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Metabolic Studies of Aminopyrine in Rat and Man by Using Stable Isotope Tracer Techniques

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The metabolites of aminopyrine (AM) were analyzed by using stable isotope tracer techniques. After the oral administration of an equimolar mixture of AM and AM-2-CD_3 (mixture 1), or an equimolar mixture of AM-3-CH_2OH and AM-3-CD_2OH (mixture 2), or either AM-3-CD_3 or AM-4-$N(CD_3)_2$ to rats, the urinary metabolites were extracted with chloroform at pH 7.0, and the extracts were subjected to gas chromatography–mass spectrometry after trimethylsilylation. Characteristic doublet peaks in the mass spectra indicated the presence of a metabolite originating from mixture 1 or 2 mentioned above.

The new metabolites detected by gas chromatography–mass spectrometry (GC–MS) were identified as 3-hydroxymethyl-4-monomethylaminoantipyrine (MAA-3-CH_2OH) and 3-hydroxymethyl-4-aminoantipyrine (AA-3-CH_2OH) from the shifts of the mass numbers of the molecular ions after the administration of AM-3-CD_3 and AM-4-$N(CD_3)_2$.

Two other metabolites were newly detected following the administration of mixture 2(AM-3-CH_2OH is an intermediary metabolite of AM) to rats. They were identified as 3-hydroxymethyl-4-acetylaminoantipyrine (AcMAA-3-CH_2OH) and 3-hydroxymethyl-4-acetylaminoantipyrine (AcAA-3-CH_2OH). MAA-3-CH_2OH was also detected in human urine after the oral administration of AM.

Keywords——metabolism of aminopyrine; GC–MS analyses; deuterated aminopyrine; stable isotope tracer; new metabolites; new metabolic route

In previous papers, we reported the metabolic fate of aminopyrine (AM) in man,[1] and in such experimental animals as rabbits, rats and guinea pigs,[2] and the detection of 4-formylaminoantipyrine (FAA), a new metabolite of AM, in the urine in all these cases.

In the present study, we attempted to detect and identify other new metabolites of AM in rats and man by using stable isotope tracer techniques.

Experimental

Chemicals——J.P.IX grade AM (Iwaki Seiyaku Co.) and reagent-grade 4-aminoantipyrine (AA, Wako Pure Chemical Ind. Ltd.) were used. 4-Monomethylaminoantipyrine (MAA), 4-acetylaminoantipyrine (AcAA) and FAA were prepared as described in a previous paper.[3] Three kinds of deuterium-labeled AM, 4-dimethylamino-3-methyl-2-trideuteromethyl-1-phenyl-3-pyrazolin-5-one (AM-2-CD_3), 4-dimethylamino-3-trideuteromethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (AM-3-CD_3), and 4-di-(trideuteromethylamino)-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (AM-4-$N(CD_3)_2$) were the same meterials as those employed in previous studies[4,5] (Fig. 1). 4-Dimethylamino-3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (AM-3-CH_2OH) was prepared according to the method of Kametani *et al.*[6] 4-Dimethylamino-3-dideuterohydroxymethyl-

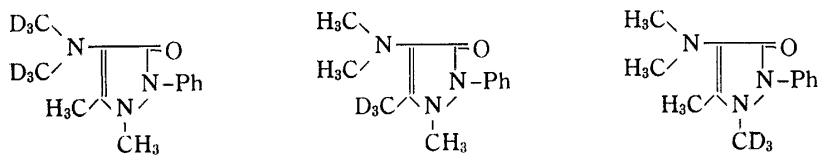

Fig. 1. Structures of Deuterium-labeled Aminopyrines

2-methyl-1-phenyl-3-pyrazolin-5-one (AM-3-CD_2OH) was prepared from AM-3-CH_2OH by the base-catalyzed hydrogen exchange method as follows.

AM-3-CD_2OH: AM-3-CH_2OH (200 mg) was dissolved in 20 ml of 1 N NaOD–D_2O. The solution was refluxed for 6 hr and extracted with chloroform. The extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized from benzene–ligroin to give 150 mg of AM-3-CD_2OH (98.6 atom D%).

