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Metabolic Fate of *d-cis*-3-Acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one Hydrochloride(CRD-401)

Teruhiko Meshi, Juko Sugihara and Yoshishige Sato

Biological Research Laboratory, Tanabe Seiyaku Co., Ltd.[1]

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Absorption, excretion and metabolism of *d-cis*-3-acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride (CRD-401) were studied in rats. CRD-401 was found to be absorbed rapidly and almost completely from the digestive tract, the half-life for absorption being 26 min.

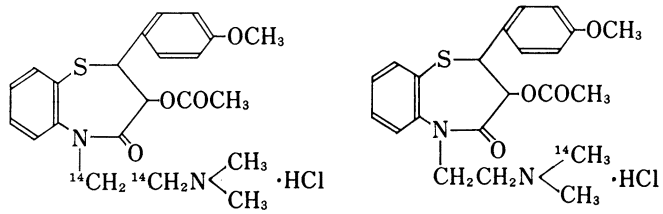
More than 90% of the radioactivity after oral administration of ^{14}C-CRD-401 was recovered from the feces and urine within 72 hours. The feces is the major excretory route since approximately 60% of the total radioactivity recovered appeared in the 72 hour feces and 65% of the administrated radioactivity was excreted in the 24 hour bile.

CRD-401 was extensively metabolized by rat since only 0.1% of the drug was recovered unchanged in the 24 hour urine and bile. Metabolic pathways of CRD-401 consisted of deacetylation, N-demethylation, O-demethylation, hydroxylation and N-oxidation. Major metabolites in urine and bile were deacetyl-O-demethyl-CRD-401, deacetyl-N,O-demethyl-CRD-401, and deacetyl-N,O-demetyl-methoxyl-CRD-401. The presence of N-oxide analogs of CRD-401 as minor metabolites was demonstrated in urine but not in bile.

d-cis-Isomer of 3-acetoxy-5-[2-(dimethylamino)-ethyl]-2,3-dihydro-2-(*p*-methoxyphenyl) 1,5-benzothiazepin-4(5H)-one hydrochloride(CRD-401)[2] has been shown by Sato, *et al.*[3] to be a potent coronary vasodilator. The investigation described in this paper was undertaken to elucidate the metabolic fate of CRD-401 in rat.

Experimental

^{14}C-CRD-401 and Reference Compounds——^{14}C-CRD-401(I), which is shown in Fig. 1, was synthesized with ^{14}C in ethyl side chain in this laboratory. The specific activity of the product was 2.5 μCi/mg, and the radiochemical purity was more than 98% as determined by thin-layer chromatography (TLC). N-$^{14}CH_3$-CRD-401 with a specific activity of 2.2 μCi/mg was used only for identification of N-oxide metabolites. The following compounds of established structure, which had been synthesized previously in this laboratory,[2] were used as reference standards: deacetyl-CRD-401(II), deacetyl-N-monodemethyl-CRD-401(III) and deacetyl-O-demethyl-CRD-401(IV).



^{14}C−CRD−401 N−$^{14}CH_3$−CRD−401

Fig. 1. Structures of ^{14}C-CRD-401 and N-$^{14}CH_3$-CRD-401

1) Location: *Kawagishi, Toda-shi, Saitama.*
2) H. Kugita, H. Inoue, M. Ikezaki, M. Konda and S. Takeo, *Chem. Pharm. Bull.* (Tokyo), **19**, 595 (1971).
3) M. Sato, T. Nagao, H. Nakajima and A. Kiyomoto, *Arzneimittel-Forsh.*, in press (1971).

Absorption——Male Wistar rats weighing about 200 g were fasted for 16 hours before dosing. An oral dose of ^{14}C-CRD-401 was 5 mg/kg. At 10, 20, 30, 60 and 120 min after the oral administration, the gastrointestinal tract was removed and the contents were washed out in 20 ml cold physiological saline. To the saline solution were added an equal volume of 1 M K_2HPO_4 and two volumes of benzene. After shaking for 5 min, an aliquot of the benzene extract was determined for ^{14}C, and then analyzed for unchanged ^{14}C-CRD-401 by TLC in a solvent system A of benzene–dioxane–water–diethylamine (70:17.5:1:7.5).

Excretion——Rats treated orally with ^{14}C-CRD-401 were housed in cages constructed to permit the separate collection of urine and feces. Urine and feces were collected at various time intervals up to 72 hours. Urine was diluted with distilled water to a fixed volume and measured for radioactivity. Feces was homogenized with 10 volumes of 0.1 N HCl, and the centrifuged supernatant was counted for ^{14}C.

For collection of bile, the bile duct was cannulated with a polyethylene tubing under anaesthesia of pentobarbital. Bile was collected for 24 hours after oral administration of ^{14}C-CRD-401.

Isolation of Urinary Metabolites——^{14}C-CRD-401 which was diluted with 10 amounts of nonradioactive CRD-401 was administered orally in a dose 100 mg/kg to 20 rats (The oral LD_{50} for CRD-401 is more than 600 mg/kg in rat and the toxic dose is more than 200 mg/kg.[3]). Twenty-four hour urine samples were used for isolation of metabolites. The procedure for the separation of urinary metabolites is outlined in Chart 1. The urine sample (105 ml) was applied on an Amberlite XAD-2 resin column (2.5 × 30 cm)[4] and the column was washed with 100 ml of water. Next, methanol was used to eluate ^{14}C-CRD-401 and its metabolites. Methanol eluate (100 ml) was adjusted to pH 3 to 4 with 1 N HCl and 2 volumes of *n*-heptane was added. After shaking, the lower layer methanol solution was recovered and concentrated to dryness *in vacuo*. The residue was dissolved in 20 ml of water, adjusted to pH 8 with NH_4OH, and extracted three times with 2 volumes of chloroform. The residual aqueous solution was retained for analysis of conjugated metabolites and N-oxide metabolites.

