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Absorption, Distribution, Excretion, and Metabolism of 1-(5-Oxohexyl)theobromine (BL 191) in Rats[1]

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The absorption, distribution, and urinary excretion of 1-(5-oxohexyl) theobromine (BL 191) after oral administration (400 mg/kg) or intravenous injection (25 mg/kg) were studied in rats, using thin-layer chromatography and colorimetry. At the same time, identification of the chemical structure on 4 metabolites of BL 191 in the urine was made.

Plasma BL 191 levels reached a maximum within 10 min–1 hr after the oral administration and declined linearly thereafter. BL 191 was no longer detected in plasma 24 hr after the administration.

After the intravenous administration, BL 191 was rapidly eliminated from plasma and could not be found 6 hr after the administration.

The experiment in rats with the ligated pylorus revealed that a considerable amount of BL 191 administered was absorbed from the stomach as well as from the duodenum and the small intestine after the oral administration.

In the organs like the brain, heart, liver, lung, and kidney and the skeletal muscle examined, BL 191 was found to approximately the same extent 10 min after the oral administration and showed the time-course similar to that of plasma levels.

BL 191 and its metabolites were rapidly excreted largely in the urine. The total amount excreted in the urine was 1.3% and 68.3% of the dose administered as unchanged BL 191 and as the metabolites, respectively, and most of the excretion was carried out within 24 hr after the administration.

1-(4,5-Dihydroxyhexyl)theobromine and 1-(3-carboxypropyl)theobromine were main urinary metabolites of BL 191.

Methylxanthine derivatives like caffeine, theophylline and theobromine have been known to exert pharmacological effects on the central nervous system, cardiovascular system and skeletal muscle. Among these derivatives, theophylline compounds are most widely used clinically and many trials have been made to reduce the irritant action on the stomach and improve the poor water-solubility of these compounds.

1-(5-Oxohexyl)theobromine (BL 191) is synthesized by Chemische Werke Albert AG, West Germany, from theobromine by introduction of oxohexyl group, a relatively long chain, at the 1-position. BL 191 possesses advantages different from those of common xanthine derivatives in that BL 191 has more potent vasodilative action than theophylline[3] and is highly soluble in water and apparently more highly soluble in lipid than theobromine.[4]

In order to study how the absorption, distribution, excretion, and metabolism are modified as compared with common methylxanthine derivatives, BL 191 was given to rats and its absorption, distribution, excretion, and metabolism were investigated.

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[1] This work was presented at the 94 th Annual Meeting of the Pharmaceutical Society of Japan at Sendai, April, 1974.

[2] Location: *1-3-2 Minamidai, Kawagoe-shi.*

[3] *a*) K. Popendiker, I. Boksay, and V. Bollmann, *Arzneim.-Forsch.*, **21**, 1160 (1971); *b*) M. Sakurai, T. Kitatani, H. Kitagawa, I. Komine, and T. Sakaguchi, *Pharmacometrics*, **10**, (5), 695 (1975).

[4] W. Mohler and A. Söder, *Arzneim.-Forsch.*, **21**, 1159 (1971).

Experimental[5]

Compound——BL 191 is white crystalline powder and has no odor. It has a molecular weight of 278.3 and a melting point of 103.5—106.5°. It is freely soluble in chloroform and soluble in water and in methylalcohol, namely, one gram of BL 191 dissolves in about 2 ml of chloroform and in about 20 ml of water or of methylalcohol. The chemical structure of BL 191 is shown in Chart 1. Authentic compounds used were 1-(5-hydroxyhexyl) theobromine, 1-(4,5-dihydroxyhexyl) theobromine and 1-(3-carboxypropyl) theobromine obtained from Chemische Werke Albert AG, West Germany.

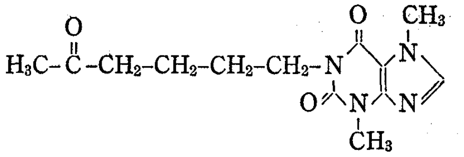

Chart 1. Chemical Structure of BL 191

Animals, Dosages and Sample Preparations——Male Sprague-Dawley rats (CLEA Japan, Inc.) weighing 200—250 g were used in this study. The animals were fasted overnight and then received an oral administration of BL 191 (400 mg/kg) dissolved in distilled water or an intravenous injection (tail vein) of BL 191 (25 mg/kg) dissolved in saline. After the drug administration, blood was collected by heart puncture using a heparinized syringe under Nembutal® anesthesia (30 mg/kg as sodium pentobarbital, *i.p.*). After the blood collection, the rats that received BL 191 by the oral route were sacrificed and the organs such as the brain, liver, heart, lung, and kidney and the skeletal muscle of hind limbs were removed for determination of tissue levels of BL 191 and its metabolites. The collected blood was centrifuged at 3000 rpm for 10 min to obtain plasma. For investigation of urinary excretion and metabolites in urine, the animals were maintained individually in metabolism cages after the oral administration and the urine was collected for 3 days.

