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Identification of Propentofylline Metabolites in Rats by Gas Chromatography/Mass Spectrometry

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Propentofylline (PPF, 3-methyl-1-(5-oxohexyl)-7-propylxanthine) has been reported to be a compound for treatment of both vascular dementia and dementia of the Alzheimer type. The short half-life (about 15 min) of PPF at the terminal elimination phase and poor bioavailability after oral administration of PPF to rabbits (Kim *et al.*, 1992) suggest in part that this drug takes the extensive first-pass metabolism in the liver. In addition, the metabolic pathway for PPF remains unclear. The objective of this experiment is to identify urinary metabolites of PPF in rats. For the identification of the metabolites, rat urine was collected after oral administration of 100 mg/kg PPF. PPF metabolite, 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine, was synthesized and confirmed by gas chromatography/mass spectroscopy (GC/MS) and ^{1}H nuclear magnetic resonance spectroscopy. The urinary metabolites of PPF were extracted with diethyl ether and identified by electron impact and chemical ionization GC/MS. One urinary metabolite was confirmed to be 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine by synthesized authentic compound. Several metabolites of monohydroxy- and dihydroxy-PPF were identified based on mass fragmentation of both intact and trimethylsilylated derivatives of PPF metabolites and the novel structure of these metabolites is suggested based on mass spectra.

Key words: Propentofylline, Hydroxypropentofylline, Metabolites, Metabolism, Gas Chromatography/mass spectrometry, Urinary metabolites

INTRODUCTION

Propentofylline (PPF, 3-methyl-1-(5-oxohexyl)-7-propylxanthine) has been reported to be a compound for treatment of both vascular dementia and dementia of the Alzheimer type (Fugi *et al.*, 1993; Nabeshima, 1995; Meilke *et al.*, 1996). PPF was synthesized to increase its solubility in lipid by substituting a methyl group to a propyl group in position 7 of the purine backbone of pentoxifylline. The pharmacological effects of PPF may be exerted via stimulation of the nerve growth factor (Meilke *et al.*, 1996), increased cerebral blood flow (Grome *et al.*, 1996), and inhibition of adenosine uptake (Fredholm *et al.*, 1994). PPF also enhances extracelullar adenosine concentrations but decreases extracellular levels of glutamate in vivo during ischemia (Andine *et al.*, 1990).

In contrast to the known pharmacological effects, few clinical pharmacokinetic and metabolism studies of PPF were reported in the literature. The elimination half-life of PPF in the β phase has been reported to be 15.3 min with very low bioavailability after oral administration in rabbits (Kim *et al.*, 1992), suggesting in part that this drug takes the extensive first-pass metabolism in the liver. It is of interest to study metabolism of PPF with long hydrocarbon chain within the structure as a model for the metabolism of long aliphatic hydrocarbon of the purine ring when compared to other compounds of xanthine derivative such as caffeine. The objective of this study is to identify metabolites in rats after the oral administration of PPF.

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MATERIALS AND METHODS

Materials

Propentofylline was kindly provided by Keunhwa Pharmaceutical Co. (Seoul, Korea) and also purchased from Sigma (MI, USA). N-Methyl-N-trimethylsilyl-trifluoroacetamide was purchased from Sigma. Helium and methane of high purity were used in gas chromato-graphy/mass spectroscopy (GC/MS). All other reagents were of analytical grade.

Sprague-Dawley rats (250 ± 20 g) were obtained from Dae-Han Laboratory Animal Research Center (Eumsung, Korea). Tap water and feed (Samyang, Korea) were provided *ad libitum*.

Urine collection in rats

Urine was collected from Sprague-Dawley rats using metabolic cages. After blank urine was collected, propentofylline (100 mg/kg in saline, 2 ml/kg) was orally administered via a gavage.

Synthesis of 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine (PPFOH)

PPFOH was synthesized from PPF by sodium borohydride reduction. Briefly, 156 mg of PPF was dissolved in 2 ml of methanol and 80 mg of sodium borohydride was reacted with PPF at 4°C and the mixture was stirred overnight. The progress of the reaction was confirmed by thin layer chromatography. The reaction product was extracted with methylene chloride. The organic layer was washed with saturated sodium chloride solution, three times, and filtered through a filter paper covered with magnesium sulfate, and the filter paper was stored in a desiccator until the crystal of PPFOH formed.