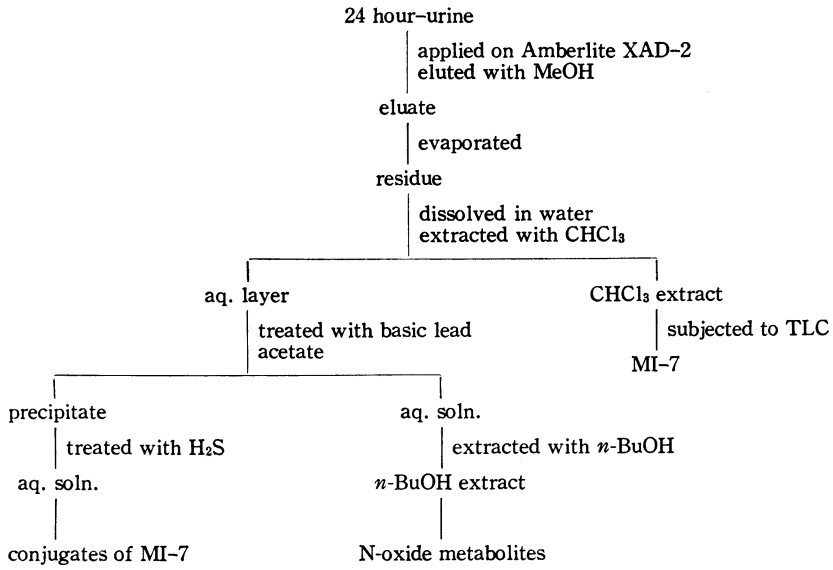


Chart 1. Major Steps used to Separate Urinary Metabolites

Chloroform extract was concentrated to a small volume and spotted on Silica gel GF_{254} plates (250 μm thick) which were prepared by the method of Stahl.[5] The plates were developed by the ascending technique with the solvent systems described in the legend to Table I. Examination of chromatogram under ultraviolet (UV) light revealed the presence of reference compounds. Radioactive metabolites were detected with an Aloka thin-layer chromatogram scanner TRM-1B. Color development of metabolites were carried out with Folin–Ciocalteau,[6] nitroprusside–acetaldehyde[7] and ninhydrin–pyridine[8] reagents. After scan-

4) J.M. Fujimoto and V.B. Haarstad, *J. Pharmacol. Exptl. Therap.*, **165**, 45 (1969).
5) E. Stahl, *Pharmazie*, **11**, 633 (1956).
6) D. Waldi, "Thin-Layer Chromatography," Springer-Verlag, Berlin, 1965, p. 498.
7) S. Walkenstein and J. Seifter, *J. Pharmacol. Exptl. Therap.*, **125**, 283 (1959).
8) I. Smith, "Chromatographic Thechniques," Interscience Publishers, Inc., New York, 1958, p. 66.

ning the chromatograms, the various radioactive zones were separately scraped from the plates, and the metabolites were eluted from the silica gel with methanol. The eluates were concentrated to dryness under vacuum, and the residues were extracted with chloroform. After removal of the solvent, the extracts were used for UV, infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy.

The aqueous solution above mentioned was treated with basic lead acetate to separate conjugated metabolites as precipitate,[9] the supernatant being retained for analysis of N-oxide metabolites. Enzymatic hydrolysis of conjugates was made with use of β-glucuronidase and aryl sulfatase, Type III (obtained from Sigma Chemical Co.). The hydrolyzed products were extracted with chloroform and then subjected to TLC.

After removal of lead salt of conjugated metabolites, the supernatant was extracted with *n*-butanol. To the butanol extract was added an equal volume of acetic acid and an excess of zinc powder, and the mixture was stirred for 1 hour at room temperature. The reaction mixture was filtered, and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in water, alkalinized with 1 M K_2HPO_4 and then extracted with chloroform. The chloroform extract was subjected to TLC in solvent system A.

For the identification of N-oxide metabolites, the urine sample of rats given N-$^{14}CH_3$-CRD-401 was treated with one-fifth volume 20% NaOH for 1 hour and then distilled to about one half of the original volume.[10] The distillate was acidified with HCl, concentrated to a small volume and then co-chromatographed with the authentic sample of N,N-dimethylhydroxylamine hydrochloride (DHM)[11] in a solvent system of *n*-butanol–acetic acid–water (4:1:1). DHM was visualized by spraying silver nitrate reagent, which was freshly prepared by mixing equal volume 17% $AgNO_3$, 25% NH_4OH and 20% NaOH.

