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Metabolism of Budralazine, a New Antihypertensive Agent. I.[1] Identification of the Metabolites of Budralazine in Rats

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The metabolism of budralazine in rats was investigated. Ten urinary metabolites were detected and identified after oral administration. Major metabolites were 3-methyl-*s*-triazolo[3,4-*a*]phthalazine and 1-(*s*-triazolo[3,4-*a*]phthalazine-3-carboxy)-β-D-glucopyranosiduronic acid. Comparison of the metabolic pattern obtained in spontaneously hypertensive rat and normotensive Wistar-strain rat was also discussed. But no significant difference was observed for the time course of biotransformation rate between them.

Budralazine (1-[2-(1,3-dimethyl-2-butenylidene)hydrazino]phthalazine, DJ-1461) is a hydrazone of 1-hydrazinophthalazine with mesityl oxide. Therapeutic effectiveness of budralazine on essential hypertension has been confirmed through a number of clinical studies. Some hydrazone derivatives[3] of 1-hydrazinophthalazine have also been found to be effective on hypertension. Recently, many authors have reported the metabolism of 1-hydrazinophthalazine hydrochloride,[4] but the metabolism of the hydrazones has not been studied. It was therefore the matter of significance to study the metabolic fate of budralazine. In order to compare the metabolism of the drug in a normal state with that in an animal model for human disease, we used normotensive rats (NR) and spontaneously hypertensive rats (SHR). A brief paper on the metabolites of budralazine was presented previously.[5] This paper reports the isolation and the structural elucidation of the metabolites of budralazine in rat urine and also comparison of the biotransformation of the orally administered drug in NR with that in SHR.

Experimental

Materials——Budralazine was synthesized according to the method of K. Ueno, *et al.*[6] Budralazine-^{14}C labelled at the 1- and 4-positions of the phthalazine ring (specific activity: 63 μCi/mg) was supplied from Daiichi Pure Chemical Co., Ltd. PPO(2,5-diphenyloxazole) and dimethyl POPOP(1,4-bis(4-methyl-5-phenyl-2-oxazole)benzene) was purchased from Packard Instrument Co., Inc.

Measurement of Total Radioactivity——The biological samples, except feces, were dissolved in MeOH and the solution (0.1 ml) was added to the scintillator (15 ml) prepared with dioxane (900 ml), dimethyl POPOP (0.3 g), PPO (7.0 g) and naphthalene (100 g). Radioactivity was determined with an Aloka LSC-652 liquid scintillation spectrometer. Corrections for counting efficiency were made by using ^{137}Cs as the external standard. The radioactivities of feces were determined by a combustion method as follows; about 50 mg sample of dry feces was oxidized by using a Intertequniq JA-101 Sample Oxidizer and the resulting $^{14}CO_2$ was absorbed in phenethylamine (18 ml). A mixture of the above phenethylamine, MeOH and a scin-

1) This work was presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya April 1975.
2) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
3) *a*) J. Druey and A. Marxer, *J. Med. Pharm. Chem.*, **1**, 1 (1959); *b*) J. Druey and B.H. Ringier, *Helv. Chm. Acta*, **34**, 195 (1951).
4) *a*) H. Zimmer, R. Glaser, J. Kokosa, A. Garteiz, V. Hess, and A. Litwin, *J. Med. Chem.*, **18**, 1031 (1975); *b*) S.B. Zak, T.G. Gilleran, J. Karliner, and G. Lukas, *J. Med. Chem.*, **17**, 381 (1974); *c*) J.M. Lesser, Z.H. Israili, D.C. Davis, and P.G. Dayton, *Drug Metabolism and Disposition*, **2**, 351 (1974).
5) M. Sano, R. Moroi, and K. Ono, *Chem. Pharm. Bull.* (Tokyo), **22**, 3006 (1974).
6) K. Ueno, R. Moroi, M. Kitagawa, K. Asano, and S. Miyazaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 1068 (1976).

tillator (3:7:9 in volume) was prepared and counted; the scintillator was prepared by dissolving PPO (15 mg) and bis-MSB [*p*-bis(methylstyryl)benzene, 1g] in toluene (1liter).

Thin-Layer Chromatography (TLC)——TLC was carried out by ascending technique on silica gel plates (Kieselgel HF_{254} Merck) activated at 110° for 60 min. The solvent systems used were solvent A; $CHCl_3$–MeOH (1: 1, v/v), solvent B; $CHCl_3$–acetone (1: 5, v/v), solvent C; $CHCl_3$–acetone (1: 9, v/v), solvent D; acetone–conc. NH_4OH (10: 0.1, v/v), solvent E; $CHCl_3$–EtOH–AcOH (8: 1: 1, v/v), solvent F; benzene–$CHCl_3$–MeOH (5: 15: 1, v/v), solvent G; $CHCl_3$–EtOH (4: 1, v/v), solvent H; cyclohexane-acetone (5: 4, v/v). The resulting chromatograms were visualized under ultraviolet (UV) light (2536 Å) or after spraying Dragendorffs reagent. The radioscannograms of TLC were obtained with an Aloka TLC Scanner Model TLC-2B or Packard Radiochromatogram Scanner Model 7201.