Determination of BL 191 and Its Metabolites in Plasma, Tissues, and Urine——A 1 ml sample of plasma was added to 7 ml of chloroform, shaken for 5 min, and centrifuged at 3000 rpm for 5 min to separate the chloroform layer. One gram samples of tissues from each organ were homogenized in 9 ml of chloroform by a potter homogenizer and the homogenate was centrifuged at 3000 rpm for 5 min to separate the chloroform layer. As regards the urine samples, a 1 ml sample of urine that had been adjusted to be neutral was added to 7 ml of chloroform and shaken for 15 min and centrifuged at 3000 rpm for 5 min to separate the chloroform layer, and 5 ml of the chloroform layer was removed. To the remaining mixture (3 ml) added was 5 ml of chloroform and extraction was carried out in the same way two times more. Moreover, the same extraction with chloroform was repeated three times after the aqueous layer was adjusted to be acidic (pH 1.0). Then, the chloroform extracts were combined. The chloroform extract thus obtained from the samples of plasma, urine or tissues was evaporated on a water bath at 65° to dryness, and the residue was dissolved in ethylalcohol and was then applied in spots on a thin-layer plate. After the development with a developing solvent A, the spots of BL 191 and its metabolites on the chromatograms were detected under ultraviolet light. The spot of BL 191 and a group of spots corresponding to its metabolites were separately removed from the TLC plate, and eluted with a solvent ($CHCl_3$: MeOH=7: 3). The eluate was evaporated to dryness, and the residue was dissolved in water and then subjected to colorimetric determination according to a modification of the assay method of caffeine.[6] To 2 ml each of the solutions, added were 4 ml of pyridine acetic acid reagent (10% pyridine solution: 3% acetic acid solution=5: 3) and 1 ml of 0.05% antiformin. The mixture was allowed to stand at room temperature for 1 min, and 1 ml of 0.1 N sodium thiosulfate and 2 ml of 1 N sodium hydroxide were added to this mixture. Within 10 min, colorimetric determinations were carried out at 460 nm.

Preliminary to this study, when recovery tests of BL 191 were performed on plasma, urine and tissues to which BL 191 was added, using the BL 191 solution in water as a standard solution according to the same assay procedure as above described, BL 191 could be recovered approximately 100%. Furthermore, when the sensitivity of the main metabolites (MI, MIII, MIV) with this assay method was compared with that of BL 191 by determining molecular extinction coefficients in the same colorimetric way as for BL 191, they showed the sensitivity in the range of 93—104%. Therefore, in this study, calibration curves were made with the BL 191 solution in water (20—50 μg/ml) according to the same procedure as the sample. The amount of the metabolites extracted from plasma, tissues, and urine with the above procedure was expressed as the amount of BL 191. However, in the present study, the sensitivity of the other metabolites with this procedure was not determined and the metabolites in plasma and tissues were not identified, because the amount of the metabolites in plasma and tissues served as a guide for the purpose of comparison with BL 191 levels.

In order to examine to what degree the urinary metabolites were extracted, the urine sample was extracted with the same procedure as previously described; the chloroform extraction was further carried out twice more, first at neutral pH and then at acidic pH; and the residue from the combined extracts was examined using TLC. As a result, no spot corresponding to the metabolites of BL 191 could be observed on the plate

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[5] Thin-layer chromatography (TLC) was carried out on silica gel plates (Kieselgel 60 F_{254}, 20 × 20 cm, 0.25 mm thick, Merck Co., Ltd.) with the developing solvents A ($CHCl_3$:EtOH:formic acid=88:10:2), B (benzene:acetone=70:30 in ammonia atmosphere), C ($CHCl_3$:EtOH=94:6 in ammonia atmosphere) and D ($CHCl_3$:EtOH:ethylacetate:acetic acid:water=75:15:10:1:1). *Rf* is the value obtained by TLC.

[6] R. Hashizume, *Yakkyoku*, 17, (11) 27 (1966).

under UV light. Based on this fact, the metabolites in the urine were considered to be almost completely extracted.