Identification of Metabolites——Identification of isolated metabolites was accomplished by co-chromatography with the authentic samples and by spectroscopic analyses including UV, IR NMR and mass spectroscopy. UV spectra were taken of CRD-401 and the isolated metabolites dissolved in 0.1 N HCl or 0.1 N NaOH. The mass spectra of CRD-401 and its metabolites were taken on a RMS-4 mass spectrometer (Hitachi Co.) with a direct solids inlet operating at 200°. NMR spectra of deacetyl-CRD-401 and its metabolites were taken on JEOL C-60 NMR spectrometer (Japan Electron Optics Laboratory Co.) in $CDCl_3$ solution, with tetramethylsilane as an internal standard.

Determination of Urinary and Biliary Metabolites——Twenty-four hour urine and bile of four rats given ^{14}C-CRD-401 (5 mg/kg) were used for quantitative determination of metabolites. Separation of metabolites was carried out according to the procedure described for isolation of urinary metabolites. Conjugated metabolites were hydrolyzed with a mixture of β-glucuronidase and sulfatase. Hydrolyzed products were extracted with chloroform and then subjected to TLC. After scanning chromatograms, radioactive spot areas were scraped out into counting vials and extracted with 1 or 2 ml methanol containing 10% 0.5 N HCl. Fifteen ml of solution of 7 g 2,5-diphenyloxazole and 50 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene in 1 liter of 50% ethanol–toluene was added and the vials were counted in an Aloka liquid scintillation spectrometer LSC-502 equipped with an automatic quenching monitor system. This procedure resulted in quantitative detection of CRD-401 and all metabolites originally spotted on the plates.

Result

Absorption

Fig. 2 illustrates the rate of disappearance of ^{14}C-CRD-401 from the gastrointestinal tract after oral administration of ^{14}C-CRD-401 to rat. Nearly one half of the administered dose had disappeared within 30 minutes and after 2 hours the recovery was less than 10%. When the data were plotted semilogarithmically a straight line was obtained; the half-life of the drug for absorption was estimated to be 26 min. The rate constant was calculated to be 2.67×10^{-2} min^{-1}, assuming first order kinetics for the relationship $K=(2.303/t)\log(C_o/C_t)$, where K is the first order constant for the disappearance of CRD-401, C_o is the concentration of CRD-401 at zero time and C_t is the concentration of CRD-401 at time t.

Excretion

Fig. 3 shows the cumulative excretion of ^{14}C in the urine and feces up to 72 hours after oral administration of ^{14}C-CRD-401. Thirty five percent of the orally administered radioactivity was excreted in the 72 hour urine, and most of the urinary excretion occurred in the

9) I.A. Kanil, J.M. Smith and R.T. Williams, *Biochem. J.*, **50**, 235 (1952).
10) J. Raaflaub, *Arzneimittel-Forsh.*, **17**, 1393 (1967).
11) T.C. Bissot, R.W. Parry and D.H. Campbell, *J. Am. Chem. Soc.*, **79**, 796 (1957).

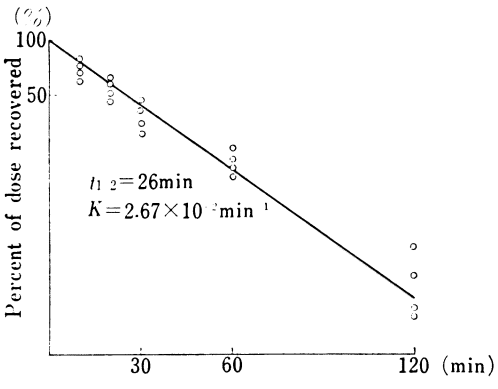


Fig. 2. Rate of Disappearance of ^{14}C-CRD-401 from Gastrointestinal Tract after Oral Administration of ^{14}C-CRD-401 to Rat

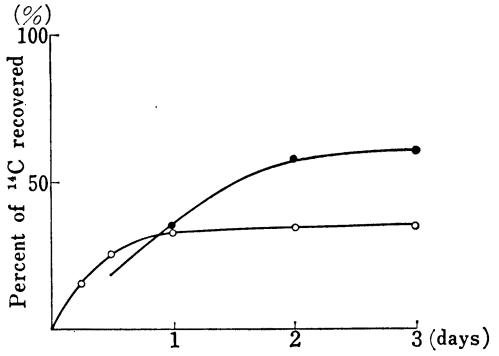


Fig. 3. Cumulative Excretion of ^{14}C in Urine and Feces after Oral Administration of ^{14}C-CRD-401 to Rat

—○—: urine —●—: feces
Each point indicates the mean of four animals.

first 24 hours. About 60% of the administered radioactivity was excreted in the 72 hour feces. Consequently, more than 90% of the administered radioactivity was excreted in the urine and feces within 72 hours.

Metabolism

When the urine sample of rats given ^{14}C-CRD-401 was applied on Amberlite XAD-2 column, about 97% of the applied radioactivity was adsorbed on the column. The radioactivity adsorbed on the column was eluted quantitatively with methanol. Methanol eluate was evaporated, dissolved in water and then extracted with chloroform at pH 8. Chloroform extract contained about 50% of the eluted radioactivity, the residual 50% in aqueous solution being retained for analysis of conjugated and N-oxide metabolites. When chloroform extract was subjected to TLC in solvent system A, seven radioactive peaks (M1—7) were usually observed, as shown in Table I.