Animal and Human Experiments——Male Wistar rats weighing 200—250 g were used. Fifty mg/kg of an equimolar mixture of AM and AM-2-CD_3 (AM: AM-2-CD_3), AM-3-CH_2OH: AM-3-CD_2OH, AM-3-CD_3 or AM-4-$N(CD_3)_2$ was orally administered in aqueous solution to the rats. Urine was collected for 24 hr after drug administration. Ten ml of the urine was diluted to 20 ml with distilled water and extracted twice with 20 ml of chloroform at pH 7.0. The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was trimethylsilylated with bis-trimethylsilylacetamide in pyridine.

A healthy male volunteer (age 37, weight 58 kg) took 100 mg of AM orally in the form of an aqueous solution. Urine samples were collected at 0—4, 4—8 and 8—12 hr after dosing. Twenty ml of the urine was extracted with chloroform and the extract was trimethylsilylated as described above for the extract from rat urine.

Gas Chromatography–Mass Spectrometry (GC–MS)——GC–MS was carried out on a Shimadzu-LKB 9000 gas chromatograph–mass spectrometer.

A coiled glass column (1 m × 3 mm i.d.) packed with 1.5% OV-17 on Chromosorb W (80—100 mesh) was used. Helium gas flow rate was 20 ml/min. The column temperature was programmed to rise from 190° to 270° at the rate of 10°/min. The temperatures of the injection port, separator and ion source were 270°, 280° and 290°, respectively.

Mass spectrometer conditions were as follows: accelerating voltage, 3.5 kV; trap current, 60 μA; ionizing energy, 20 eV.

Results and Discussion

In order to detect the urinary metabolites of AM by the ion cluster technique, an equimolar mixture of AM and AM-2-CD_3 was administered to rats. The total ion chromatogram of the trimethylsilylated extract from urine is shown in Fig. 2. Ion clusters were observed in the mass spectra obtained from peaks a, b, c, d, e and f. By comparing the retention times and mass spectra with those of authentic samples, peaks a, b, e and f were identified as trimethylsilylated AA, AM-3-CH_2OH, AcAA and FAA, respectively. The compounds detected as peaks c and d were demonstrated to be the new metabolites of AM from their mass spectra (Fig. 3).

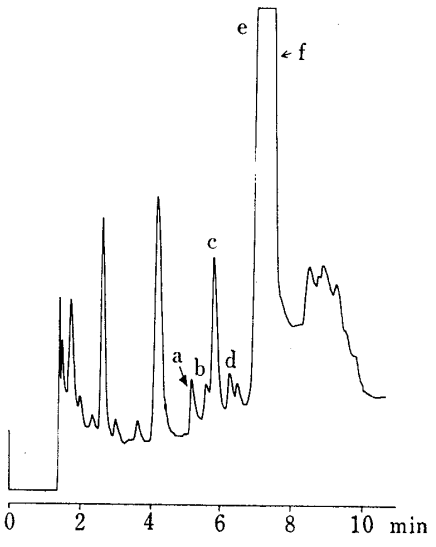

Fig. 2. Total Ion Chromatogram of the Trimethylsilylated Extract from Rat Urine after Administration of an Equimolar Mixture of AM and AM-2-CD_3

a: AA-TMS, b: AM-3-CH_2OH-TMS, c: unknown, d: unknown e: AcAA-TMS, f: FAA-TMS.

In the mass spectrum of peak c, the molecular ions appeared at *m/z* 377: 380, and the presence of ion clusters separated by three mass units indicated that the 2-methyl group was not metabolized. In order to determine the metabolized position, the administration of either AM-3-CD_3 or AM-4-$N(CD_3)_2$ was performed. The appearance of the molecular ion of peak c at *m/z* 379 or 380 after the administration of AM-3-CD_3 or AM-4-$N(CD_3)_2$, respectively, revealed that a deuterium atom from the 3-methyl group and three deuterium atoms from the 4-dimethylamino group had been lost. Thus, the metabolite in peak c was identified as 4-monomethyl amino-3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (MAA-3-CH_2OH), which was formed by the concomitant oxidation of the 3-methyl group to a hydroxymethyl group and the 4-dimethylamino group to a monomethylamino group.