Absorption of BL 191 from the Stomach and the Intestine——In the experiment on the absorption from the stomach, the rats that had been fasted overnight were anesthetized with ether, and after the abdominal section the pylorus was ligated; and then BL 191 dissolved in water was orally given to the rats at dosage levels of 400 and 100 mg/kg.

In the experiment on the absorption from the intestine, the rats were treated in the same way and the pylorus and the ileum near the cecum were both ligated; and then BL 191 dissolved in water was injected into the duodenum at the dosage levels of 400 and 100 mg/kg. The bile duct of all animals was ligated simultaneously when the pylorus and the ileum were ligated, in order to avoid the possible influx of BL 191 and its metabolites into the intestine *via* the bile duct.

One hour after the drug administration, blood was collected by heart puncture under Nembutal® anesthesia (30 mg/kg as sodium pentobarbital, *i.p*), and then the stomach and the intestine were removed, and the remaining amount of BL 191 in the contents of the stomach or the intestine was determined according to the same method as used in the determination of plasma levels. BL 191 absorbed from the stomach or the intestine was calculated by subtracting the remaining amount of BL 191 in the stomach or in the intestine from the dose administered, and the absorption rate was expressed as per cent of the dose administered.

Separation and Identification of Metabolites in Urine——Urine samples collected for 3 days after the oral administration of BL 191 were extracted with chloroform according to the method of extraction from urine above described. Metabolites in the urine were separated using two-dimensional TLC with solvents A and C (Fig. 3). The metabolites extracted from the spots on the chromatogram according to the procedure above described were determined colorimetrically, and expressed as per cent of the total amount of BL 191 and its metabolites recovered from the urine collected for 3 days (Table IV).

Each metabolite (Mu, MI, MII, MIV), eluted from more than 50 chromatograms, was confirmed to give a single spot on the TLC plate, and then subjected to determination of the ultraviolet (UV) absorption spectrum, infrared (IR) spectrum, and mass spectrum, or elementary analysis. The UV absorption spectrum was taken with a spectrophotometer (Type 124, HITACHI). The IR spectrum was determined in the KBr tablet with an IR spectrophotometer (Type 521, Perkin-Elmer). Elementary analysis was performed with an analyzer (Type 240, Perkin-Elmer), and mass analysis was carried out with a mass spectrometer (Type JMS-O1SG, HITACHI) and the mass spectrum evaluation was made according to Hinze, *et al.*[7]

Mu: UV $\lambda_{max}^{H_2O}$ nm: 207, 274. UV $\lambda_{min}^{H_2O}$ nm: 246. *Rf*: 0.96 (solvent C). *Rf*: 0.77 (solvent D).

MIV: UV $\lambda_{max}^{H_2O}$ nm: 207, 274. UV $\lambda_{min}^{H_2O}$ nm: 246. *Rf*: 0.52 (solvent D). *Rf*: 0.02 (solvent C).

MIII: UV $\lambda_{max}^{H_2O}$ nm: 207, 274. UV $\lambda_{min}^{H_2O}$ nm: 246. *Rf*: 0.30 (solvent B). *Rf*: 0.43 (solvent D).

MII: UV $\lambda_{max}^{H_2O}$ nm: 207, 274. UV $\lambda_{min}^{H_2O}$ nm: 246. *Rf*: 0.03 (solvent B). *Rf*: 0.41 (solvent D). *Anal.* Calcd. for $C_{13}H_{20}O_4N_4$: C, 50.91; H, 6.75; N, 18.08. Found: C, 51.25; H, 6.50; N, 18.08.

MI: UV $\lambda_{max}^{H_2O}$ nm; 207, 274. UV $\lambda_{min}^{H_2O}$ nm: 246. *Rf*: 0.92 (solvent C). *Rf*: 0.66 (solvent D).

IR spectra and mass spectra are presented in Fig. 4 and Fig. 5, respectively.

Results

The Time-Course of Plasma Levels of BL 191 and Its Metabolites

As shown in Fig. 1, levels of BL 191 in the plasma after the oral administration of 400 mg/kg of BL 191 reached a maximal level within 10 min—1 hr after the administration, and declined linearly thereafter. BL 191 was no longer detected 24 hr after the drug administration. The metabolites of BL 191 were present in the plasma within 10 min and were not detected at 24 hr after the administration, with the time-course being similar to that of BL 191 levels in the plasma.