Administration of Budralazine——1) Twenty male rats (Wistar-strain, age 7—8 weeks, body weight approximately 190 g) were used for the separation of the metabolites. Budralazine-^{14}C suspended in 0.5% CMC administered orally in a dose of 15 mg/kg. The urine free from feces was collected during 48 hr after administration. For the collection of large amount of metabolites, thirty rats were given 400 mg/kg of non-labelled budralazine by oral administration and the urine was collected similarily.

2) Five male rats were used for the separation of 1-hydrazinophthalazine. Budralazine-^{14}C was administered in a dose of 15 mg/kg. The urine free from feces was collected during 3—8 hr.

3) NR and SHR were used for the comparative study of the biotransformation rate of the drug. Budralazine-^{14}C was administered in a similar way as mentioned above. The urine was collected in five periods during 0—2, 0—4, 0—8, 0—16 and 0—24 hr after administration, and the feces were also collected during 24 hr.

Determination of Spectra——UV spectra were taken in MeOH on a Hitachi 323 Spectrophotometer and infrared (IR) spectra in KBr on a Hitachi 285 Spectrometer. Nuclear magnetic resonance (NMR) spectra were measured in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard on a Hitachi R-20B Spectrometer; chemical shifts from TMS were given in ppm. Mass spectra were determined at 75 eV on a JEOL JMC-OISG-2 Spectrometer or Hitachi RMU-6M/CI.

Results

Isolation of the Metabolites from Rat Urine

In a preliminary experiments, a few ml aliquots of the 48 hr urine obtained after the administration of the labelled compound were examined by TLC on which the metabolites were detected by using Dragendorffs reagent, I_2, $FeCl_3$, UV light and their radioactivity. The radioscannogram of TLC developed by solvent D is shown in Fig. 1. The *Rf* value of each metabolite was smaller than that of the unchanged compound. Based on the radioscannograms of pooled urine, AcOEt extract from alkalinized urine and $CHCl_3$ extract from acidified urine shown in Fig. 1, a separation method was established as shown in Chart 1.

Five hundred ml of urine obtained from 20 rats administered the labelled drug were filtered and adsorbed onto a column of Amberlite XAD-2 resin (300 ml). After the column was washed with water (600 ml), the metabolites were eluted with $CHCl_3$–MeOH (1: 1) 600 ml, and the recovery of radioactivity was 94%. The eluate was concentrated to dryness in a stream of nitrogen. The residue was dissolved in H_2O (250 ml) and adjusted to pH 10—11 with 1N NaOH (25 ml). The aqueous solution was extracted continuously for 20 hr with AcOEt (extracted ratio: 14.1%). After concentration of the AcOEt extract, the residue (oil, 1.7 g) was chromatographed over a basic alumina column (30 g, Merck). The column was eluted with benzene 300 ml, benzene–$CHCl_3$ (1: 1) 300 ml, $CHCl_3$ 300 ml and $CHCl_3$–MeOH (98: 2) 300 ml in succession. The radioactivity was mainly found in the fractions eluted with benzene and $CHCl_3$, and was negligible in other eluents. The radioactive substance contained in the benzene fraction was further purified by repeated preparative TLC (pTLC) developed with solvent A (*Rf*: 0.42), and was obtained as crystals named M-2. Metabolite M-1 was separated from $CHCl_3$ fraction by a similar method as in the case of M-2, and was characterized by TLC (*Rf*: 0.32, solvent A). The above alkaline aqueous layer after being extracted with AcOEt was adjusted to pH 2—3 with 1N HCl and extracted continuously for 20 hr with $CHCl_3$ (extracted ratio: 17%). After evaporation of $CHCl_3$ of the extracts, the residue (1.9 g, oil) was chromatographed over neutral alumina column (40 g, Merck). The column was eluted with benzene 300 ml, benzene–$CHCl_3$ (9: 1) 200 ml, benzene–$CHCl_3$ (1: 1) 300 ml, $CHCl_3$ 300 ml, $CHCl_3$–

