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The Measurement of Plasma Concentration of Aminopyrine and Its Metabolites in Man

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By means of mass fragmentography using the isotope dilution method, the measurement of the plasma concentration of aminopyrine (AM) and its metabolites was performed successfully.

It was clarified from the plasma concentration–time curve that the individual differences were observed in two processes which are demethylation of AM to 4-monomethylaminoantipyrine (MAA) or MAA to 4-aminoantipyrine (AA) and acetylation of AA to 4-acetylaminoantipyrine.

Furthermore, a quantitative correlation between the plasma concentration and the amount of urinary excretion was discussed.

Keywords——aminopyrine and its metabolites; plasma concentration; mass fragmentography; individual difference; demethylation; acetylation

In the previous papers, we have reported the metabolism and urinary excretion of aminopyrine (AM) in man. As a new metabolite of AM, 4-formylaminoantipyrine (FAA) was detected in the urine of all subjects, and there were individual differences in the urinary excretion behavior of the subjects.[2–5] Gas chromatography–mass spectrometry (GC–MS) using an isotope dilution method is a new analytical technique to measure the precise plasma concentration of a drug and its metabolites, and enables us to examine in detail the metabolic behavior of a drug. The method has been applied successfully to measure the plasma concentration of AM and its metabolites in man in the present study.

Materials and Methods

Materials——Aminopyrine (d_0-AM), 4-aminoantipyrine (d_0-AA), 4-monomethylaminoantipyrine (d_0-MAA), 4-acetylaminoantipyrine (d_0-AcAA), 4-formylaminoantipyrine (d_0-FAA) and N,O-bistrimethylsilylacetamide (BSA) used in the experiment were obtained as described in the previous paper.[3]

The Syntheses of Deuterium-labeled AM (d_3-AM) and Its Metabolites——In order to obtain deuterium-labeled pyrazolone derivatives, deuterium labeled antipyrine (d_3-AN) was prepared from 1-phenyl-3-methyl-5-pyrazolone and d_6-dimethylsulfate (99% D). The synthetic procedures of d_3-AM, d_3-FAA and d_3-AcAA from d_3-AN are shown in Chart 1.

Gas Chromatography-Mass Spectrometry (GC–MS)——Instrument: A JEOL model JMS-D100 mass spectrometer equipped with a JGC-20K gas chromatograph was combined with a JEOL model MS-PD-1 multiple ion detector for the mass spectrometric analysis.

Analysis: GC–MS as well as GC was performed under the conditions described in Table I. A reverse dilution method was applied for the mass fragmentography. The mass spectrometer was operated in its specific ion detection mode focused on masses 231 and 234 for AM, 217 and 220 for MAA, and 203 and 206 for AA.

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2) S. Iguchi, T. Goromaru, and A. Noda, *Chem. Pharm. Bull.* (Tokyo), **23**, 932 (1975).
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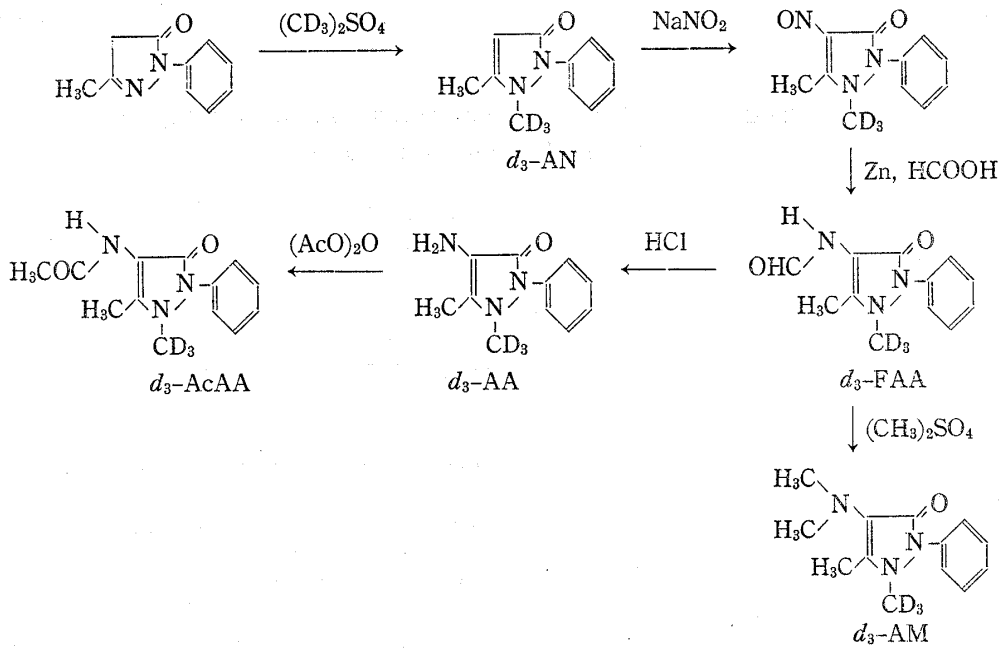