The time-course of plasma levels of BL 191 and its metabolites after the intravenous administration of 25 mg/kg of BL 191 is illustrated in Fig. 2. Levels of BL 191 in the plasma declined rapidly following the administration and could not be detected after 6 hr. The metabolites of BL 191 in the plasma were detected as early as 3 min after the administration, increased till 30 min after the administration, declined thereafter, and could not be detected after 6 hr, as was with BL 191 levels in the plasma after the intravenous administration.

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[7] *a*) H.-J. Hinze, *Arzneim.-Forsch.*, 21, 1456 (1971); *b*) H.-J. Hinze, G. Bedessem, and A. Söder, *ibid.*, 22, 1144 (1972).

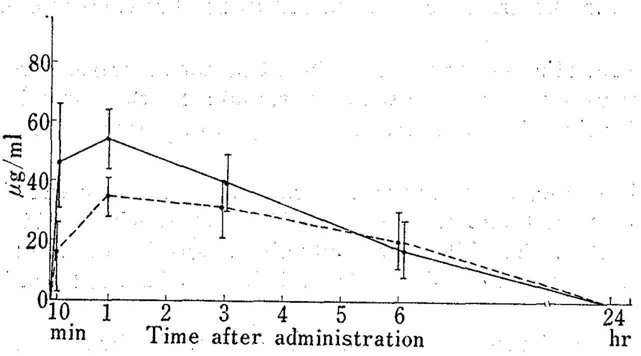

Fig. 1. The Plasma Levels of BL 191 and Its Metabolites after Oral Administration of BL 191 in a Dose of 400 mg/kg ($n=6$)

Vertical bars represent the mean ±S.D.
—●—: BL 191 ---●---: metabolites

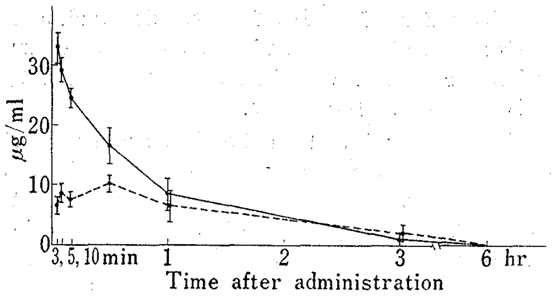

Fig. 2. The Plasma Levels of BL 191 and Its Metabolites after Intravenous Administration of BL 191 in a Dose of 25 mg/kg ($n=5$)

Vertical bars represent the mean ±S.D.
—●—: BL 191 ---●---: metabolites

Absorption of BL 191 from the Stomach and the Intestine

As previously described, BL 191 and its metabolites were detected quite early following the oral administration of BL 191. These results suggest that BL 191 is rapidly absorbed from the stomach and the intestine. Then, the absorption of BL 191 from the stomach and the intestine was examined in rats with the ligated gastrointestinal tract.

As shown in Table I, when 100 and 400 mg/kg of BL 191 were given orally to rats with the ligated pylorus, BL 191 was absorbed from the stomach within 1 hr after the administration by 43.7% and 28.6%, respectively. In the case of rats with the ligated pylorus and ileum that received 100 mg/kg of BL 191 intraduodenally, BL 191 was absorbed by 94.5% within 1 hr. When the dose of BL 191 given intraduodenally was increased to 400 mg/kg, the rats all died. It seemed that the surgical manipulation of the ligature and the rapid absorption of the large amount of BL 191 were affected by each other and were responsible for these fatalities. Therefore, the experiment could not be continued with the dose of 400 mg/kg.

TABLE I. Absorption of BL 191 from Gastro-Intestinal Tract in 60 min after Administration

Dose	Absorption from the stomach (%)	Plasma level (μg/ml)	Absorption from the small intestine (%)	Plasma level (μg/ml)
400 mg/kg	28.6±5.1	35±11	a)	
100 mg/kg	43.7±6.3	11± 6	94.5±0.9	41±11

Values represent the mean ± S.D. of 5 rats.
a) The rats died.

Tissue Levels of BL 191 and Its Metabolites

Tissue levels of BL 191 and its metabolites after the oral administration of 400 mg/kg of BL 191 are shown in Table II.

As was in the case of plasma levels, BL 191 was distributed in each organ examined to approximately the same extent within 10 min, then gradually declined, and was not found at all at 24 hr after the administration.

The metabolites of BL 191 were found in the tissues 10 min or 3 hr after the drug administration. The levels of the metabolites in the brain, heart, lung, kidney, and skeletal muscle were approximately the same, and the time-course of the levels in these organs was similar to that of plasma levels. The levels of the metabolites in the liver were higher than in the other organs, but were the same as the levels in the other organs at 6 hr after the administration.