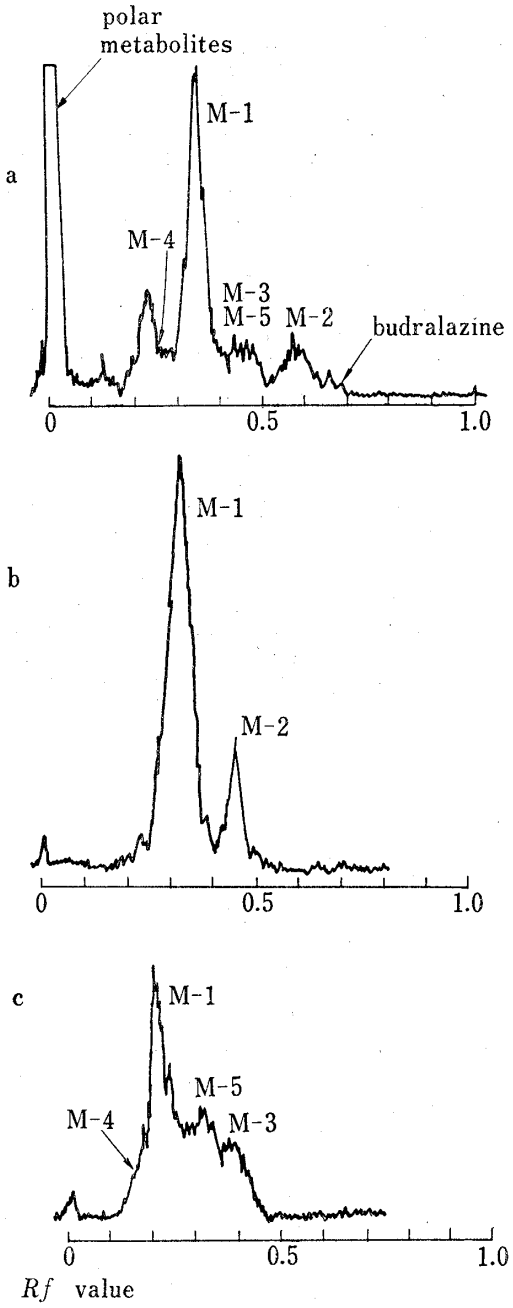


Fig. 1. Radioscannogram of TLC

a: pooled urine, solvent D.
b: AcOEt extract obtained from alkalinized urine, solvent A.
c: $CHCl_3$ extract obtained from acidified urine, solvent B.

MeOH (98:2) 300 ml and $CHCl_3$–MeOH (95:5) 300 ml in succession. Most of the radioactivity was present in the fractions eluted with benzene–$CHCl_3$ (1:1), $CHCl_3$–MeOH (98:2) and $CHCl_3$–MeOH (95:5). The individual radioactive fraction was purified again by column chromatography and pTLC. Metabolites obtained from benzene–$CHCl_3$ (1:1), $CHCl_3$–MeOH (98:2) and $CHCl_3$–MeOH (95:5) fractions were characterized by TLC as M-3 (*Rf*: 0.40, solvent B), M-4 (*Rf*: 0.17, solvent C) and M-5 (*Rf*: 0.29, solvent C), respectively. The remaining acidic aqueous layer after being extracted with $CHCl_3$ was applied to an ion exchange resin (IRA 400 OH^-, 50 ml) column, and the column was washed with water 300 ml. Materials eluted with 0.1N HCl 300 ml were found to be radioactive and purified by using pTLC in solvent E, where *Rf* values of radioactive spots were 0.53—0.60 (M-6, M-7 and M-8) and near zero (M-9). M-9 was further chromatographed over Sephadex LH-20 (50 ml) and eluted with $CHCl_3$–MeOH (1:1).

In order to confirm the presence of 1-hydrazinophthalazine or acetylated 1-hydrazinophthalazine as a metabolite, identification of these compounds, which are known to be labile,[7] was carried out according to the method of Zak, *et al.*[8] by converting the metabolites in freshly collected urine to the anisaldehyde derivatives. Ten ml of the urine collected during 3—8 hr was mixed with ascorbic acid (250 mg), 1N HCl (2 ml), EtOH (10 ml) and 1% anisaldehyde (1 ml) and heated at 65° for 15 min. After readjustment to pH 9 with 1N NaOH, the reaction mixture was extracted with benzene. The extract was purified by TLC (*Rf*: 0.75, solvent H). This was named AM-10; original metabolite was named M-10.

Quantitative Determination of the Metabolites

The rats used in this experiment were NR and SHR. The urine sample, after measurement of radioactivity, was adjusted to pH 1 with 1N HCl, mixed with ascorbic acid (50 mg), 1% anisaldehyde (0.2 ml) and EtOH (5 ml) and incubated at 65° for 15 min. After readjustment to pH 9 with 1N NaOH, the mixture was extracted with AcOEt. The aqueous layer was adjusted to pH 7 with 1N HCl followed by a extraction with AcOEt. The both AcOEt extracts and the aqueous layer were examined by TLC with solvent D, F and H. Each

7) *a*) H. Zimmer, J. McManus, T. Novison, E.V. Hess, and A.H. Litwin, *Arzneim-Forsch.* (Drug Res.), **20**, 1586 (1970); *b*) A.R. Schulert, *Arch. Int. Pharmacodyn.*, **132**, 1 (1961).
8) S.B. Zak, M.F. Bartlett, W.E. Wagner, T.G. Gillera, and G. Lukas, *J. Pharm. Sci.*, **63**, 225 (1974).