Chart 1. Synthetic Procedures of d_3-AM and Its Metabolites

Table I. GC–MS Conditions

Metabolite	MID focus	Column	Oven Temp.	Derivative
AM	*m/e* 231 (d_0) *m/e* 234 (d_3)	1% XE-60/Shimalite W (80—100 mesh), 2 mm × 1 m	190°	—
MAA	*m/e* 217 (d_0) *m/e* 220 (d_3)	1% XE-60/Shimalite W (80—100 mesh), 2 mm × 1 m	195°	—
AA	*m/e* 203 (d_0) *m/e* 206 (d_3)	1% XE-60/Shimalite W (80—100 mesh), 2 mm × 1 m	200°	—
FAA	*m/e* 303 (d_0) *m/e* 306 (d_3)	1.5% OV-17/Shimalite W (80—100 mesh), 2 mm × 1 m	230°	TMS
AcAA	*m/e* 317 (d_0) *m/e* 320 (d_3)	1.5% OV-17/Shimalite W (80—100 mesh), 2 mm × 1 m	230°	TMS

In the cases of FAA and AcAA, trimethylsilylation was necessary to obtain appropriate peaks for GC, and the molecular ion peaks (*m/e* 303 and 306 for FAA–TMS, *m/e* 317 and 320 for AcAA–TMS) were utilized for the analyses. Mass spectrometer conditions were: accelerating voltage, 3 kV; ionizing current, 300 μA; ionizing energy, 23 eV; separator temperature, 240°.

Isotope Effect of d_3-AM——In order to investigate the isotope effect on the metabolism of AM, the urine was collected during periods of 0—2, 2—4, 4—6, 6—8, 8—12, 12—24 and 24—36 hr after the oral administration of an equimolar mixture of d_0-AM and d_3-AM (total 100 mg) to man. The amount of AM or each metabolite was calculated from the peak height ratio (d_3/d_0).

Human Experiment——Human subjects were the same healthy volunteers as used in the previous experiment. The experiment was peformed under similar conditions described in the previous paper on the study of the urinary excretion.[3] An aqueous solution of d_3-AM (100 mg) was orally administered to the subject before breakfast. The blood samples were taken at 0, 1, 2, 4, 6 and 8 hr after administration.

Assay Procedure——The procedure for the sample preparation is shown in Chart 2. A 2.0 ml portion of plasma sample was gently shaken in a stoppered 50 ml centrifuge tube with hand for 10 sec with 1 ml of phosphate buffer (pH 7.4) containing d_0-AM (5 μg), d_0-MAA (10 μg), d_0-AA (50 μg), d_0-FAA (5 μg) and d_0-AcAA (5 μg) as the internal standards. To the mixture was then added 1.8 g of $(NH_4)_2SO_4$ and the tube was shaken again. The mixed solution was extracted twice with 10 ml of chloroform. The combined extract was dried over anhydrous sodium sulfate and evaporated to dryness. A pyridine solution of the residue was used for the analyses of AM, MAA and AA. A portion of the pyridine solution was used for the analyses of FAA and AcAA after trimethylsilylation with 50 μl of BSA for 1.5 hr at 60°. Two μl of the sample solution was injected into the gas chromatograph under the conditions described in Table I.