Table II. The Tissue Levels of BL 191 and Its Metabolites after Oral Administration of BL 191 in a Dose of 400 mg/kg

Tissue	10 min		3 hr		6 hr		24 hr	
	BL 191	Met.	BL 191	Met.	BL 191	Met.	BL 191	Met.
	Concentration (μg/g tissue)							
Brain	30±17	33± 6	18± 8	18± 8	12± 9	11± 4	—a)	5
Heart	48±18	24± 6	48±20	36±17	20± 6	20±10	—	5
Lung	43±10	36±12	29±13	38±21	16± 5	20±10	—	—
Liver	57±30	123±54	28±25	84±50	14± 9	28±23	—	5
Kidney	39±19	32±23	33±16	42±23	15± 7	25±20	—	—
Skeletal muscle	47±14	16± 3	38±21	36±17	24±14	19±11	—	—

Values represent the mean ± S.D. of 6 rats.
a) not detected

At 24 hr after the administration, however, the metabolites were hardly observed in any of the organs examined including the liver.

Urinary Excretion of BL 191 and Its Metabolites

Table III shows the amount and time-course of excretion of BL 191 and its metabolites into the urine after the oral administration of 400 mg/kg of BL 191.

Table III. Excretion of BL 191 and Its Metabolites in the Urine after Oral Administration of BL 191 in a Dose of 400 mg/kg

Time (hr)	BL 191 (dose %)	Metabolites (dose %)	Total (dose %)
0—6	0.8±0.3	33.0±7.9	33.8±8.2
6—24	0.4±0.1	29.2±3.5	29.6±3.5
24—48	0.1±0.0	5.2±1.3	5.3±1.3
48—72	—a)	0.9±0.4	0.9±0.4
72—96	—	—	—

Values represent the mean ± S.D. of 4 rats.
a) not detected

The amount of unchanged BL 191 excreted in the urine was quite small, and, the excretion being rapid, BL 191 could not be detected 2 days after the administration and afterwards. The total amount of BL 191 excreted unchanged in the urine was only 1.3% of the dose administered. Regarding the metabolites of BL 191 excreted in the urine, 33.0% and 62.2% of the dose administered were excreted within 6 hr and 24 hr, respectively. The urinary excretion of the metabolites of BL 191 declined rapidly after 2—3 days, and any metabolite was not found 3 days after the administration and thereafter. The total excretion amount of BL 191 and its metabolites in the urine during 3 days after the administration was 69.9% of the dose administered.

Separation and the Amount of the Metabolites of BL 191 in the Urine

The urinary metabolites of BL 191 were separated by two-dimensional TLC and, as shown in Fig. 3, 12 metabolites could be separated. The urinary excretion ratio of the metaboilites is shown in Table IV. These results showed that MII, MIII, and MIV were the main urinary metabolites of BL 191.

Identification of the Metabolites in the Urine

Of the metabolites of BL 191 in the urine, Mu (BL 191), MIV, MIII, MII, and MI were examined to identify their chemical structures.

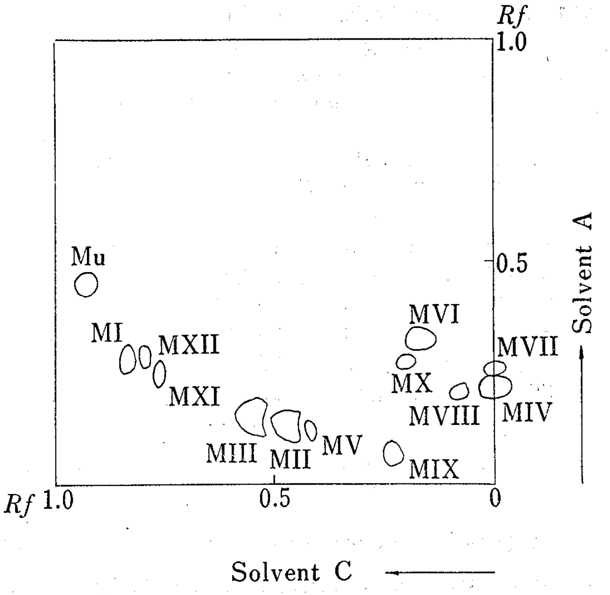

Fig. 3. Separation of Metabolites of BL 191 in Urine

Mu——In IR spectrum (Fig. 4), this showed IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 2960 and 2940 (CH), 1660 (amide group of xanthine), 1700 (CO), and 755 (side-chain skeleton). In mass spectrum (Fig. 5), this substance gave the molecular ion at *m/e* 278 and showed the same fragment ion pattern as authentic BL 191. UV absorption peaks, *Rf* values and IR spectrum of this substance were the same as those of BL 191. Thus, Mu was identified to be BL 191.

