of the authentic sample of *trans*-COOH. t_R Values of M-II, M-VI and M-VII were also found to be identical with those of unchanged pentazocine, *trans*-OH and norpentazocine, respectively.

Characterization and Identification of Urinary Metabolites

(1) **Fraction I**——Mass spectrum (Table III) of the trimethylsilylated M-I showed the molecular ion peak at *m/e* 387. The fragment ions at *m/e* 332, 319 and 304, which include the aromatic ring of this metabolite, indicate a shift to higher levels by 30 amu which is equivalent to a methoxy group as compared with those of the authentic pentazocine (*m/e* 302, 289 and 274). The fragment ions of the side chain at *m/e* 178 and 68 are characteristic of pentazocine. In NMR spectrometry (Fig. 2), the signal of three hydrogen atoms of a methoxy group appeared at 3.83 ppm in addition to the signals caused by protons of 6- and 11-methyl groups and 3'-dimethyl group at 1.37, 0.89 and 1.79 ppm, respectively. In addition, the number

of protons on the aromatic ring was reduced to two which were shown as singlet at 6.70 and 6.75 ppm. From these evidences, it was concluded that M-I is 9-methoxy pentazocine (or its 8-methoxy-9-hydroxy isomer).

The mass (Table III), NMR and IR spectra of M-II were found to be identical with those of the authentic pentazocine; therefore it was determined to be unchanged pentazocine.

In the mass spectrum (Table III), M-III showed the molecular ion peak at *m/e* 475. From this and the fact that a methoxy group was added onto the aromatic ring as in the case of M-I and the fragment ions of the side chain at *m/e* 266 and 156 were trimethylsilylated, it was concluded that M-III is a methoxy hydroxy metabolite oxidized on the side chain to an alcohol. NMR spectrum (Fig. 2) of this metabolite indicates the loss of a methyl group at 3'-position (1.81 ppm), and the signal of protons of methoxy group appeared at 3.86 ppm and that of two hydrogen atoms of $-CH_2-$ of $-CH_2OH$ side chain appeared at 4.13 ppm. Based on these evidences, and by comparing with the results of NMR spectrometry, TLC and GLC of M-V which will be shown later, M-III was concluded to be 8, 9 (or 9, 8)-methoxy hydroxy metabolite oxidized on the side chain to *cis*-OH.

Table III. Mass Spectra of the Trimethylsilylated Metabolites of Pentazocine

Metabolites	Prominent and important fragment ions (*m/e*)
M-I	387, 372, 332, 319, 304, 275, 178, 113, 110, 73, 71, **70**, 69, 68, 45, 41
M-II (pentazocine)	357, 342, 302, 289, 274, 178, 110, 73, 70, 69, 68, 47, **45**, 41
M-III	475, 460, 332, 319, 304, 266, 156, 110, **73**, 70, 45
M-IV	445, 430, 302, 289, 274, 266, 156, 110, 73, 70, **45**
M-V	475, 460, 332, 319, 304, 266, 156, 110, **73**, 70, 45
M-VI (*trans*-OH)	445, 430, 302, 289, 274, 266, 156, 110, **73**, 70, 45
M-VII (norpentazocine)	289, 274, 110, 73, 70, **45**
M-VIII (*trans*-COOH)	459, 444, 302, 289, 280, 274, 170, 110, **73**, 70, 45

The mass spectra of the trimethylsilylated metabolites of pentazocine are tabulated beginning with the molecular ion followed by the fragment ions necessary for characterization or identification of the structures of metabolites, and the base peak is shown in boldface. The mass spectra of M-II, M-VI, M-VII and M-VIII were identified with the trimethylsilylated authentic samples. See Chart 2 for the structure of these metabolites.