TABLE I. *Rf* Values of CRD-401 and Its Related Compounds

Compound	*Rf* values			
	Solvent A	Solvent B	Solvent C	Solvent D
CRD-401[a)]	0.75	0.95	0.85	0.87
Deacetyl-CRD-401[a)]	0.66	0.94	0.82	0.78
Deacetyl-N-monodemethyl-CRD-401[a)]	0.54	0.87	0.76	0.52
Deacetyl-O-demethyl-CRD-401[a)]	0.29	0.72	0.59	0.27
$CHCl_3$ extract of urine sample[b)]	0.66 (M1)			
	0.54 (M2)			
	0.41 (M3)			
	0.29 (M4)			
	0.20 (M5)			
	0.16 (M6)			
	0.03 (M7)			

Chromatography was performed on Silica gel GF_{254} with solvent systems: A, benzene–dioxane–water–diethylamine (70:17.5:1:7.5); B, chloroform–methanol–NH_4OH (80:15:5); C, benzene–methanol–diethylamine (75:15:5); D, chloroform–acetone–diethylamine (88:2:10).

a) detected under UV light

b) Urine sample was collected for 24 hours after oral administration of ^{14}C-CRD-401 to rats and urinary metabolites were detected by thin-layer chromatogram scanning.

M1 and M2——Table II shows prominent peaks of UV spectra and reaction to spray reagents for CRD-401 and its metabolites. The UV spectra taken at acid and alkaline pH for M1 and M2 were the same as found for deacetyl-CRD-401. The *Rf* values of both metabolites had not been changed by treating them with 1N HCl for deacetylation. M2 was positive to ninhydrin reagent, but M1 was not. This fact suggested that M2 was N-demethylated. M1 and M2 had the same *Rf* values (in solvent systems A, B, C, and D) as the authentic samples of II and III. Therefore, it may be concluded that M1 and M2 are deacetyl-CRD-401(II) and deacetyl-N-monodemethyl-CRD-401(III), respectively.

TABLE II. Prominent Peaks of UV Spectra and Reaction to Color Reagents for CRD-401 and Its Metabolites

Compound	UV spectra		Color reaction	
	in 0.1N HCl mμ	in 0.1N NaOH mμ	Folin–Ciocalteau	Ninhydrin
CRD-401 (I)	238	238	−	−
Deacetyl-CRD-401 (II)	241	242	−	−
Deacetyl-N-demethyl-CRD-401 (III)	241	241	−	+
Deacetyl-O-demethyl-CRD-401 (IV)	240	249	+	−
M1	241	242	−	−
M2	241	241	−	+
M3	239	246	+	−
M4	240	249	+	−
M5	239	246	+	+
M6	238	248	+	+
M7	240	248	+	+

M3, M4, M5, M6, and M7——The treatment of these metabolites with 1N HCl for deacetylation did not change their chromatographic mobilities. This fact suggested that all of these metabolites had been deacetylated. The UV spectra taken at acid pH for M3—7 resembled that found for deacetyl-CRD-401. The individual prominent peak of M3—7 was at 238—240 mμ, whereas that of deacetyl-CRD-401 was at 241 mμ. However, at alkaline pH, there was a bathochromic shift to about 246—251 mμ for M3—7, but not for deacetyl-CRD-401. These data suggested that phenolic hydroxyl groups were present in M3—7. The reaction to the spray reagent supported the above suggestion, since these metabolites responded positively to the phenol reagent by giving a pale blue color on a white background with Folin–Ciocalteau reagent. The reaction to ninhydrin reagent was positive for M5—7, but not for M3 and M4. This fact suggested that M5—7 had been N-demethylated.

M4 had the same *Rf* values (in solvent systems A, B, C, and D) as the authentic deacetyl-O-demethyl-CRD-401(IV). The methanol eluate containing M4 from the chromatogram was concentrated to dryness, and the residue was extracted with chloroform. After removal of the solvent, the extract was crystallized from ethanol. IR spectrum of M4 was identical with that of the authentic IV. Furthermore, the mass spectrum of M4 was identical with that of the authentic IV. From these data, it could be concluded that M4 was deacetyl-O-demethyl-CRD-401(IV).

M6 which was excreted most largely in urine was assumed to be both O- and N-demethylated by UV spectra and reaction to the spray reagents. Table III shows mass spectral analysis of deacetyl-O-demethyl-CRD-401(M4) and urinary metabolites. The mass spectrum of M6 indicated its molecular weight to be 344 which is 14 lesser than that of M4. This fact suggested that this metabolite was N-demethylated derivative of M4. The fragment ion at 270, which is characteristic for M4 and M6, is formed probably by the loss of the side chain with rearrangement of a proton to oxygen of carbonyl group, as shown in Fig. 4. Further-

Table III. Mass Spectral Analysis of Urinary Metabolites of CRD-401

Compound	Molecular ion composition *m/e*	Difference from O-CRD-401	Prominent ions common to metabolites *m/e*
Deacetyl-O-demethyl-CRD-401(O-CRD-401)	358 ($C_{19}H_{22}O_3N_2S$)		270 and 178
M3	388 ($C_{20}H_{24}O_4N_2S$)	$+OCH_2$	300, 284 and 178
M4	358 ($C_{19}H_{22}O_3N_2S$)		270 and 178
M5	374 ($C_{19}H_{22}O_4N_2S$)	$+OCH_2$ and $-CH_2$	300, 284 and 178
M6	344 ($C_{18}N_{20}O_3N_2S$)	$-CH_2$	270 and 178
M7	360 ($C_{18}H_{20}O_4N_2S$)	+O and $-CH_2$	284 and 178

more, the NMR spectrum of M6 indicated that there was a singlet near at 7.7 τ which probably belongs to a N-CH_3 group and that numbers of proton near at 7.7 τ decreased to one half (three protons) of that of M4, as shown in Table IV. From these data, it is clear that M6 is N-monodemethylated derivative of M4, deacetyl-N,O-demethyl-CRD-401. When M7 (*Rf* 0.03) was rechromatographed in solvent system C, it was separated into two components (*Rf* 0.20 and 0.35). One of them (*Rf* 0.35), which was small in amount than the another (*Rf* 0.20), was supposed to be N-methylated derivative of the latter component on the basis of chromatographic behavior and color reaction.