Extraction of propentofylline and its metabolites in urine

An aliquot of urine (5 ml) was added to a 15 ml centrifuge tube and pH was adjusted to 11.5 using 1 N sodium hydroxide (0.2 ml). After vortex mixing, 5 ml of distilled diethyl ether was added and the mixture was shaken on a shaker for 20 min. The tubes were centrifuged and put in a freezer (-25°C, 5 min; refrigerating circulator, Lauda, Germany) for separation of the organic layer. The organic layer was evaporated using drying block (Dri-Block, Techne Inc., Princeton, NJ, USA) under the stream of nitrogen, and dried in a desiccator with P_2O_5/KOH. An aliquot of the residue was either dissolved in 100 μl of methanol, or trimethylsilylated with 50μl of N-methyl-N-trimethylsilyltrifluoroacetamide at 60°C for 10 min (Schanzer *et al.*, 1991). This solution (2-3 μl) was injected into GC/MS.

Gas chromatograph/mass spectrometry (GC/MS)

Gas chromatograph: A gas chromatograph (HP 5890A) equipped with a HP capillary column Ultra-2 (cross-linked 5% phenylmethylsilicone; 25 m × 0.2 mm × 0.11 μm film thickness) was used for analysis of propentofylline metabolites. The flow rate of carrier gas (He) was 1.08 ml/min. The split ratio was 10:1 and septum purge rate was 5 ml/min. Both the injector and detector were set at 300°C. The oven temperature gradient was used; Temperature was increased from 180°C of initial value to 220°C by 10 per minute without holding time, and finally increased to 280°C by 3°C per minute with holding for 1 minute at final temperature.

Electron impact (EI) mode: HP 5890A GC was coupled with HP 5970 B mass selective detector. Ionization potential was 70 eV. Transfer line temperature was 300°C.

Chemical ionization (CI) mode: HP 5890A GC was coupled with HP5988A mass spectrometer. Methane was used as reagent gas at 1 torr. Electron energy was 230 eV and emission current was 300 A.

^{1}H Nuclear magnetic resonance spectroscopy (^{1}H NMR)

^{1}H NMR spectra were recorded on a Varian Gemini 300 spectrophotometer. Chemical shift (δ) in ppm and coupling constants in Hz were presented.

RESULTS AND DISCUSSION

Synthesis of 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine (PPFOH)

The structure of PPFOH was confirmed by NMR and GC/MS.

^{1}H NMR ($CDCl_3$, 300 MHz); δ (ppm) 0.94 (t, 3H, $NCH_2CH_2CH_3$), 1.17 (d, J=6.2, 3H, $CH_3CH(OH)$), 1.35-1.75 (m, 6H, $CH_3CH(OH)$ $CH_2CH_2CH_2CH_2N$), 1.89 (sextet, 2H, $NCH_2CH_2CH_3$), 3.47 (s, 1H, OH), 3.57 (s, 3H, N-CH_3), 3.76-3.82 (m, 1H, methine), 4.01 (t, 2H, $CH_2CH_2CH_2N$), 4.24 (t, 2H, $NCH_2CH_2CH_3$), 7.53 (s, 1H, vinyl CH).

GC/MS (Table I); Molecular weight (underivatized, m/z 308; TMS-derivatized, m/z 380).

Underivatized urinary metabolites of PPF

Underivatized metabolites of PPF extracted from rat urine were analyzed by a gas chromatography/mass spectrometer equipped with either electron impact (EI) or chemical

Table I. Characteristic mass ions (m/z) of propentofylline (PPF) and 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine (PPFOH) by GC/MS/EI[a]

	Mass ions (m/z),	M^+ [b]
Underivatized		
PPF[c]	166 180 209 221 249 263	306(M^+)
PPFOH[d]	166 180 209 221 247 264 293	308(M^+)
Trimethylsilylated		
PPF	143 170 209 248 281 363(M^+-15)	378(M^+)
PPFOH	117 166 209 247 265 365(M^+-15)	380(M^+)

[a]GC/MS/EI, gas chromatography/mass spectrometry/electron impact; [b]M^+, molecular ion of chemicals in mass spectrum; [c]PPF, propentofylline; [d]PPFOH, 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine.