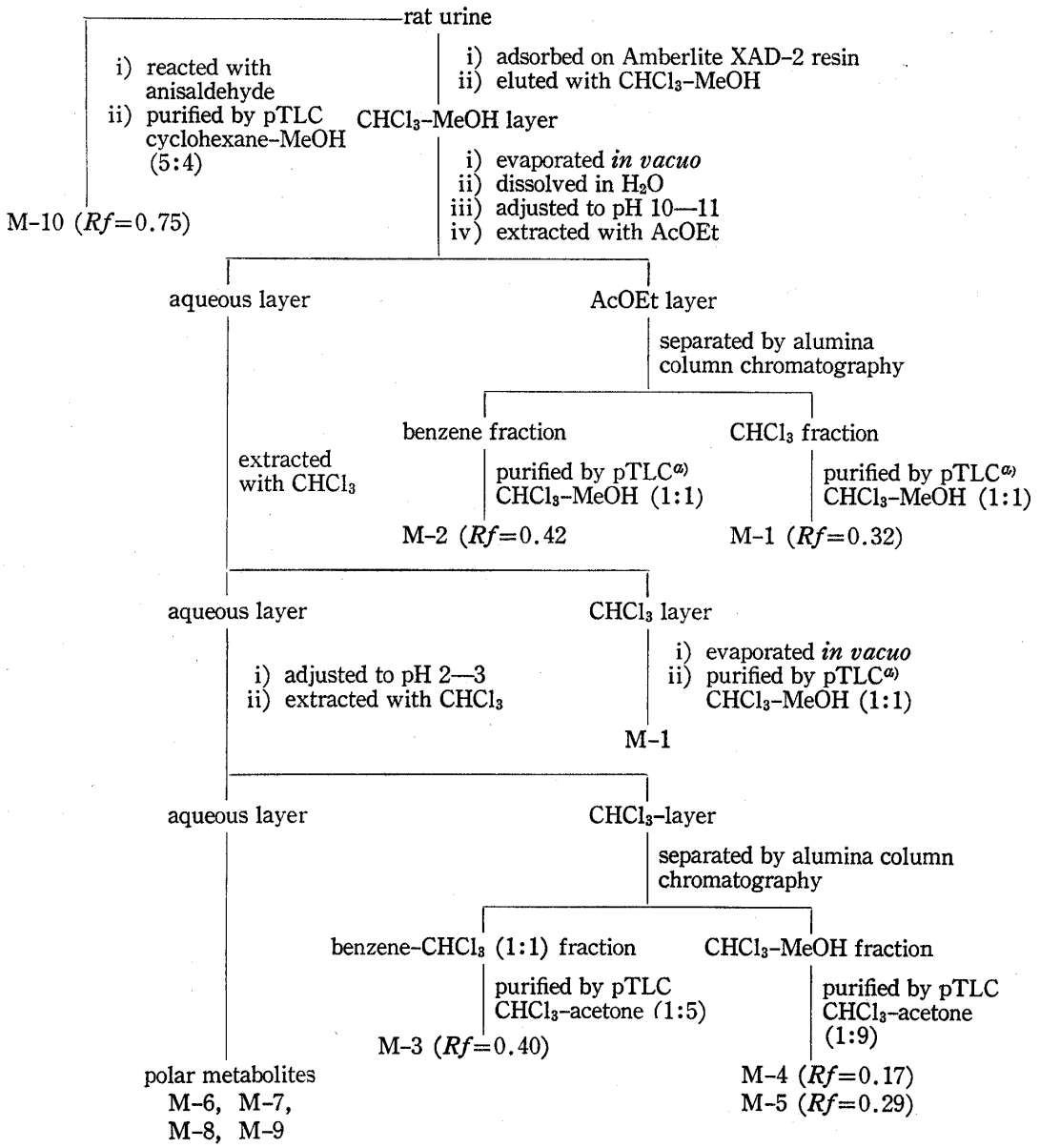
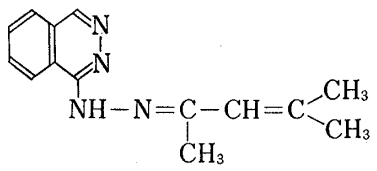
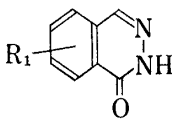


Chart 1. Separation of Metabolites from Rat Urine

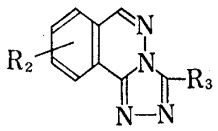
a) pre-coated TLC plates Silica Gel 60 F_{254} (Merck)



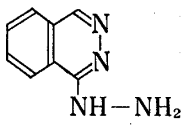
Budralazine



M-2 : R_1=H
M-8 : R_1=OH



M-1 : R_2=H, R_3=CH_3
M-3 : R_2=H, R_3=H
M-4 : R_2=H, R_3=CH_2OH
M-5 : R_2=H, R_3=CO_2H
M-6 : R_2=OH, R_3=CH_3
M-7 : R_2=OH, R_3=H
M-9 : R_2=H, R_3=$CO_2C_6H_9O_6$



M-10

Chart 2

spot corresponding to the metabolite was scraped off under UV light and extracted with $CHCl_3$–MeOH (1: 1). The radioactivity of the extracts was measured with a scintillation counter.