Table IV. NMR Data of Metabolites of CRD-401

Compound	Signal and number of proton		
	Aromatic proton τ[a)]	N-Methyl proton τ	O-Methyl proton τ
Deacetyl-CRD-401	2.23—3.18 (multiplet) (8)	7.76 (6)	6.23 (3)
M4	2.25—3.28 (multiplet) (8)	7.71 (6)	(0)
M5	2.24—3.13 (multiplet) (7)	7.62 (3)	6.12 (3)
M6	2.25—3.30 (multiplet) (8)	7.69 (3)	(0)

a) chemical shift in τ from tetramethylsilane, solvent $CDCl_3$
Figures in parentheses indicate number of protons.

The mass spectra of the latter component of M7 gave molecular ion at 360, as shown in Table III. The increase in weight of 16 mass units of the molecular ion of M7 as compared with M6 indicated that this compound was oxygenated derivative of M6. The presence of the prominent common ion at *m/e* 178 (C_9H_8ONS), which is formed probably by the loss of the side chain and 2-phenyl ring, as shown in Fig. 4, suggested that the oxygen was not substituted on the heterocyclic ring and must be substituted on the 2-phenyl ring. The assignment of the hydroxyl group to 2-phenyl ring was established from a comparison of selected peaks of M6 and M7, which differed by 16 mass units. Therefore, it seems that M7 is a hydroxylated derivative of M6.

Prominent peak common to M3, M5, and M7 appeared at *m/e* 284, which is formed probably by the loss of the side chain, (methylene for M3 and M5) and two protons, as shown in Fig. 4 (The probable structure of *m/e* 284 in Fig. 4 was tentatively postulated to be catechol derivative). This suggested that M3 and M5, like M7, were hydroxylated at the 2-phenyl ring.

The mass spectra of M3 and M5 gave molecular ions at 388 and 374, respectively. The increase in weight of 14 and 28 mass units of the molecular ions of M5 and M3 as compared with M7 suggested that the compounds were the mono- and di-methylated derivatives of M7. The ion at 300, which is probably formed by the loss of side chain, is observed characteristi-

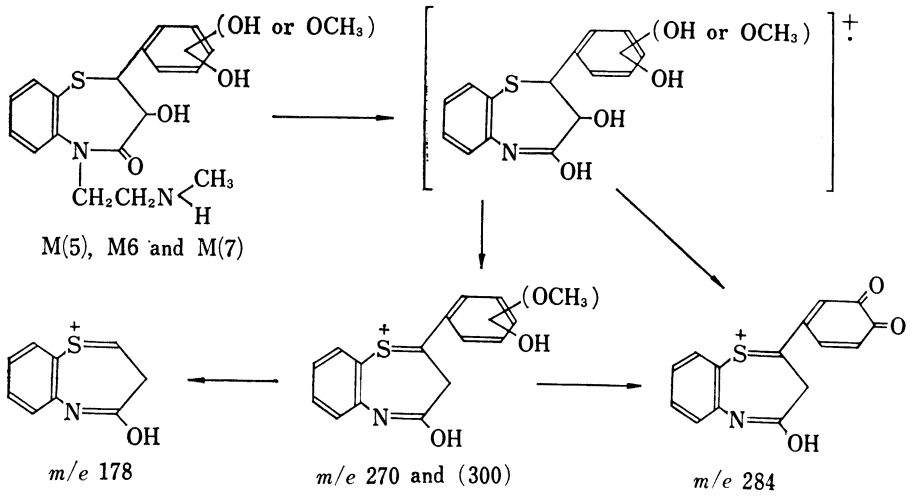


Fig. 4. Structures of Selected Fragments of M5, M6, and M7

cally for M3 and M5. The NMR spectrum of M5 had a prominent peak near at 6.2 τ which probably belongs to -OCH_3 and that near at 7.7 τ which belongs to -NCH_3. The proton count of M5 near at 6.2 τ corresponded to three protons, and that near at 7.7 τ corresponded to three protons, as shown in Table IV. Therefore, it seems reasonable to conclude that M5 is a O-methylated derivative of M7 or a methoxylated derivative of M6. Since M3 was excreted in a small amount, NMR spectrum could not be taken. However, the molecular ion composition of M3 showed an addition of CH_2 when compared with that of M5 (Table III). This suggested that this metabolite must be a N-methylated analog of M5.

Conjugated Metabolites——When the eluate from Amberlite XAD-2 column was extracted with chloroform to remove lipophilic metabolites and then the aqueous residue was treated with basic lead acetate, about 80% of the radioactivity in the aqueous residue was precipitated as lead salt, residual 20% being retained for analysis of N-oxide metabolites. Conjugated metabolites were liberated by treating the lead salt with H_2S and then incubated with β-glucuronidase and sulfatase. More than 80% of the incubated radioactivity was extracted with chloroform after incubation with β-glucuronidase and sulfatase. When the hydrolyzed products were subjected to TLC with solvent system A, seven radioactive peaks were observed. The *Rf* values of these metabolites were identical with those of M1—7, respectively.