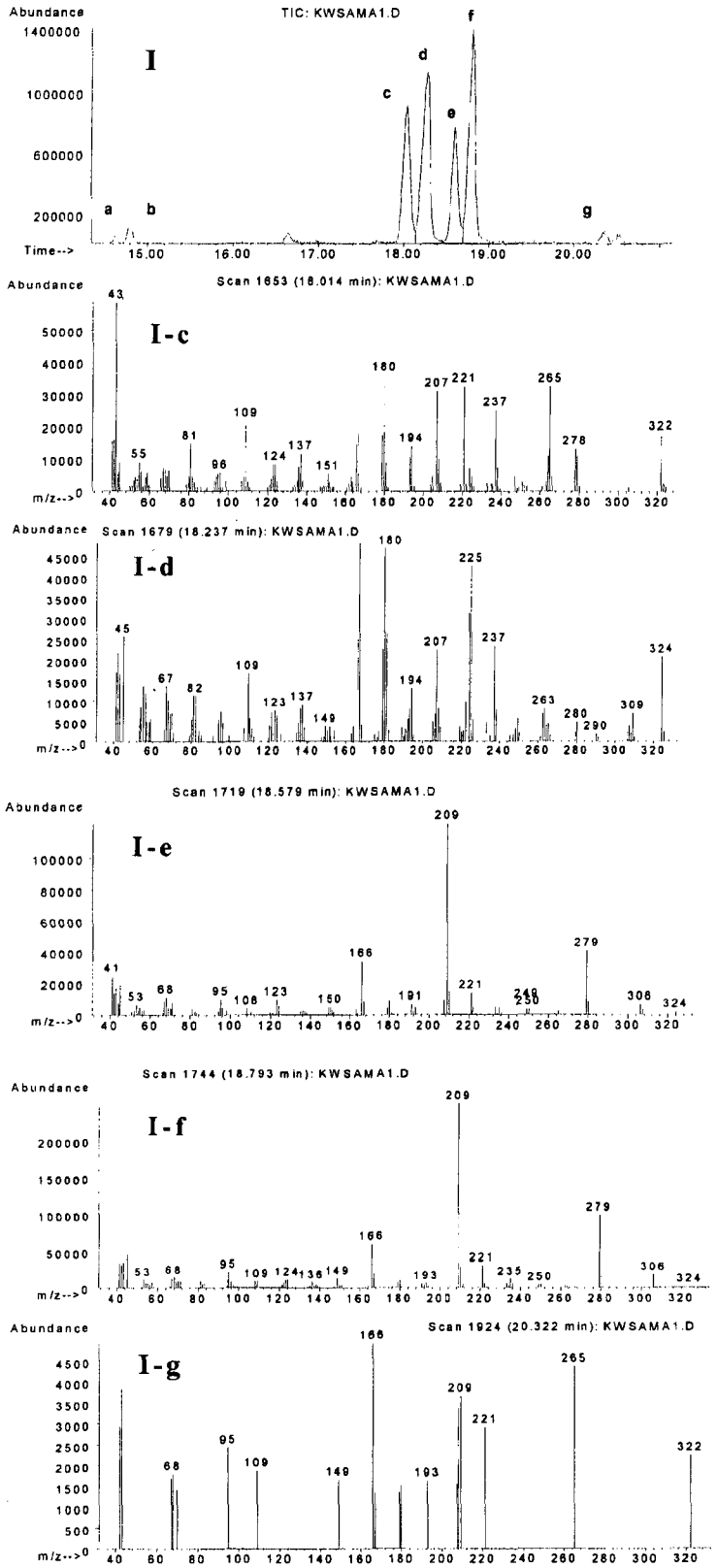

Fig. 1. Total ion chromatogram (**I**) and mass spectra (**I-c~I-h**) of underivatized metabolites extracted from urine of rats after oral administration of propentofylline. Total ion chromatogram and mass spectra were obtained by EI mode of GC/MS. Peaks **a** and **b** in the total ion chromatogram **I** were identified as PPF and PPFOH by authentic and synthesized compounds, respectively