Structure of the Metabolites

The structure of the metabolites (Chart 2) was deduced from their IR, NMR, UV and mass (MS) spectra.

Metabolite M-1——M-1, which was one of the major metabolites, was obtained as crystals, mp 170.5—172.5° (AcOEt). The NMR spectrum in $CDCl_3$ showed signals due to the methyl group attached to the aromatic ring (2.85 ppm, s: 3H). The MS spectrum indicated the presence of a molecular ion at *m/e* 184 ($C_{10}H_8N_4$ Obsd.: 184.0749, Calcd.: 184.0748). The UV spectrum in MeOH showed a maximum at 240 nm (Fig. 2),[7a] indicating the presence of a *s*-triazolo[3,4-*a*]phthalazine ring. The experiments by the isotope dilution method and measurement of the mixed melting point with an authentic sample[4b] proved that M-1 is 3-methyl-*s*-triazolo[3,4-*a*]phthalazine.

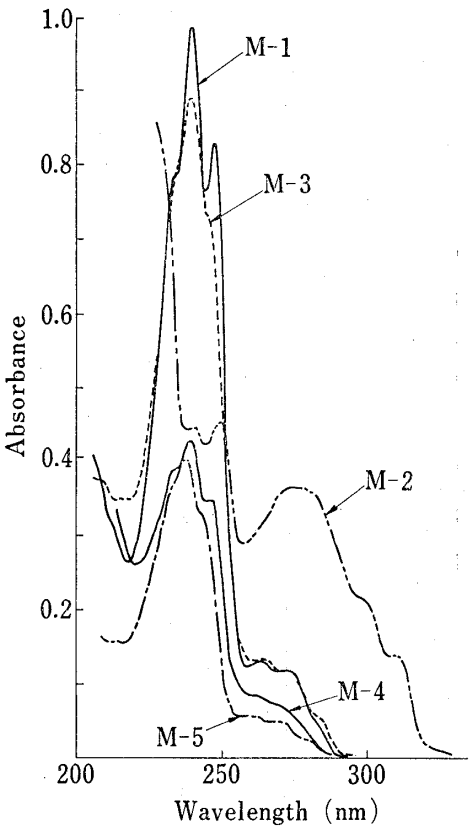


Fig. 2. Absorption Spectra of the Metabolites in MeOH Sample

M-1, M- 2 and M-3: E 1%
M-4 and M-5: E 1/5%

Metabolite M-2——M-2 was obtained as crystals, mp 178—180° (MeOH). The MS spectrum, obtained by combination with gas chromatography, showed a molecular ion at *m/e* 146 ($C_8H_6ON_2$ Obsd.: 146.0480, Calcd.: 146.0485) accompanying a M^+-CO fragment. The NMR spectrum in $CDCl_3$ indicated the presence of an aromatic ring (7.75—8.0 ppm m: 3H, 8.2 ppm s: 1H, 8.45 ppm m: 1H). The IR spectrum (KBr) had a characteristic band of the amide group at 1660 cm^{-1}. The UV spectrum in MeOH (Fig. 2) showing absorptions at 251.5 and 283 nm differs from that of *s*-triazolo[3,4-*a*]phthalazine or phthalazine rings. These data appeared to correspond to the structure of phthalazine-1-one. The structure of M-2 was finally determined by the isotope dilution method.

Metabolite M-3——The UV spectrum in MeOH (Fig. 2) indicated the presence of a *s*-triazolo[3,4-*a*]phthalazine ring. The spectrum was similar to that of M-1. In the NMR spectrum of M-3, only the aromatic ring protons were observed (Fig. 3). The MS spectrum afforded a molecular ion at *m/e* 170 ($C_9H_6N_4$ Obsd.: 170.0628, Calcd.: 170.0592). The structure of M-3 is *s*-triazolo[3,4-*a*]phthalazine and finally proved by comparison with an authentic sample.[4b]

Metabolite M-4——This metabolite was obtained as crystals, mp 208—209° (EtOH). The UV spectrum in MeOH (Fig. 2) showed the presence of a *s*-triazolo[3,4-*a*]phthalazine ring. The NMR spectrum in CD_3OD (Fig. 3) suggested the presence of a hydroxymethyl group (5.15 ppm s: 2H). Based on the data, it was suggested that the methyl group of M-1 was oxidized to an alcohol. The MS spectrum exhibited a molecular ion peak at *m/e* 200 ($C_{10}H_8O_1N_4$ Obsd.: 200.0690, Calcd.: 200.0698). Thus, the structure of M-4 is 3-hydroxymethyl-*s*-triazolo[3,4-*a*]phthalazine. This was identified by comparison with an authentic sample.[9]

9) Synthesis will be reported in the near future.