MIV——In IR spectrum (Fig. 4), this substance showed IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1704 (CO). Mass spectrum (Fig. 5) showed a molecular ion at *m/e* 266. UV absorption peaks, *Rf* values and IR and mass spectra of MIV were identical to those of the authentic compound. Subsequently, MIV was identified to be 1-(3-carboxypropyl) theobromine.

Table IV. Urinary Excretion Rates of BL 191 and Its Metabolites

Metabolites	Structure	% of total excreted amounts
Mu	BL 191	3.8
MI	1-(5-hydroxyhexyl) theobromine	2.6
MII	stereoisomer of MIII[a]	23.9
MIII	1-(4,5-dihydroxyhexyl) theobromine	33.9
MIV	1-(3-carboxypropyl) theobromine	18.5
MV		2.9
MVI		6.0
MVII-MXII		9.2

[a] assumed chemical structure

MIII——IR spectrum (Fig. 4) showed IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3280—3370 (OH), and mass spectrum (Fig. 5) showed a molecular ion at *m/e* 296. *Rf* values and UV, IR and mass spectra of this substance were well coincided with those of the authentic compound. Thus, MIII proved to be 1-(4,5-dihydroxyhexyl) theobromine.

MII——IR spectrum (Fig. 4) showed IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3380 (OH). Mass spectrum (Fig. 5) showed a molecular ion at *m/e* 296 and exhibited that the fragment ion pattern of this substance was the same as that of MIII. Elementary analytical data of MII were identical to the theoretical values of MIII and UV absorption spectrum of MII was identical to that of MIII. But its *Rf* values were different from those of MIII. Accordingly, MII can be a stereoisomer of MIII.

MI——IR spectrum (Fig. 4) showed IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3370 (OH), and mass spectrum (Fig. 5) exhibited a molecular ion at *m/e* 280. *Rf* values, UV spectrum and IR and mass spectral data of this substance coincided well with those of the authentic compound. Based on the results, MI was identified to be 1-(5-hydroxyhexyl) theobromine.