ionization (CI). The total ion chromatogram showing these metabolites is shown in Fig. 1 (**I**). This chromatogram was obtained by GC/MS/EI. Peaks **a** and **b** were identified as PPF and its metabolite PPFOH (3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine), respectively. These two compounds were confirmed by comparing these mass spectra to those of either commercially available PPF (M^+, 306) or synthesized PPFOH (M^+, 308), as shown in Table I. The peaks from **c** to **g** in total ion chromatogram **I** (Fig. 1) were considered metabolites originating from PPF, which were not detected in drug-free urine blank (blank chromatogram not shown). The total ion chromatogram **II** (Fig. 2) was obtained by GC/MS/CI to further provide evidence of the molecular weights of these compounds. Peaks of the total ion chromatogram **I** in Fig. 1 were eluted in the same order as in peaks of the total ion chromatogram **II** in Fig. 2 because the same kind of column with the different length was used. Therefore, peaks **c**, **d**, **e**, and **f** of the total ion chromatogram **I** (Fig. 1) should be consistent with those of the total ion chromatogram **II** (Fig. 2). Peaks **a**, **b**, and **g** shown in the total ion chromatogram **I** were not detected in the total ion chromatogram **II**. The molecular weight ion (M^+) of metabolite **c** was confirmed to be m/z 322 based on the masses of the

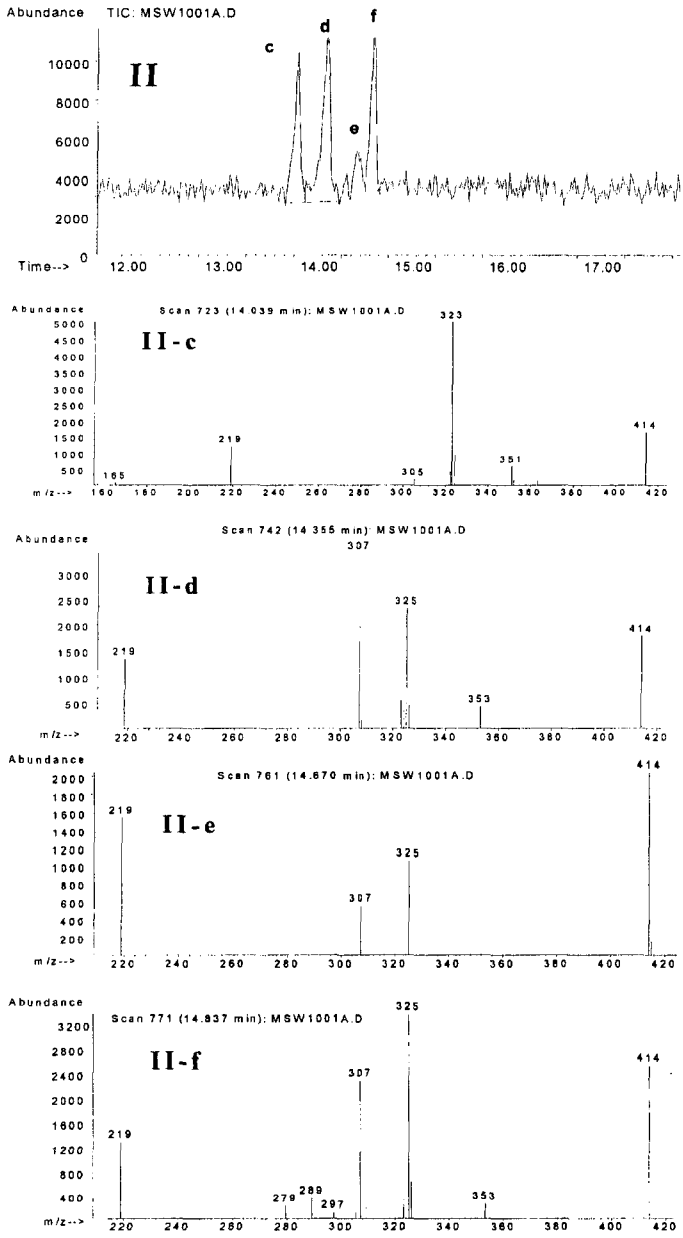

Fig. 2. Total ion chromatogram (**II**) and mass spectra (**II-c**~**II-f**) of underivatized metabolites extracted from urine of rats after oral administration of propentofylline. The total ion chromatogram and mass spectra were obtained by CI mode of GC/MS