Metabolite M-5——This metabolite was obtained as crystalline form, mp 240—245° (EtOH–H_2O). The UV spectrum (Fig. 2) indicated the presence of a *s*-triazolo[3,4-*a*]phthalazine ring. The IR spectrum (KBr) had a characteristic band at 2500—3000 cm^{-1} and at 1730 cm^{-1} suggesting the presence of a carboxy group, whereas the MS spectrum showed no significant peaks having oxygen function in higher mass region. The presence of a fragment ion (*m/e* 170, $C_9H_6N_4$) in the highest region and the similar mass pattern with that of M-3 indicated that decarboxylation of M-5 resulted from the electron impact, and thus gave no molecular ion at *m/e* 214. As a result, M-5 is *s*-triazolo[3,4-*a*]phthalazine-3-carboxylic acid. The assignment was proved by comparison with an authentic sample.[9]

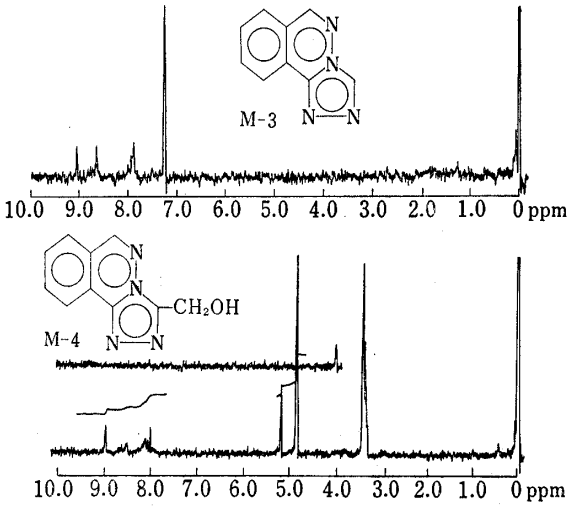


Fig. 3. NMR Spectra of M-3 and M-4

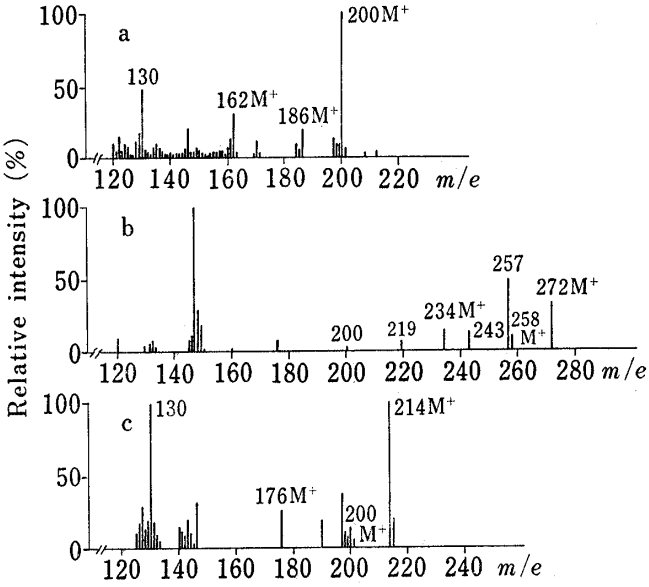


Fig. 4. Mass Spectra of the Mixed Metabolites (M-6, M-7 and M-8)

a: original mixture
b: derivatives of TMS
c: derivatives of methylation

Metabolite M-6, M-7, M-8——These metabolites were detected on TLC, but not isolated in a completely pure form, because of their small amounts. These metabolites were mainly identified employing a shift technique and fragmentation in MS spectrometry. The chemical ionization MS spectra showed ions at *m/e* 200, 186 and 162, which were presumed as molecular ion of the metabolites. These ion peaks were confirmed by electron impact high resolution mass spectrometry ($C_{10}H_8ON_4$ Obsd.: 200.0663, Calcd.: 200.0698, $C_9H_6ON_4$ Obsd.: 186.0547, Calcd.: 186.0541, $C_8H_6O_2N_2$ Obsd.: 162.0458, Calcd.: 162.0429). These facts indicated the presence of an oxygen atom in each of M-1, M-3 and M-2. Fig. 4 shows the MS spectra. Molecular ion peaks were shifted to 72 mass units by treatment with N,O-bis(trimethylsilyl)-acetamide and to 14 mass units by treatment with diazomethane. The results suggested the presence of a phenolic hydroxyl group in each metabolite. The structure of fragment ion *m/e* 130 in original mixture was deduced as shown in Chart 3. The position of hydroxyl group was presumed at the benzene ring of *s*-triazolo[3,4-*a*]phthalazine ring. The exact position of hydroxyl group of M-6 was proved at position 8 of *s*-triazolo[3,4-*a*]phthalazine ring by comparison of the behavior on TLC with that of an authentic sample.[9] But the position of hydroxyl group of M-7 and M-8 could not be confirmed. As a result, M-6 is 3-methyl-8-hydroxy-*s*-triazolo[3,4-*a*]phthalazine. M-7 and M-8 are 8 or 9-hydroxy-*s*-triazolo-[3,4-*a*]phthalazine and 6 or 7-hydroxyphthalazine-1-one, respectively.