(2) **Fraction II**——The mass spectrum (Table III) of M-IV showed the molecular ion peak at *m/e* 445, indicating that this metabolite is an isomer of M-VI which will be described later. Ions of the prominent fragments containing the aromatic ring were exhibited at *m/e* 302, 289 and 274 the same as those of the authentic pentazocine, and the peaks of ions due to the side chain of this metabolite at *m/e* 266 and 156 showed trimethylsilylation of the side chain. In NMR spectrum, the loss of a methyl group at 3'-position was confirmed, and the signal caused by $-CH_2-$ of the side chain's $-CH_2OH$ appeared at 4.13 ppm. By comparing these data with those of the authentic sample of *trans*-OH (M-VI), M-IV was speculated to be 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq), 11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*cis*-2-buten-1-ol (*cis*-OH).

The mass spectrum (Table III) of M-V indicated that it is an isomer of M-III as the molecular ion appeared at *m/e* 475. In NMR spectrum, the signal of a methoxy group appeared at 3.86 ppm and of two hydrogen atoms of $-CH_2OH$ at 4.06 ppm in addition to those of methyl groups of 3'-, 6- and 11-positions at 1.79, 1.37 and 0.89 ppm, and of two protons on the aromatic ring at 6.70 and 6.75 ppm, respectively. From these results, together with the results of TLC and GLC, M-V was concluded to be 8,9 (or 9,8)-methoxy hydroxy metabolite oxidized on the side chain to *trans*-OH.

By comparison of the mass (Table III), NMR (Fig. 2) and IR spectra of M-VI with those of the authentic sample, this metabolite was identified to be 1,2,3,4,5,6-hexahydro-8-hydroxy-α-6(eq), 11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*trans*-2-buten-1-ol (*trans*-OH).

The mass spectrum showing the molecular ion peak at *m/e* 289 (Table III) and the chromatographic properties of M-VII were identical with those of the authentic sample of norpentazocine.

(3) **Fraction III**——The metabolite (M-VIII) in this fraction, unlike the above metabolites, was not extractable into organic solvents but was recoverable with XAD-2 column, suggesting an increase in the polarity. The mass spectrum (Table III) showed the molecular ion peak at *m/e* 459 and the fragment ions necessary for identification of the metabolite at *m/e* 444, 302, 289, 280, 274, 170 and 110. The absorption band which appeared near 1700 cm^{-1} in IR spectrum supported the presence of a carboxy group in the structure. NMR spectrum showed the loss of three methyl protons of the terminal methyl groups of dimethylallyl side chain at 1.79 ppm. Additionally, these spectra and chromatographic properties were identical with those of the authentic sample of *trans*-COOH. From these evidences, it was concluded that M-VIII is 1,2,3,4,5,6-hexahydro-8-hydroxy-α, 6(eq), 11(ax)-trimethyl-2,6-methano-3-benzazocine-crotonic acid (*trans*-COOH).

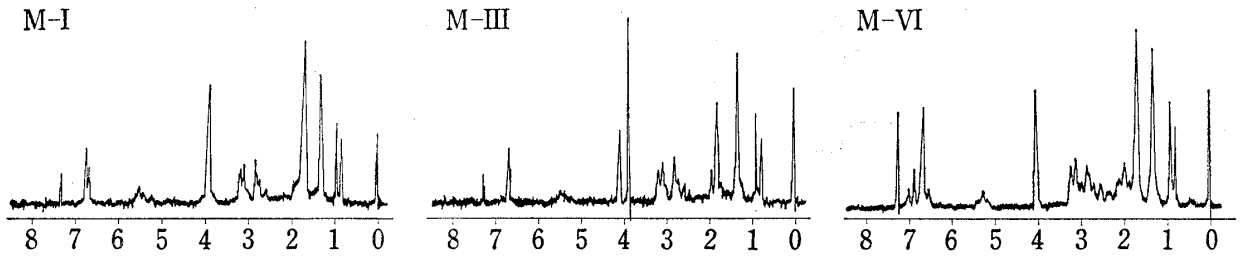

Fig. 2. NMR Spectra of M-I, M-III and M-VI

Discussion

Pittman, *et al.* reported that the amount of the metabolites in human and monkey represent 61%[10] and 70%,[3] respectively, of the administered dose when determined at 24 hr after pentazocine was given orally. These results are in sharp contrast to the studies in which about 20%[5,7] of the administered dose was recovered from urine in the rat. These facts suggest that the metabolism of pentazocine in the rat differs quantitatively or qualitatively from that in the primates.