$M^+ + H$ and $M^+ + C_2H_5$ ions (spectrum **II-c**, in Fig. 2). The molecular weight ion (M^+) of metabolite **g** was assumed to be m/z 322 when interpreted based on only GC/MS/EI mass spectrum in the sight of other metabolite spectra obtained by two mode of GC/MS. The molecular weight ions (M^+) of metabolites **d**, **e** and **f** in the total ion chromatograms **I** and **II** were all m/z 324 as supported by in their mass spectra (**I-d**~**I-f** in Fig. 1 and **II-d**~**II-f** in Fig. 2). Fragment ions at m/z 81, 109, 137 or 166 might occur from cleavage of the purine ring in the metabolites as reported for xanthine analogs (Hignite, 1980).

Trimethylsilylated metabolites of PPF

Total ion chromatograms of trimethylsilylated derivative of these metabolites and their mass spectra were obtained by EI (**III**, as shown in Fig. 3) or CI (**IV**, as shown in Fig. 4) mode of GC/MS. Trimethylsilylated PPFOH was found at 14.85 min in the total ion chromatogram **III**. This peak was identified by extracted ions since it overlapped with an interfering peak of urine. The molecular weight ion (M^+) of peak **a** was found to be m/z 394 and the peaks **b**, **c**, **d**, **e**, and **f** all gave the same molecular weight ion (M^+) of m/z 468, from the interpretation of total ion chromatogram **III** (Fig. 3) and total ion chromato-