Metabolite M-9——The metabolite which was one of the major metabolites was not isolated in a pure form and assumed to be a conjugate because its *Rf* value was close to zero in any

solvent system. Its various color reactions for glucuronide were positive. Whereas, enzymatic hydrolysis of M-9 with bacterial β-glucuronidase gave no obviously positive result. The MS spectrometry by the shift technique indicated no information except for a fragment ion at *m/e* 197, which was assigned as a *s*-triazolo[3,4-*a*]phthalazine-3-carbonium ion ($C_{10}H_5ON_4$ Obsd.: 197.0463, Calcd.: 197.0457). The occurrence of the fragment ion at *m/e* 197 suggested that the metabolite might be an ester type compound of M-5. These findings led us to perform ammonolysis of M-9. The degradated compound was identified as the amide of M-5 by comparison of its MS spectrum with that of an authentic sample. Therefore it was suggested that M-9 is 1-(*s*-triazolo[3,4-*a*]phthalazine-3-carboxy)-β-D-glucopyranosid uronic acid.

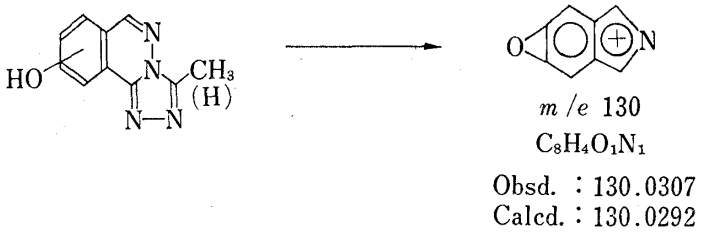


Chart 3. Fragment Ion Structure of Hydroxylated M-6 and M-7

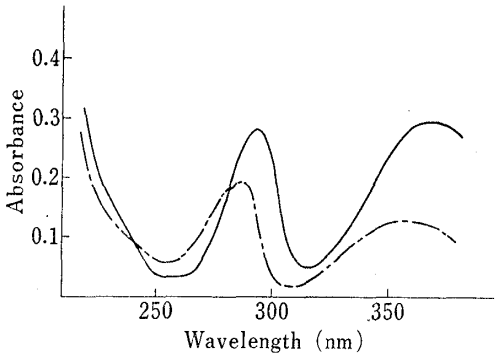


Fig. 5. Absorption Spectra of the Metabolite in MeOH

sample, ———: AM-10 E 1/5*
– – – –: budralazine E 1/5*

Metabolite M-10——The UV spectrum of AM-10 (Fig. 5) was similar to that of budralazine and indicated the presence of a hydrazone of 1-hydrazinophthalazine with *p*-methoxybenzaldehyde. The MS spectrum showed a molecular ion at *m/e* 278 ($C_{16}H_{14}ON_4$ Obsd.: 278.1171, Calcd.: 278.1167) and a fragment ion at *m/e* 171 which is produced by ring closure of the hydrazone. As a result, AM-10 was assigned as 1-[2-(4-methoxyphenylmethylidene)hydrazino]phthalazine. This was further proved by an isotope dilution method with an authentic sample. Thus M-10 was 1-hydrazinophthalazine.

Metabolites M-2, M-3, M-4, M-5, M-7, M-8 and M-9 have already reported as new metabolites of budralazine in previous communication.[5] During the course of our study, Zak, *et al.*[4a] reported that the M-2 and M-3 were new metabolites of hydralazine. Hydralazine was found as M-10·HCl in the metabolites of budralazine. Therefore the above results implied that the metabolic fate was resemble between budralazine and hydralazine. However, Akashi, *et al.*[10] have reported that budralazine caused a sustained reduction in systolic blood pressure without any change in heart rate after single oral administration. The side effects, such as tolerance and tachycardia which are undesirable ones, were weaker than those of hydralazine. These facts show that budralazine is different from hydralazine somewhat on pharmacological character. The active compounds were budralazine itself and M-10. But the reason why there were different mode of pharmacological action between the drugs would depend on different absorption rate. These problems will be discussed in a subsequent paper.

The Urinary and Fecal Excretion Rates of the Radioactivity

The urinary and fecal excretion of radioactive materials for 24 hr after single oral administration of budralazine were studied in NR and SHR. The urinary excretion rate of radioactivity gradually increased in both strains as shown in Table I. NR eliminated slightly more radioactivity in the urine in this period than SHR. On the contrary, the fecal excretion

10) A. Akashi, T. Chiba, and A. Kasahara, *Europ. J. Pharmacol.*, **29**, 161 (1974).