N-Oxide Metabolites——The hydrophilic metabolites excluding conjugated metabolites, which were present at the origin of chromatograms in all solvent systems studied, could be extracted almost completely with *n*-butanol. However, these metabolites were unstable and hence could not be isolated. When these metabolites were treated with zinc powder in the presence of acetic acid, and then chromatographed in solvent system A, three radioactive spots (*Rf* 0.74, 0.66 and 0.41) were observed. Their *Rf* values were corresponded to those of CRD-401, deacetyl-CRD-401 and deacetyl-O-demethyl-CRD-401, respectively. This suggested that oxide metabolites (N-, S-, and N,S-oxides) which can be reduced by zinc, were present in the urine of rat given CRD-401. However, the unstability of these metabolites led us to supposition that most of the metabolites might be N-oxides. To confirm this supposition, when the 24 hour urine sample of rats given N-$^{14}CH_3$-CRD-401 was treated with alkaline and then distilled to about one half of the original volume, it was found that approximately 5% of the urinary radioactivity was distilled off and almost all of the radioactivity in the distillate was present as ^{14}C-DMH, which was identified by cochromatography with

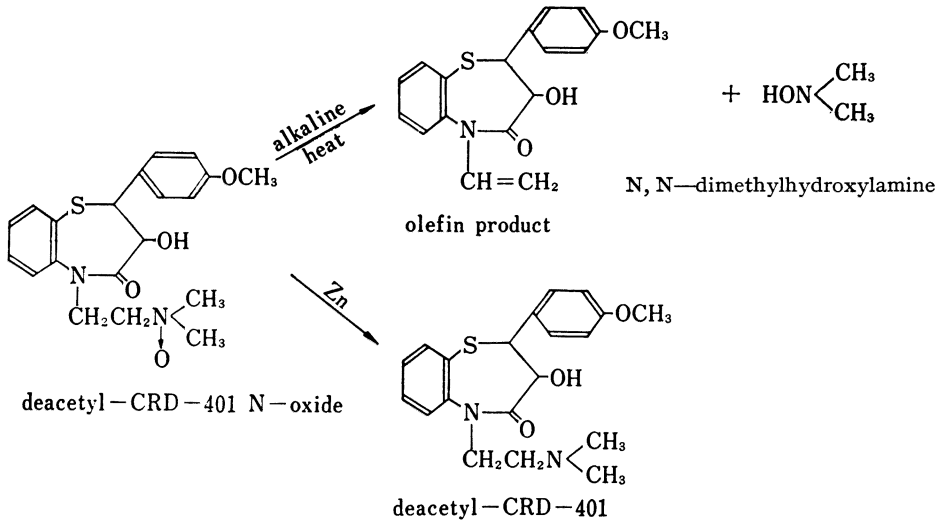


Fig. 5. Decomposition and Reduction of Deacetyl-CRD-401 N-Oxide

the authentic sample of DMH (*Rf* 0.22) in a solvent system of *n*-butanol–acetic acid–water (4:1:1). Since it was demonstrated that N-oxides of aliphatic tertiary amines were decomposed to N,N-dialkylhydroxylamines and olefin products by alkaline or heat,[12,13] it may be considered that N-oxide analogs of CRD-401 are decomposed into DMH and olefin products, as shown in Fig. 5. However, in the present study olefin products could not be isolated probably because of polymerization.[10] From these data described above, it seems likely to conclude that N-oxide analogs of CRD-401 are formed by rats.

Table V. Quantitative Determination of Urinary and Biliary Metabolites of ^{14}C-CRD-401

Compound	Urine[a] (31.4 %)		Bile[b] (64.8%)	
	Free form %	Conjugate form %	Free form %	Conjugate form %
CRD-401	0.1	—	0.1	—
M1	0.2	0.7	0.3	1.0
M2	1.1	0.8	0.6	2.5
M3	1.5	2.0	0.7	4.2
M4	6.3	2.1	3.4	26.7
M5	11.4	4.4	1.5	17.8
M6	27.0	17.1	2.3	22.6
M7	3.9	5.0	1.8	8.5
N-Oxide metabolites[c]	13.4	0	0	0
Total	64.9	32.1	10.7	83.3

Quantitative determination of metabolites was accomplished by combining column chromatography, solvent extraction and TLC. Data were expressed as the percentages of the total radioactivity excreted in urine or bile. Values in parentheses indicate per cent recovery of the administered radioactivity.

a) Urine samples were collected for 24 hours after oral administration of ^{14}C-CRD-401 to four rats.

b) Bile samples were collected for 24 hours after oral administration of ^{14}C-CRD-401 to four rats.

c) N-Oxide metabolites consisted of CRD-401 N-oxide, deacetyl-CRD-401 N-oxide and deacetyl-O-demethyl-CRD-401 N-oxide.

12) A.C. Cope, T.T. Foster and P.H. Towle, *J. Am. Chem. Soc.*, **71**, 3929 (1949).
13) A.C. Cope, D.C. Mclean and N.A. Nelson, *J. Am. Chem. Soc.*, **77**, 1628 (1955).