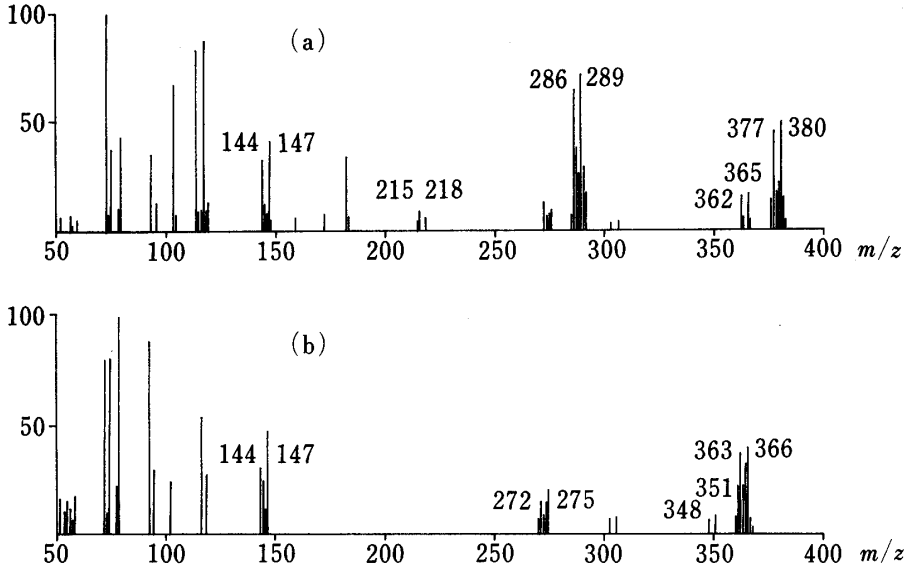

Fig. 3. Mass Spectra of the Metabolites in Peak c (a) and Peak d (b)

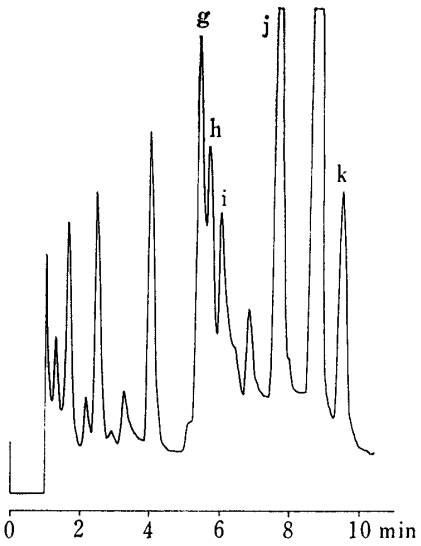

Fig. 4. Total Ion Chromatogram of the Trimethylsilylated Extract from Rat Urine after Administration of an Equimolar Mixture of AM-3-CH_2OH and AM-3-CD_2OH

g: AM-3-CH_2OH-TMS, h: MAA-3-CH_2OH-diTMS, i: AA-3-CH_2OH-diTMS, j: unknown, k: unknown.

As for the metabolite from peak d, the molecular ions appeared at *m/z* 363: 366, indicating no metabolism of the 2-methyl group. After the administration of AM-3-CD_3 or AM-4-N$(CD_3)_2$, the molecular ion appeared at *m/z* 365 or 363, respectively. A deuterium atom from the 3-position of AM-3-CD_3 and six deuterium atoms from the 4-position of AM-4-N$(CD_3)_2$ had been lost. These results elucidated the structure of the metabolite in peak d as 4-amino-3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (AA-3-CH_2OH).

Yoshimura *et al.*[7] have already reported that two unknown metabolites were detected by thin-layer chromatography in rat urine after the administration of AM-3-CH_2OH, but they did not identify them. We suggest that the unidentified metabolites reported by Yoshimura *et al.* are MAA-3-CH_2OH and AA-3-CH_2OH.

Further analysis of metabolites was performed following the oral administration of AM-3-CH_2OH, an intermediary metabolite of AM, to rats. The total ion chromatogram of the trimethylsilylated extract from the urine after the administration of an equimolar mixture of AM-3-CH_2OH and AM-3-CD_2OH (AM-3-CH_2OH: AM-3-CD_2OH) is shown in Fig. 4.

Ion clusters were observed in the mass spectra of peaks g, h, i, j and k. The metabolites corresponding to peaks g, h and i were identified as trimethylsilylated AM-3-CH_2OH, MAA-3-CH_2OH and AA-3-CH_2OH, respectively, by comparison of the mass spectra with those obtained in the case of AM dosing. The mass spectra obtained from peaks j and k are shown in Fig. 5.