Table I. Urinary and Fecal Excretion of Radioactivity after Oral Administration of Budralazine-^{14}C in NR and SHR

Tine (hr)	NR			SHR		
	Urinary excretion	Fecal excretion	Total	Urinary excretion	Fecal excretion	Total
2	6.28±1.52			4.05±1.93		
4	16.17±2.97			14.12±1.64		
8	20.69±4.12			26.84±0.33		
16	36.91±6.06	36.07±3.25	72.98±9.31	31.73±0.20	40.34±0.30	72.07±0.50
24	44.60±2.93	37.40±3.70	82.00±6.63	37.82±2.01	42.85±2.18	80.67±4.19

(percent of dose; mean±standard error)

rate of the radioactivity in SHR was slightly higher than that in NR. But the difference in the two results in each case could not be regarded as significant. The total excretion rate in SHR was similar to that in NR. The total excretion ratio was approximately 80% of the dosed radioactivity at 24 hr after the administration in both rats.

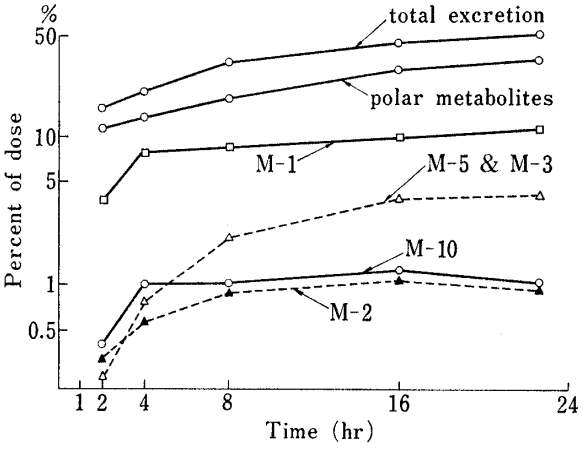


Fig. 6. Time-Course of Urinary Metabolites in NR

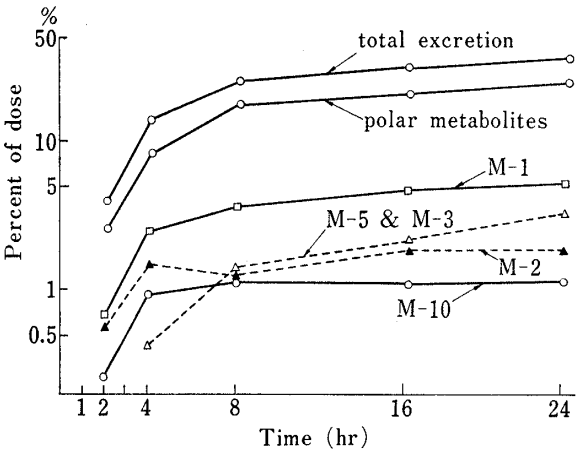


Fig. 7. Time-Course of Urinary Metabolites in SHR

Comparison of the Metabolites in NR and SHR

The urinary metabolite pattern in NR and SHR is shown in Fig. 6 and 7. The major urinary metabolites were water soluble compounds (M-6, M-7, M-8 and M-9), which comprised 40—50% of the dose at 24 hr in both NR and SHR. In the water soluble compounds, M-9 was contained as a major metabolite. Excretion of M-1, the second major metabolite increased rapidly until 4 hr after administration in NR and SHR. But the excretion ratio of M-1 was 5% in SHR and 11% in NR at 24 hr. This fact suggests that acetyltransferase activity of SHR may be smaller than that of NR. The excretion of M-3 and M-5 were about 4—5% of the administered dose in NR and SHR. Its excretion rate was increased until 8—16 hr after administration. M-3 and M-5 showed the same *Rf* value of TLC in solvent D, but could be separated by means of an ion exchange resin. As a result, M-5 was much more than M-3 at a ration of 6: 4 at any period. M-2 comprised 1—2% of the dose both in NR and SHR. But the excretion rate of M-2 in SHR was remained relatively constant for 4 hr after administration. M-10 became a constant between 8—16 hr after administration both in NR and SHR. Only a trace of M-4 and unchanged budralazine was excreted in the urine (below 0.1%). From these results, no evidence of difference of the biotransformation rate was observed in NR and SHR after single oral administration of budralazine, though there was slightly difference in the distribution ratio of M-1 between NR and SHR.

Vainionpaa, *et al.*[11] reported that the *in vitro* activity of the microsomal enzymes and the concentration of the cytochrome P-450 did not differ significantly between NR and SHR. So the results obtained here may be reasonable. The similarity of the biotransformation rate both in NR and SHR may cause from the same origin of the strain.

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11) V. Vainionpaa, E.R. Heikkinen, and H. Vepaaralo, *Pharmacol. Res. Commun.*, **6**, 343 (1974).