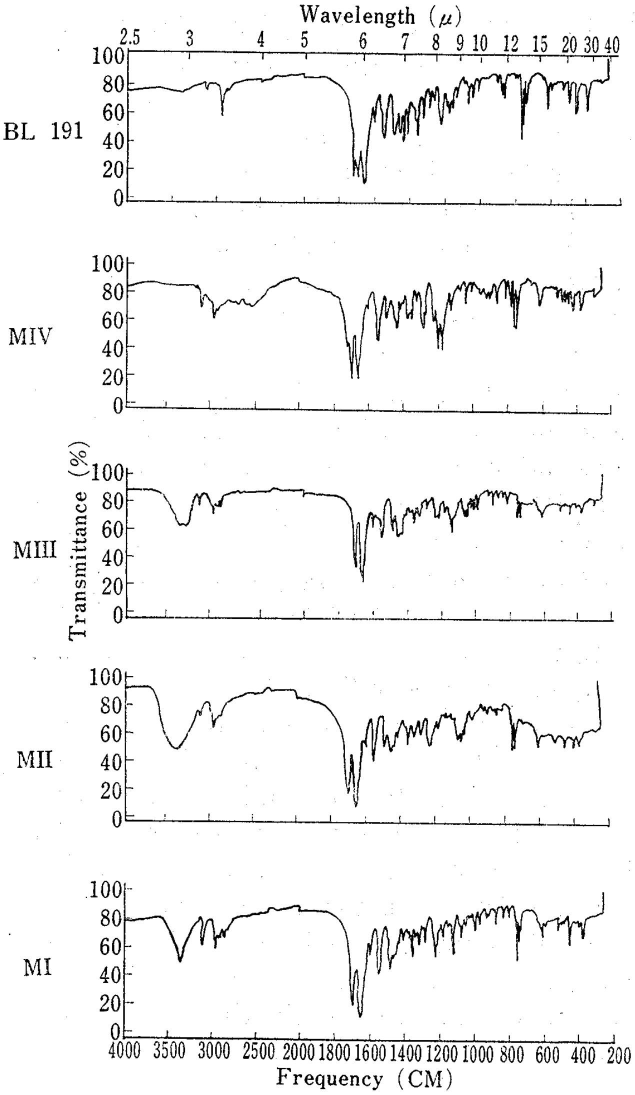

Fig. 4. IR Spectra of BL 191 and Its Metabolites

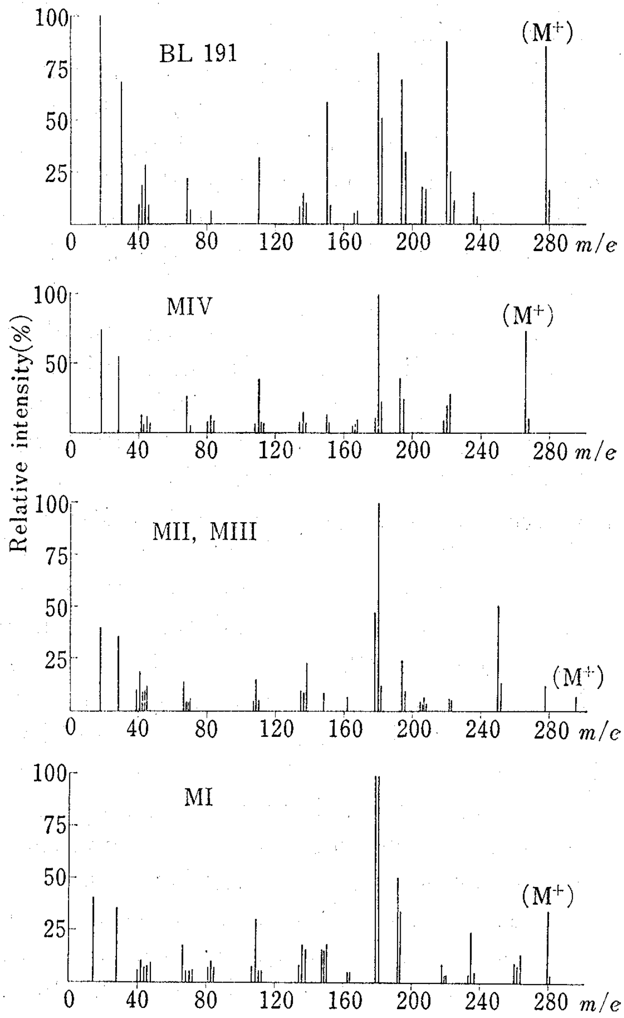

Fig. 5. Mass Spectra of BL 191 and Its Metabolites

Discussion

BL 191 was detected in the plasma in relatively high concentrations as early as 10 min after the oral administration. This result shows that BL 191 may be readily absorbed from the stomach as well as from the intestine. In fact, the experimental result of this study disclosed that BL 191 was absorbed to a considerable extent from the stomach as well when given to the ligated stomach of the rat. This result, together with the facts that caffeine[8a,b] and theophylline compounds[8c] are readily absorbed from the gastrointestinal tract and also from the stomach,[8d,e] suggests that the absorption of BL 191 from the gastrointestinal tract is basically similar to that of common xanthines.

Schanker, *et al.* reported that there was a parallel relationship between the rate of absorption of some drugs from the gastrointestinal tract and the degree of their lipid-solubility.[8d,9]

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8) *a*) J. Axelrod and J. Reichenthal, *J. Pharmacol. Exptl. Therap.*, **107**, 519 (1953); *b*) A.W. Burg and E. Werner, *Biochem. Pharmacol.*, **21**, 923 (1972); *c*) E.B. Truitt Jr., V.A. Mckusick, and J.C. Krantz Jr., *J. Pharmacol. Exptl. Therap.*, **100**, 309 (1950); *d*) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957); *e*) S.H. Waxler and H.B. Moy, *J. Am. Pharm. Assoc.* (*Scinet. Ed.*), **49**, 619 (1960).

9) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).

The water: chloroform partition coefficient of BL 191 was more than 100 (at both pH 2.0 and 7.4),[10] and this may be one of the explanations for the rapid absorption of BL 191 from the gastrointestinal tract.

The results that BL 191 was detected in the organs and the skeletal muscle examined to approximately the same extent as early as 10 min after the administration suggest that BL 191 was easily distributed by the blood stream to various tissues at similar rates. This is in agreement with the findings[8a,11] that caffeine and theophylline, methylxanthines, were distributed by the blood stream evenly to various tissues, and it is considered that there may be no difference between BL 191 and other methylxanthines with respect to distribution to tissues.