In agreement with the results obtained by Pittman, *et al.*,[3] we have confirmed the presence of *trans*-COOH (M-VIII) as well as unchanged pentazocine (M-II), *cis*-OH (M-IV) and *trans*-OH (M-VI) in the rat urine after oral administration of pentazocine. Additionally, we have detected norpentazocine (M-VII), 9-methoxy pentazocine (or its 8-methoxy-9-hydroxy isomer) (M-I) and 8,9 (or 9,8)-methoxy hydroxy metabolites oxidized on the side chain to *cis*-OH (M-III) and to *trans*-OH (M-V).

On the other hand, Lynn, *et al.*[6] in a comparative study of biotransformation products of a series of benzomorphan compounds, found that oxidation occurred on both the dimethylallyl substituent and the aromatic ring in the case of pentazocine. The former oxidation forms alcoholic and carboxylic acid metabolites and the latter, hydroxypentazocine and N-dealkylhydroxypentazocine. Furthermore, their report demonstrated that these hydroxylated metabolites on the aromatic ring were further metabolized by methylation on one hydroxy group to form methoxypentazocine and N-dealkylmethoxypentazocine. Methoxyhydroxypentazocine, which was oxidized on the side chain of methoxypentazocine, was also detected. However, they could not clarify the site of hydroxylation or methoxylation on the aromatic ring and oxidation on the dimethylallyl side chain.

In our experiments, NMR spectra of the methoxy hydroxy metabolites (M-I, M-III and M-V) provided evidence that the site of hydroxylation on the aromatic ring was at 9-position,

but did not afford conclusive evidence which one of hydroxy groups at 8-and 9-positions was methylated. When M-III was developed in solvent system IV, it was revealed that M-III was overlapped by an unidentified metabolite (*Rf* values expressed as the relative to the *Rf* of the authentic pentazocine; M-III 0.75, unidentified metabolite 0.78). Based on the *Rf* value, it is presumably a catechol metabolite of pentazocine, and further investigation is being undertaken to support this view.

The average recovery from 24 hr urine was that unchanged pentazocine was 9% of the administered dose, *cis*-OH 11% and *trans*-COOH 40% in man.[10] In the monkey,[3] unchanged pentazocine was 26% of the excreted amount, *cis*-OH 18%, *trans*-OH 12% and *trans*-COOH 28%. However, Pittman, *et al.* did not report on the presence of M-I, M-III, M-V and M-VII metabolites in these species.