Quantitative Determination of Urinary and Biliary Metabolites

Table V shows quantitative determination of urinary and biliary metabolites of ^{14}C-CRD-401. Approximately 31% of the administered radioactivity was excreted in 24 hour urine of four rats. About 50% of the urinary radioactivity was present as chloroform extractable metabolites, about 40% was conjugates of former metabolites and the remainder was N-oxide metabolites. The largest metabolite in the urine was M6, which contained more than 40% of the urinary radioactivity. M4 and M5 were excreted largely in urine. M2 and M3 were excreted in minor amounts. About 50% of N-oxide metabolites was present as deacetyl-CRD-401 N-oxide, and the remainder was CRD-401 N-oxide and deacetyl-O-demethyl-CRD-401 N-oxide. Urinary excretion of unchanged ^{14}C-CRD-401 was only 0.1% of the urinary radioactivity.

Approximately 65% of the administered radioactivity was excreted in 24 hour bile after oral administration of ^{14}C-CRD-401. Only about 10% of the biliary radioactivity was present as chloroform extractable metabolites and the remainder was conjugated metabolites. None of N-oxide metabolites was detected in the bile. The largest metabolite in the bile was M4. M5 and M6 were major metabolites whereas M1, M2, and M3 were minor metabolites. Biliary excretion of unchanged ^{14}C-CRD-401 was only 0.1% of the biliary radioactivity.

Discussion

Rapid absorption of CRD-401 from the digestive tract was demonstrated by rapid disappearance of the drug from the gastrointestinal tract. It is assumed that this drug might be absorbed almost completely from the digestive tract, since approximately 95% of the administered dose was disappeared from the gastrointestinal tract within 120 min.

Studies of the radioactivity of the excreta of rats after a oral dose of ^{14}C-CRD-401 have shown that more than 90% of administered ^{14}C can be recovered from urine and feces within 72 hours. Excretion in the feces seemed to be the main route of elimination of CRD-401 and its metabolites, since approximately 60% of ^{14}C excreted within 72 hours was recovered from the feces and more than 60% of the orally administered radioactivity was excreted into the bile within 24 hours. The high recovery of the administered radioactivity in the bile suggested that an enterohepatic circulation occurred. This circulation was confirmed using pairs of rats with the bile duct cannulated, in which the cannula from one rat (A) was inserted into the duodenum of the second (B) (When bile was collected from rat B after rat A was given an intravenous dose of ^{14}C-CRD-401, about 15% of the dose given to rat A was excreted after 24 hours).

The results of the study showed that very little unchanged CRD-401 was excreted by rats in urine and bile. Only about 0.1% of a dose of CRD-401 was recovered from urine and bile unchanged. This fact suggested that CRD-401 was extensively metabolized by the rat.

The largest metabolite in the urine was M6, deacetyl-N,O-demethyl-CRD-401, as shown in Table V. Therefore, it was assumed that the main metabolic pathways of CRD-401 were deacetylation, N-demethylation and O-demethylation. Deacetylation, hydrolysis of acetyl ester, was the largest metabolic pathway, since it was shown that almost all of the isolated metabolites excluding a small part of N-oxides were deacetylated.

M1, deacetyl-CRD-401, was the smallest metabolite (0.2% of the urinary metabolites). This suggested that most of M1 formed from CRD-401 was metabolized further by N-demethylation and O-demethylation.

N-Dealkylation and O-dealkylation are common metabolic reactions during drug metabolism. In the metabolism of CRD-401, the presence of N-demethylated, O-demethylated and N,O-demethylated metabolites was demonstrated. It was noted that M4, deacetyl-O-demethyl-CRD-401, was excreted more largely in urine and bile than M2, deacetyl-N-mono-

demethyl-CRD-401, as shown in Table V. Therefore, it seems likely that CRD-401 is more readily O-demethylated than N-demethylated.

M5 which was excreted in a large amount was methylated derivative of M7. At present, the pathway by which M5 is formed is uncertain. However, it may be considered that there are two pathways for the formation of M5; one is direct hydroxylation of M2, and the other is hydroxylation of M6 to produce catechol derivative, followed by subsequent O-methylation. The results of the present study showed that dihydroxylated metabolite was isolated as M7 but the position of the hydroxyl groups on 2-phenyl ring of M7 could not be established. Axelrod, *et al.*[14] reported that a number of sympathomimetic amines with monophenolic structures could form catecholamines such as dopamine, norepinephrine and epinephrine when incubated with an enzyme in the microsomes of the liver. Furthermore, Daly and Manian[15] reported that 7- or 8-hydroxychlorpromazine was metabolized to 7,8-dihydroxychlorpromazine. Tatsumi, *et al.*[16] reported that phenacetylurea underwent successive hydroxylation at the 4 position and then at the 3 position of benzene nucleus. Taking these facts into consideration, it seems most likely that M5 is formed by hydroxylation of M6, followed by O-methylation.

M3, N-methylated derivative of M5, was one of minor metabolites. This metabolite, like M5, was supposed to be formed by hydroxylation of M4, followed by O-methylation, since intermediate catechol derivative was detected by chromatographic behavior (*Rf* 0.35 in solvent system C) and color reaction (positive to Folin–Ciocalteau reagent and negative to ninhydrin reagent).