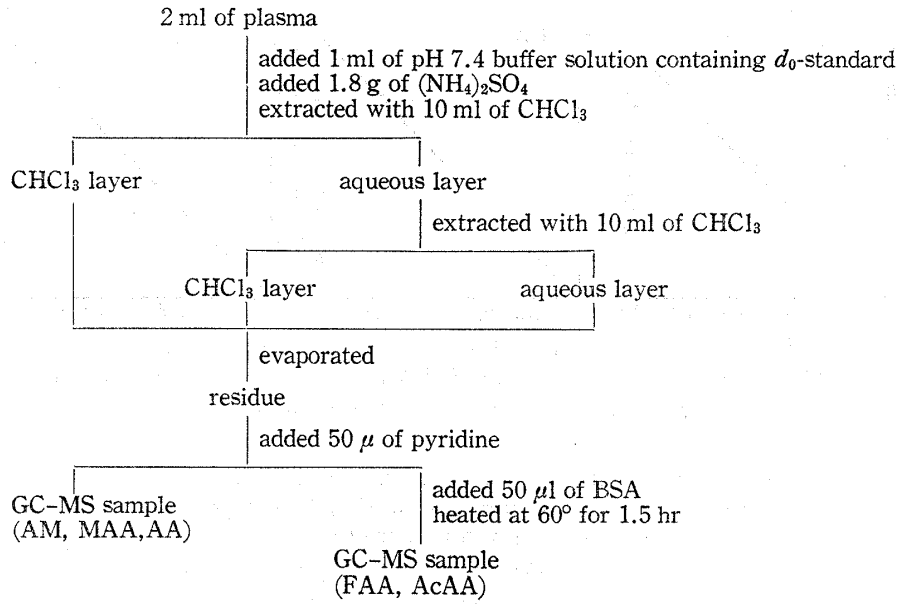

Chart 2. Preparation of Sample for Gas Chromatography-Mass Spectrometric Determination of AM and its Metabolites from Human Plasma

Results and Discussion

A reverse dilution method was used for the quantitative mass fragmentographic analysis of AM and its metabolites in plasma. Partial mass spectra adjacent to the molecular ion peak of each compound are shown in Fig. 1. In order to calculate the peak height ratio (d_3/d_0), one of the intensive peaks which do not overlap mutually with the peaks of labeled and non-labeled compounds should be selected. As shown in Fig. 1, it was the most appropriate to measure the intensity of the molecular ion peak for the analysis of each compound except MAA. In the case of MAA, the sample was diluted with an excess amount of d_0-MAA to minimize the interference of the fragment ion peak at m/e 217 (M^+ of d_0-MAA or M^+-3 of d_3-MAA). The standard curves of AM, FAA and AcAA were prepared. As shown in Fig. 2, the peak height ratio of AM, FAA or AcAA was directly proportional to its molar ratio (d_3/d_0).