Among the ion clusters that appeared at *m/z* 405: 407, 390: 392, 333: 335, 291: 293 and 144: 146 in peak j, *m/z* 405: 407 indicates the molecular ions. The peaks of $(M-15)^+$, $(M-72)^+$ and $(M-114)^+$ observed in the case of trimethylsilylated AcAA suggested the presence of a

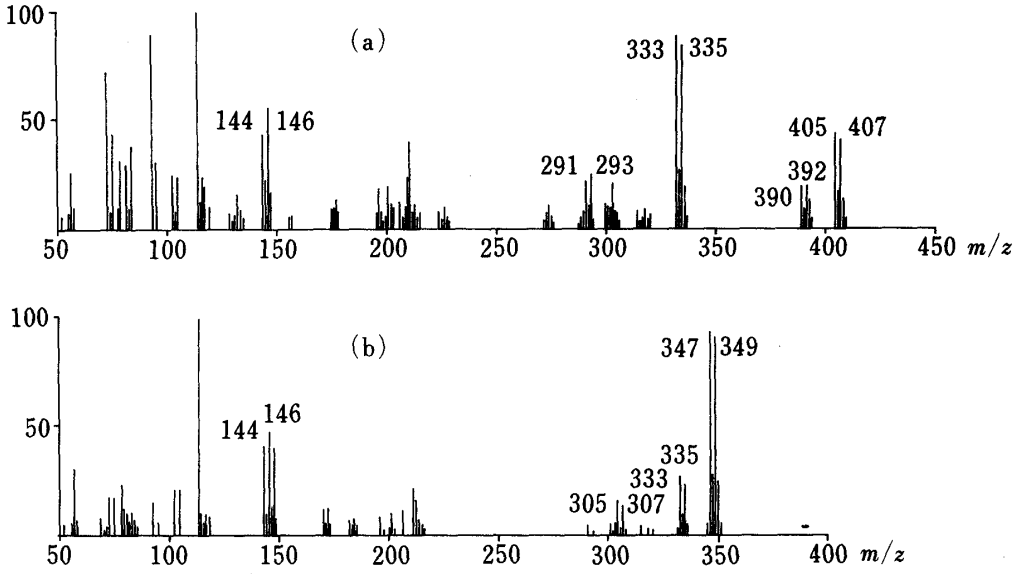

Fig. 5. Mass Spectra of the Metabolites in Peak j and Peak k

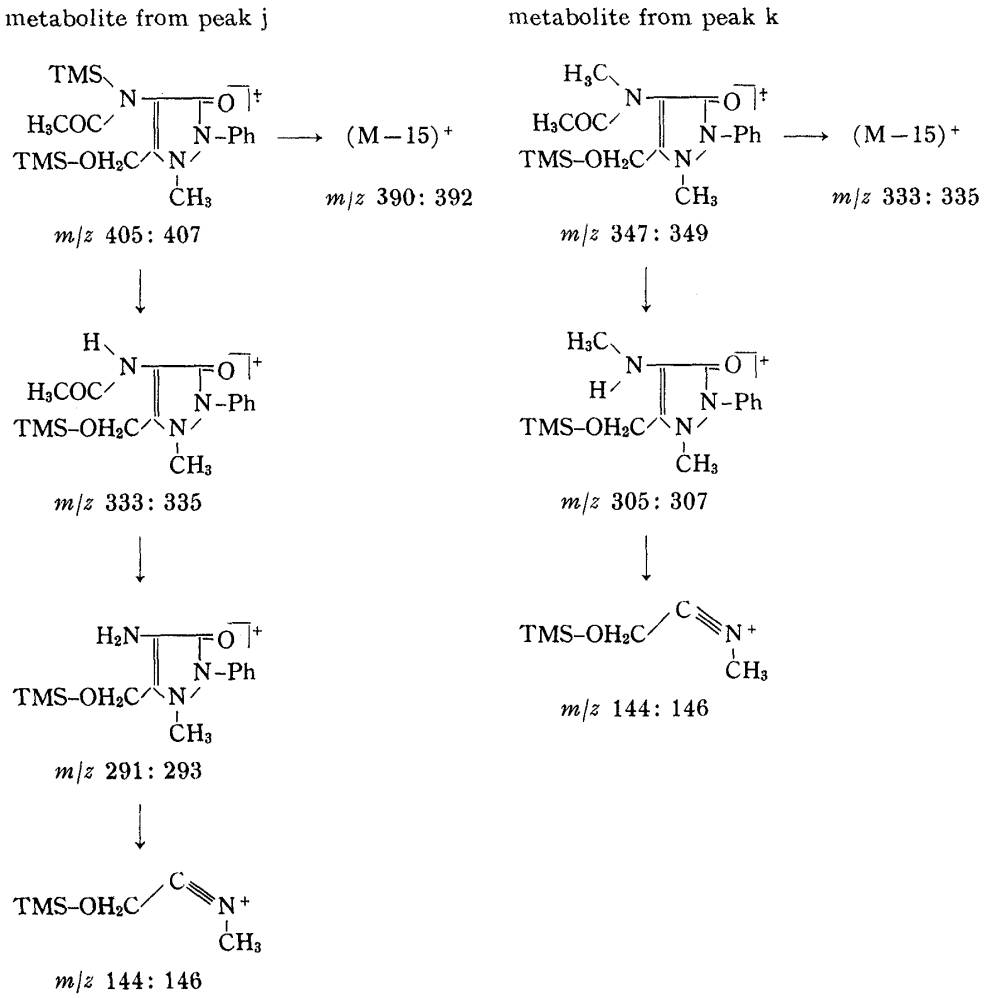

Chart 1. Postulated Fragmentation Pathways of the Metabolites in Peak j and Peak k