N-Oxide metabolites, which were excreted in urine but not in bile, were shown to consist of CRD-401 N-oxide, deacetyl-CRD-401 N-oxide and deacetyl-O-demethyl-CRD-401 N-oxide by reducing them with zinc powder. The biological N-oxidation of aliphatic tertiary amines has already been shown to occur in several drugs.[17–21] N-Oxide metabolites of CRD-401 were very water-soluble and hence scarcely extracted with chloroform. Beckett and Hewick[22] reported that chlorpromazine N-oxide was not extracted completely with ether but done with *n*-butanol. N-Oxide metabolites of CRD-401 were found to be unstable, and hence it is difficult to isolate them. Fishman, *et al.*[17] reported that chlorpromazine N-oxide was decomposed to chlorpromazine, N-monodemethyl-chlorpromazine and a third substance after standing in the desiccator for 6—12 weeks.

S-Oxidation is one of the well known metabolic pathways of S containing drugs. However, the present study showed that S-oxide metabolites could not be detected. This was in good agreement of the report of Dreyfuss, *et al.*[23] that thiazesim [5-(2-dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5H)-one], a structural analogue of CRD-401, did not undergo S-oxidation *in vivo*. The inability to detect S-oxidation may be attributed to the possibility that S-oxidation may not actually have occurred, since it was shown that CRD-401 was N-oxidized but not S-oxidized when heated with H_2O_2 and acetic acid.[24] It may be supposed that 2-phenyl ring of CRD-401 becomes steric hindrance for attack of S-oxidation enzyme on the S atom position.

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From a consideration of the facts described above, it seems most reasonable to conclude that the metabolic fate of CRD-401 include 5 different pathways of deacetylation, N-demethylation, O-demethylation, hydroxylation followed by O-methylation, and N-oxidation. Therefore, reactions shown in Fig. 6 may be proposed for the metabolism of CRD-401.

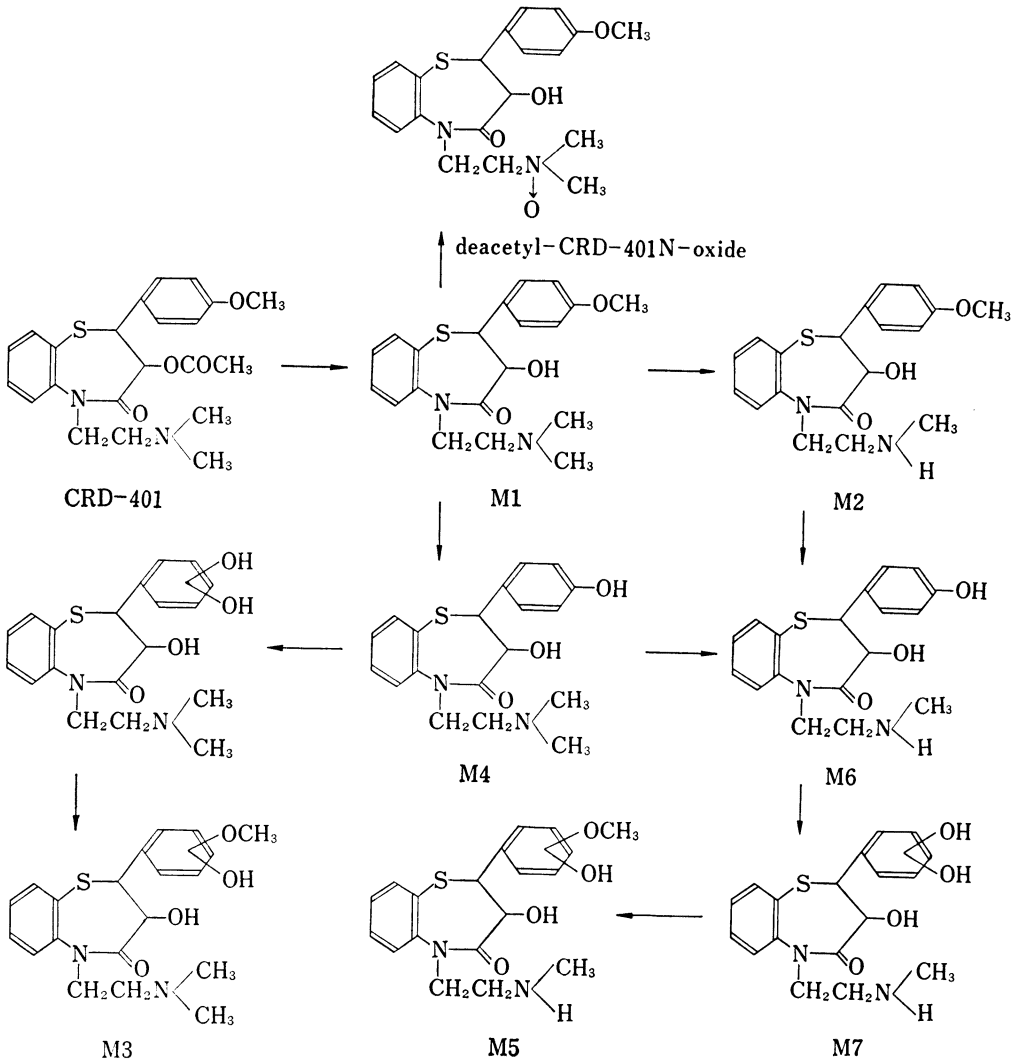

Fig. 6. Possible Metabolic Pathways of CRD-401

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