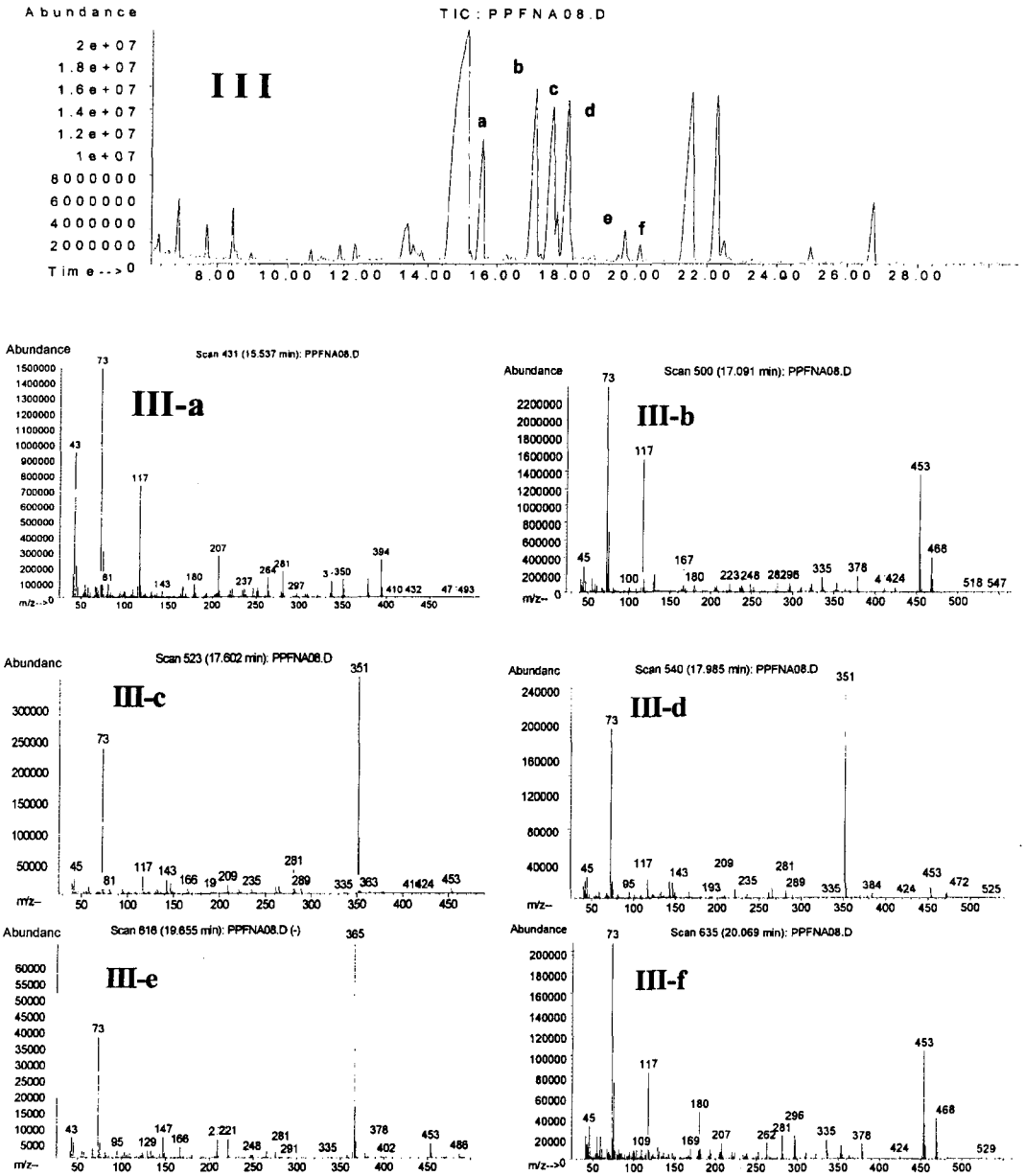

Fig. 3. Total ion chromatogram (**III**) and mass spectra (**III-a**~**III-f**) of trimethylsilylated metabolites extracted from urine of rats after oral administration of propentofylline. The total ion chromatogram and mass spectra were obtained by EI mode of GC/MS