A considerable amount of the metabolites of BL 191 was detected in the plasma and tissues immediately after the oral or intravenous administration of BL 191, and 12 urinary metabolites were separated. Only 1.3% of the dose administered was excreted in the urine as unchanged BL 191. These results suggest that BL 191, as other methylxanthines,[12] is readily metabolized after administration to a large extent. From the result that the liver had the highest level of the metabolites of BL 191 among the organs examined, it seems that BL 191 is metabolized to a large extent in the liver, as is the case with a number of other drugs.

The urinary metabolites of BL 191 were investigated and 3 main metabolites were found. The chemical structures and the amounts of these metabolites indicate that the biotransformation of BL 191 was not due to the xanthine skeleton but remarkably due to the oxohexyl side chain. In the present study, the metabolites demethylated on the xanthine skeleton and the derivatives of uric acid were not identified as the main metabolites of BL 191. According to Hinze,[13] when BL 191 was given to man, the demethylated form was detected but derivatives of uric acid were not found as the urinary metabolites of BL 191. Mohler, *et al.*[14] reported that the metabolism of 1-hexyl-theobromine, which is structurally similar to BL 191, in rats was pronounced in the hexyl side chain, and this result is in well accord with ours on BL 191. When caffeine, theophylline or theobromine was given orally to man[12b] or to animals,[12a] the demethylated form of the original compound and the derivatives of uric acid were present as the main urinary metabolites, indicating the difference in metabolism between BL 191 and other methylxanthines such as caffeine, theophylline and theobromine. For this difference there might be some possible explanations: (1) as indicated by the result of the present study that BL 191 and its metabolites were rapidly eliminated from the tissues, most of BL 191 and its metabolites might be eliminated from the tissues before they undergo demethylation at the 3- and/or 7-position or oxidation at the 8-position because of the high water-solubility of BL 191 and its metabolites; (2) owing to the relatively long side chain at the 1-position, the demethylating and oxidizing reactions might be depressed possibly through steric hindrance of the binding between the substrates and enzymes responsible for the reactions; and (3) the above-described rapid elimination and depression of the reactions might occur simultaneously and affect each other. However, no firm conclusion can be drawn only from the data obtained in the present study.

When BL 191 was given intravenously or orally, BL 191 and its metabolites in plasma rapidly disappeared, and 33.8% of the dose administered was excreted in the urine within 6 hr and 63.4% within 24 hr. According to Christ, *et al.*,[15] when labelled BL 191 was given

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10) S. Yoshida and Y. Amano, unpublished data.

11) J.A. Schack and S.H. Waxler, *J. Pharmacol. Exptl. Therap.*, **97**, 283 (1949).

12) *a)* A.W. Burg and M.E. Stein, *Biochem. Pharmacol.*, **21**, 909 (1972); *b)* H.H. Cornish and A.A. Christman, *J. Biol. Chem.*, **228**, 315 (1957).

13) H.-J. Hinze, *Arzneim.-Forsch.*, **22**, 1492 (1972).

14) W. Mohler, K. Popendiker, and M. Reiser, *Arzneim.-Forsch.*, **16**, 1524 (1966).

15) *a)* H.-M. Kellner and O. Christ, "BL 191 Symposion," Frankfurt/Main, May, 1971, p. 20; *b)* O. Christ, K. Gleixner, H.-M. Kellner, R. Müller, and W. Rupp, *Arzneim.-Forsch.*, **22**, 1933 (1972).

to rats more than 96% of the total radioactivity was excreted from the body within 2 days. These results suggest that BL 191 and its metabolites are readily excreted from the body. Moreover, the data obtained by Christ, *et al.*[15] using labelled BL 191 in rats revealed that about 90% of the total radioactivity was excreted in the urine and about 6% in the feces, and the present study shows that about 70% of the dose administered was excreted in the urine as BL 191 and its metabolites. The amount of the urinary excretion obtained here by us was slightly less than that reported by Christ, *et al.* probably because (1) there was an approximately 100 fold difference between the dose employed by Christ, *et al.* (3 mg/kg of ^{14}C-BL 191, *p.o.*) and ours, and this difference in the dose level might have come out as the difference in the ratio of urinary to fecal excretion; and (2) with the assay procedure used in this study there might have occurred a difference in the sensitivity between BL 191 and its metabolites other than the main ones. However, our results may be essentially in agreement with those by Christ, *et al.* Therefore, taking the above-described facts into consideration, it may be said that BL 191 and its metabolites are excreted largely through the urinary organs, as other methylxanthines.

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