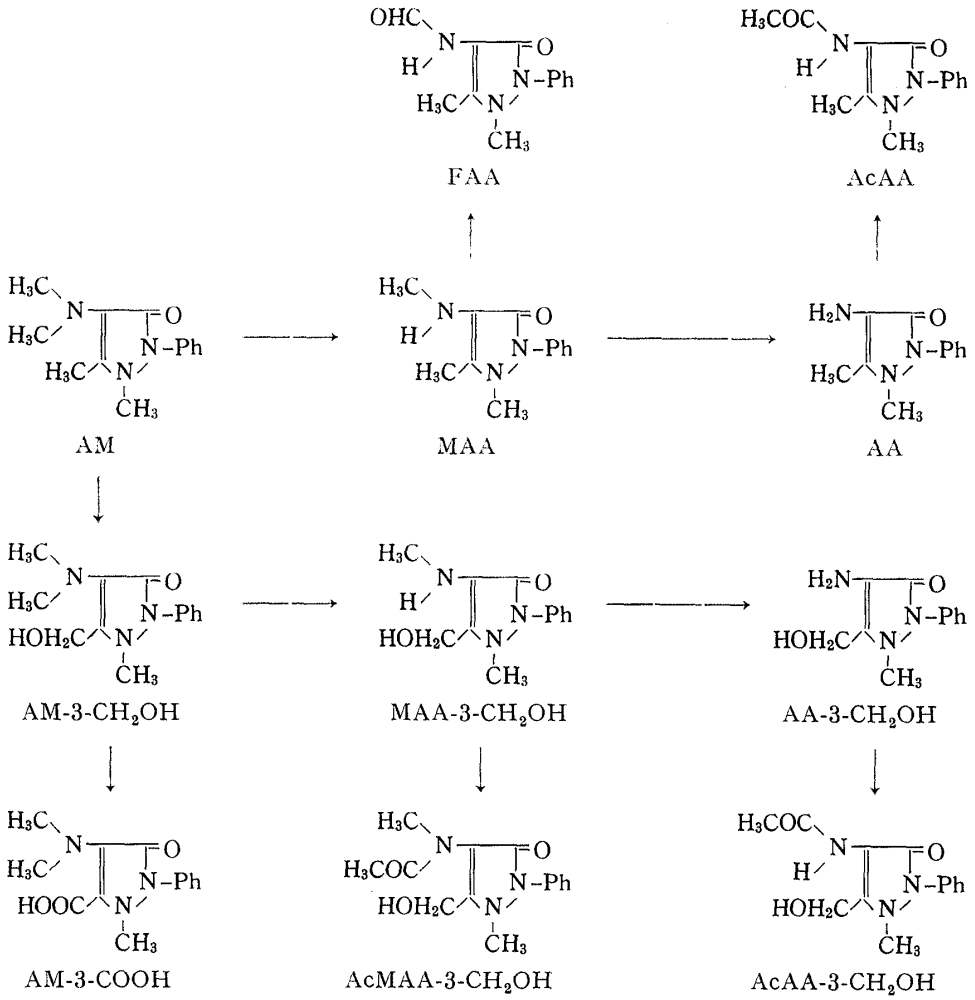

Chart 2. Metabolic Pathways of AM in Rats

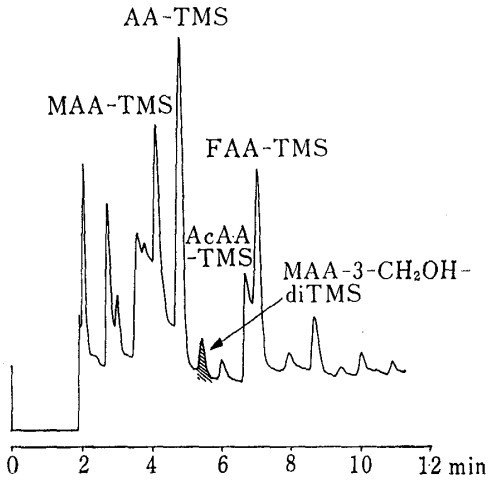

Fig. 6. Total Ion Chromatogram of the Trimethylsilylated Extract from Human Urine after AM Administration

trimethylsilyl-acetylamino moiety.

In the case of peak k, the ion clusters appeared at *m/z* 347: 349, 333: 335, 305: 307 and 144: 146. The molecular ions were found at *m/z* 347: 349. The peaks of $(M-14)^+$ and $(M-42)^+$, like those observed in the case of 4-acetylmethylaminoantipyrine,[3] suggested the presence of an acetylmethylamino moiety.

Fragment ions at *m/z* 144: 146 were also observed in the spectra of trimethylsilylated MAA-3-CH_2OH: MAA-3-CD_2OH and AA-3-CH_2OH: AA-3-CD_2OH, while corresponding fragment ions were observed at *m/z* 144: 147 after dosing AM: AM-2-CD_3.

The fragmentation pathways of these metabolites are presumed to be as shown in Chart 1. The metabolites in peaks j and k were identified as trimethylsilylated 4-acetylamino-3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (AcAA-3-CH_2OH) and 4-acetylmethylamino-3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (AcMAA-3-CH_2OH), respectively.