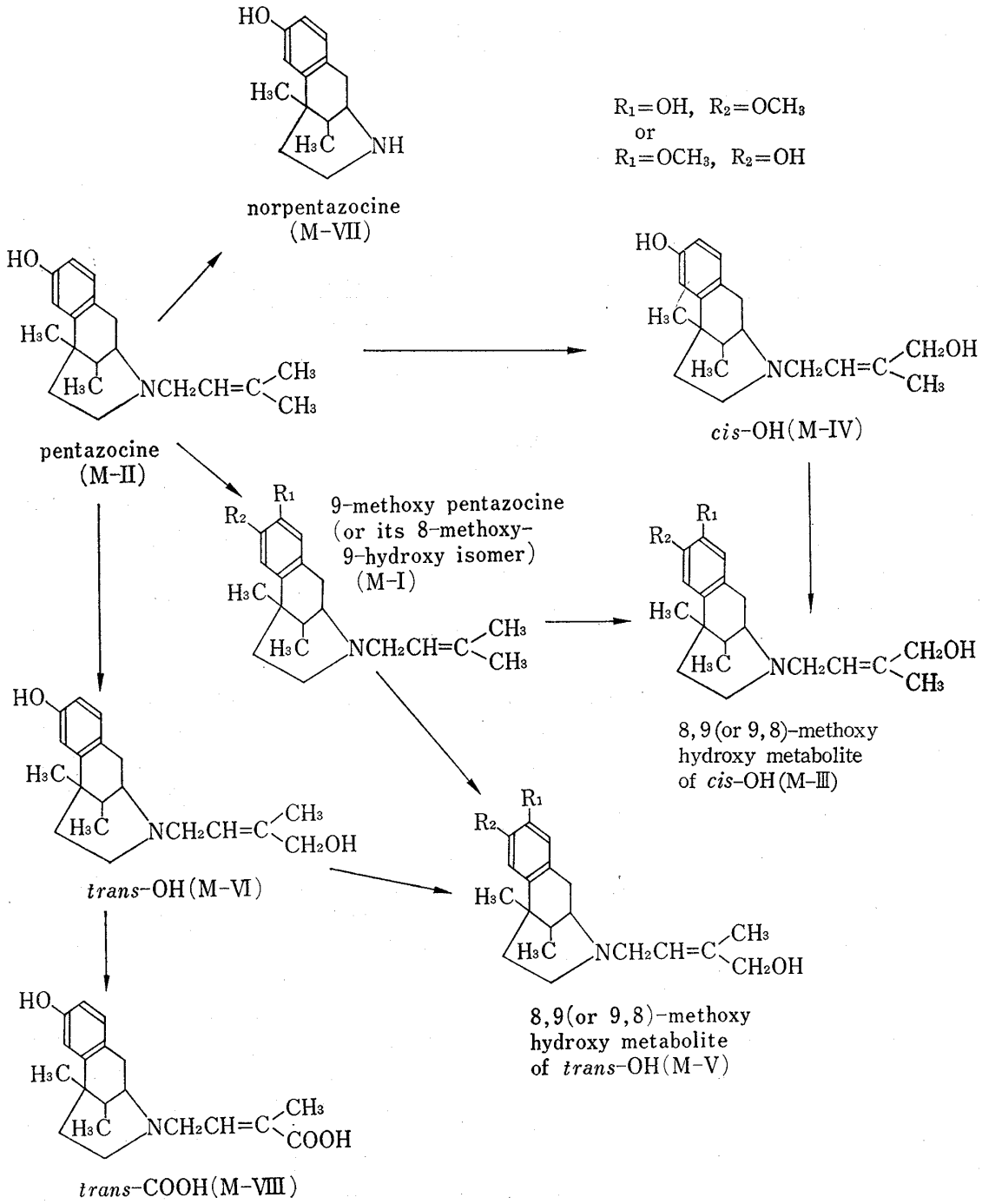

Chart 2. Postulated Metabolic Pathways of Pentazocine in the Rat

In this experiment, thin-layer and gas-liquid chromatographic data clearly indicate the main metabolite to be unchange pentazocine of the nonconjugated and conjugated forms (about 65% of the metabolites in urine). Although the other identified metabolites are only about 20%, the amount of these metabolites is in the following order: M-IV>M-VII, M-VIII>M-III>M-V, M-I>M-VI. In the primates, pentazocine was mainly metabolized through oxidation of the dimethylallyl side chain. However, our results in the rat indicated that pentazocine was metabolized through hydroxylation or methoxylation on the aromatic ring as well as oxidation of the dimethylallyl side chain, though the main metabolite excreted in the rat was as unchanged pentazocine. We were unable to explain the reason why the recovery of the metabolites was low after oral administration in the rat.

We postulate the metabolic pathway of pentazocine in the rat as illustrated in Chart 2.

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