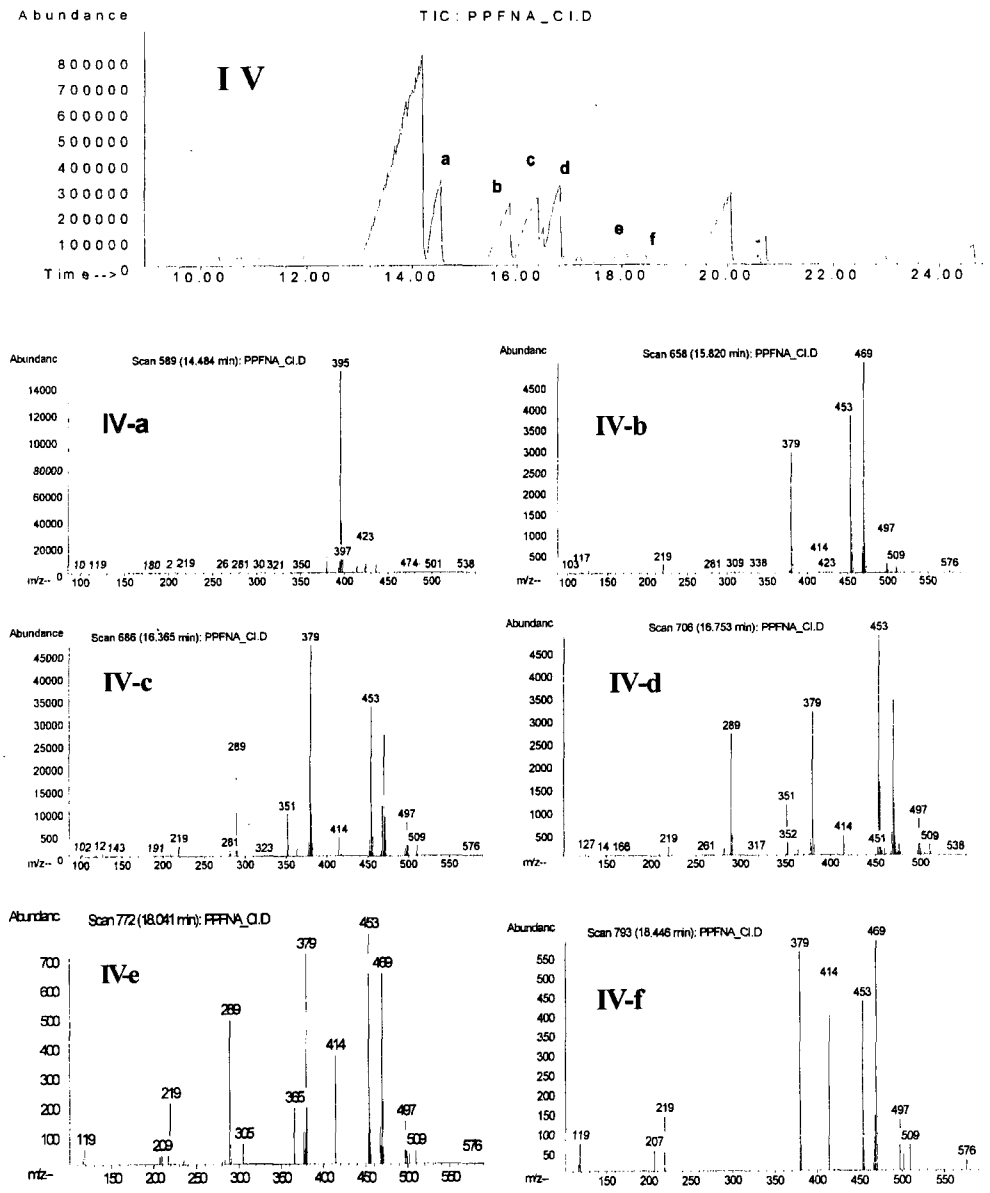

Fig. 4. Total ion chromatogram (**IV**) and mass spectra (**IV-a IV-f**) of trimethylsilylated metabolites extracted from urine of rats after oral administration of propentofylline. The total ion chromatogram and mass spectra were obtained by CI mode of GC/MS

gram **IV** (Fig. 4). The ions of m/z 73 from the mass spectra **III-a~III-f** in Fig. 3 are the characteristic ions of trimethylsilylated compounds. The characteristic ions of m/z 117 are present in all mass spectra **III-a~III-f** (with exception in mass spectra **III-e**). These ions are produced from fragment of $[CH_3\text{-}CH\text{-}OTMS]^+$ in the 1 position of the purine ring. The presence of these ions suggests occurrence of reduction at C5 of 5-oxohexyl group and presence of CH_3-CH(OH)- within the structure of metabolites. Fragmentations of $M^+ + H$ and $M^+ + C_2H_5$ were observed in mass spectra **IV-a~IV-f** of Fig. 4. The molecular weight ion of m/z 394 (322 + TMS; **III-a** in Fig. 3 and **IV-a** in Fig. 4) is considered mono-hydroxylated PPF that is different from PPFOH (380; 308 + TMS). PPF metabolites with two hydroxyl groups in the 5-oxohexyl chain of the purine ring have molecular weight ion of m/z 468 (324 + 2TMS; as observed in EI mass spectra **III-b~III-f** in Fig. 3 and CI mass spectra **IV-b~IV-f** in Fig. 4).

Six major metabolites of PPF including PPFOH are derived and presented in Fig. 5 based on CI and EI mass spectra. Metabolite, **ii**, is PPFOH that was confirmed by synthesized authentic standard. Dihydroxy metabolites of PPF may have the structures of **v~ix**. The mass spectrum **III-e** are corresponded to the structure of **vi** in Fig. 5 since the ion of m/z 365 was produced by the cleavage of fragment $[TMSO\text{-}CH_2]^+$ at $HO\text{-}CH_2\text{-}CH_2(OH)\text{-}(CH_2)_4$- from the 1 position of the purine ring.