The metabolic pathways of AM in rats established by the above procedure are summarized in Chart 2. A new metabolic route of AM, involving oxidation at 3-methyl, demethylation at 4-dimethylamine and acetyl conjugation successively, was clarified. Since no 3-hydroxymethyl metabolites, such as MAA-3-CH_2OH, AA-3-CH_2OH and AcAA-3-CH_2OH, were detected in the urine after the administration of MAA, AA or AcAA, it was proved that 3-hydroxymethyl metabolites were not formed from the corresponding 3-methyl metabolites.

Finally, metabolism of AM *via* this new route was examined in man. The total ion chromatogram of the trimethylsilylated extract from 0—4 hr urine after the administration of AM to man is shown in Fig. 6. Trimethylsilylated MAA-3-CH_2OH was detected by GC–MS in the urine extracted 12 hr after dosing.

References and Notes

1) S. Iguchi, T. Goromaru, and A. Noda, *Chem. Pharm. Bull.*, **23**, 932 (1975).
2) S. Iguchi, T. Goromaru, A. Noda, and N. Tsubone, *Chem. Pharm. Bull.*, **23**, 1889 (1975).
3) T. Goromaru, A. Noda, K. Matsuyama, and S. Iguchi, *Chem. Pharm. Bull.*, **24**, 1376 (1976).
4) T. Goromaru, K. Matsuyama, A. Noda, and S. Iguchi, *Chem. Pharm. Bull.*, **26**, 33 (1978).
5) T. Goromaru and A. Noda, *Chem. Pharm. Bull.*, **26**, 2258 (1978).
6) T. Kametani, K. Kigasawa, N. Ikari, T. Iwata, M. Saito, and H. Yagi, *Chem. Pharm. Bull.*, **15**, 1305 (1967).
7) H. Yoshimura, H. Shimeno, and H. Tsukamoto, *Yakugaku Zasshi*, **90**, 1406 (1970).