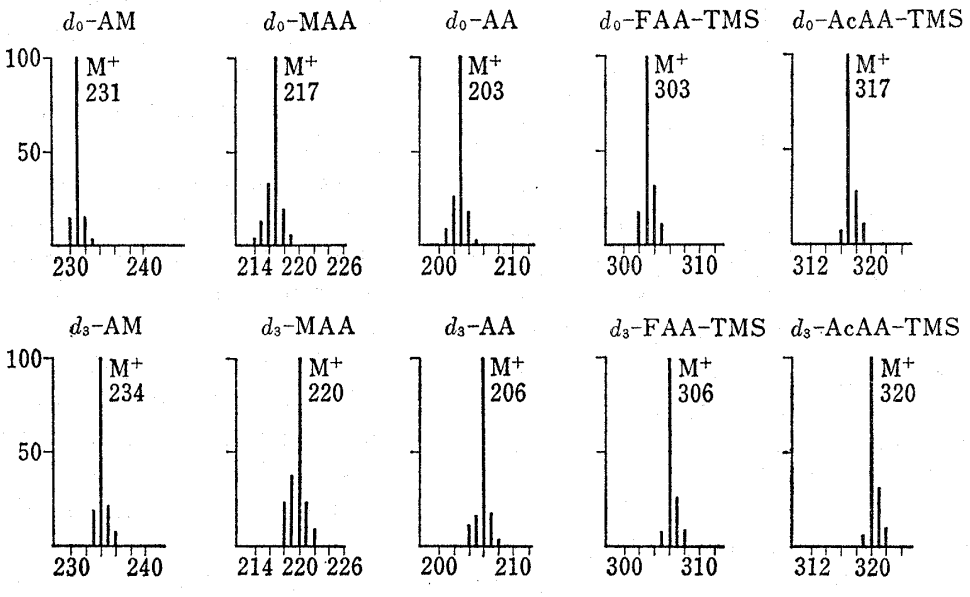

Fig. 1. Mass Spectra around Molecular Ion Peaks of d_0- and d_3- Compounds

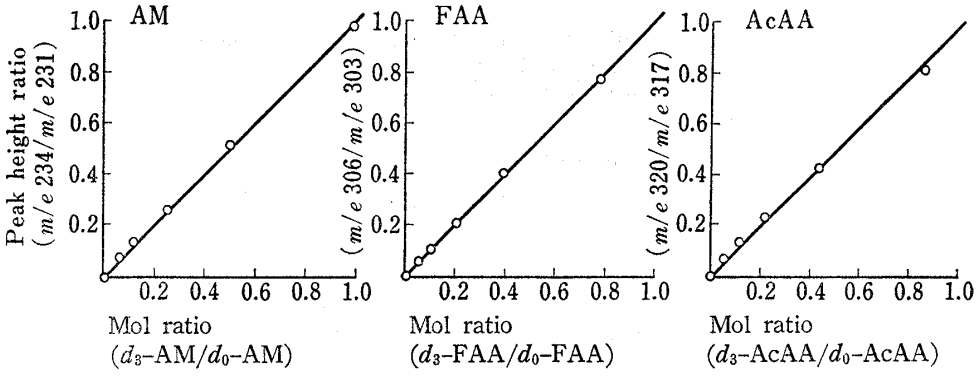

Fig. 2. Standard Curves of AM, FAA and AcAA

Table II. Excreted Amount Ratio of Undeuterated and Deuterated Metabolite after Administration of d_0-Aminopyrine and d_3-Aminopyrine Mixture (1 : 1)

Time (hr)	Excreted amount ratio (d_0/d_3)				
	AM	MAA	AA	FAA	AcAA
0— 2	1.01	1.02	—	—	—
2— 4	0.94	1.05	—	—	—
4— 6	—	1.01	—	0.98	1.03
6— 8	—	0.99	1.03	1.04	0.97
8—12	—	1.02	—	1.00	0.99
12—24	—	—	—	1.04	0.97
24—36	—	—	—	—	1.02
Ave.	0.98	1.02	1.03	1.02	1.00

In the human experiment, an aqueous solution containing an equimolar mixture of d_0-AM and d_3-AM (total 100 mg) was orally administered. The amount ratio (d_3/d_0) of AM and its metabolites in urine was nearly one in each case as shown in Table II. This indicates that the isotope effect was not observed for d_0- and d_3-compounds. It is then concluded that the metabolic behavior of d_3-AM in man is similar to that of d_0-AM. Therefore, 100 mg of d_3-AM in an aqueous solution was administered to man in order to measure the plasma concentration. Double ion monitering mass fragmentograms of the plasma extract were shown in Fig. 3. The plasma concentration was also calculated from the peak height ratio of d_0- and d_3-compounds.