In conclusion, PPF was metabolized for the most part

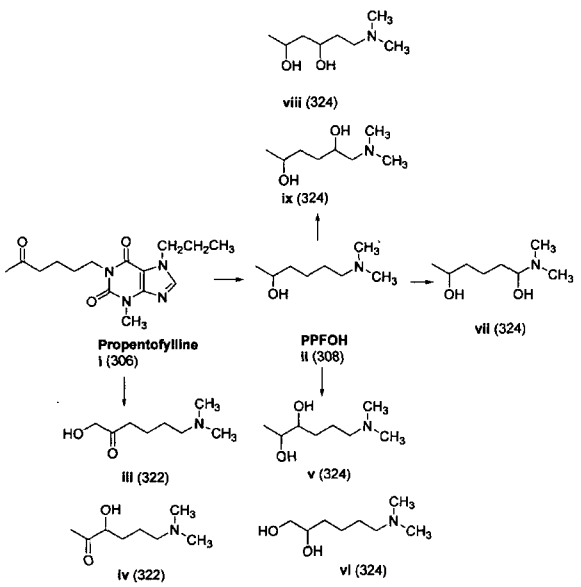

Fig. 5. The possible metabolites of propentofylline based on EI and CI mass spectra of GC/MS. The metabolite **ii** (PPFOH) was confirmed by synthesized compound as described in Experimental.

to monohydroxy and dihydroxy forms and excreted through urine in rats. PPFOH (**ii**), one of the PPF metabolites, was confirmed by synthesized authentic standard. The novel structures of other metabolites were suggested based on EI and CI mass spectra although the structural elucidation of metabolites requires their synthesis. Further studies are progressing to identify metabolites of PPF and elucidate the metabolic pathways.

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REFERENCES

Andine, P., Rudolphi, K. A., Fredholm, B. B., Hagberg, H. Effects of propentofylline (HWA 285) on extracellular purines and excitatory amino acids in CA1 of rat hippocampus during transient ischaemia. *Br. J. Pharmacol.* 100 (4), 814-818 (1990).

Fredholm, B. B., Lindstrom, K., Wallman-Johansson, A. Propentofylline and other adenosine transport inhibitors increase the efflux of adenosine following electrical or metabolic stimulation of rat hippocampal slices. *J. Neurochem.*, 62 (2), 563-573 (1994).

Fugi, K., Hiramatsu, M., Kameyama, T., Nebeshima, T. Effects of repeated administration of propentofylline on memory impairment produced by basal forebrain lesion in rats. *European J. Pharamacol.*, 236 (3), 411-417 (1993).

Grome, J. J., Hofmann, W., Gojowczyk, G., Stefanovich, V. Effects of a xanthine derivative, propentofylline, on local cerebral blood flow and glucose utilization in the rat. *Brain Res.* 740 (1-2), 41-46, 1996.

Hignite, C., Chapter 16. Nucleic acids and derivative. In *Biochemical applications of mass spectroscopy, Vol. 1*; Waller, G. R. and Dermer, O. C. (Eds.). John Willey & Sons, pp 527-566 (1980).

Kim, N. S., Ito, T., Kawata, M., Uchida, T., Goto, S. Preparation and evaluation of Eudragit gels. IV: Rectal gel preparations for sustained release and avoidance of first-pass metabolism of propentofylline. *J. Pharm. Sci.*, 81 (9), 904-907 (1992).

Meilke, R., Kittner, B., Ghaemi, M., Kessler, J., Szelies, B., Herholz, K., Hiess, W. D. Propentofylline improves regional cerebral. *J. Neurol. Sci.*, 141 (1-2), 59 (1996).

Nabeshima, T. Nerve growth factor strategy and preparation of animal model for Alzheimer-type senile dementia. *Yakugaku Zasshi*, 115 (7), 499-512 (1995).

Schanzer W., Geyer H. and Donike, M. Metabolism of methandienone in man: identification and synthesis of conjugated excreted urinary metabolites, determination of excretion rates and gas chromatographic-mass spectrometric identification of bis-hydroxylated metabolites. *J. Steroid. Biochem. Mol. Biol.* 38 (4), 441-464 (1991).