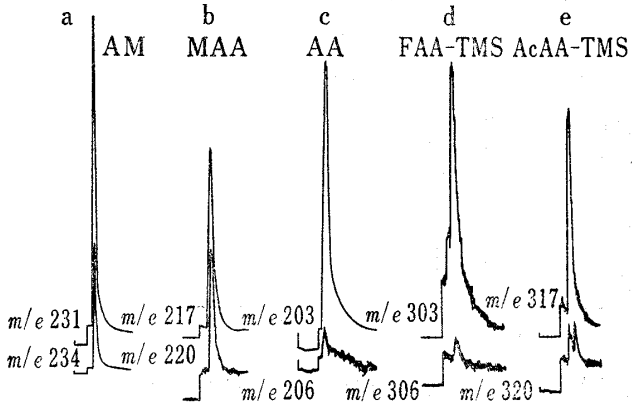

Fig. 3. Mass Fragmentogram of Plasma Extract after Oral Administration of d_3-Aminopyrine

a: 1% OV-60 190°, b: 1% XE-60 195°, c: 1% XE-60 200°, d: 1.5% OV-17 230°, e: 1.5% OV-17 230°.

The metabolic pattern of AA acetylation was classified into two types according to the urinary excretion data described in the previous paper;[3] (i) excretion of total metabolites of more than 50% to the dose and a large amount of AcAA more than 90% to the total amount of metabolites (*e.g.* subject K.M.), (ii) excretion of AcAA in less than 30% of the total amount of metabolites and a larger amount of AA (*e.g.* subject T.G.). As a similar

tendency described above was noticed in the plasma concentration of all the subjects, two subjects, K.M. and T.G. were selected as a representative of each group to appear in Fig. 4. The results of blood level examination demonstrated a marked individual difference not only in the urinary excretion but also in the plasma level. From the plasma concentration–time curve shown in Fig. 4 and Fig. 5, it was found that in the elimination rate or demethylation rate from AM to MAA, the rate of K. M. was more rapid than that of T. G. It seems that

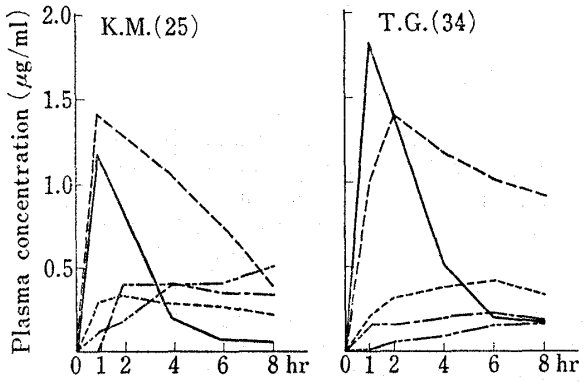

Fig. 4. Plasma Concentration of AM and Its Metabolites after Oral Administration of d_3-AM

——: MAA ——: AM ——: FAA
——: AA ——: AcAA

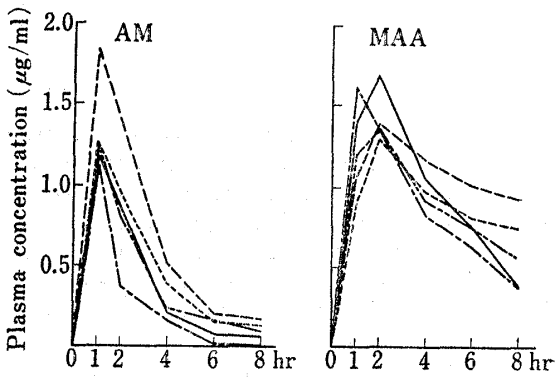

Fig. 5. Plasma Concentration of AM and MAA after Oral Administration of d_3-AM

——: T.G. ——: O.T. ——: N.T.
——: K.M. ——: S.I.

the distribution of AM into plasma takes place immediately after the oral administration and reach the maximum within 1 hr following MAA formation. A remarkable difference between K. M. and T. G. existed in the acetylation of AA to AcAA. In the case of T. G., the amount of AcAA in plasma was very small, whereas a remarkable amount of AA was detected through the experiment. On the contrary, K. M. showed a higher concentration of AcAA in plasma from the initial stage. A similar tendency was observed in the urinary excretion of AM or AA. In the case of FAA, it appeared in plasma of both subjects at a relatively early stage in definite amounts without significant individual differences.

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