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In Vivo Formation of 4-Formylaminoantipyrine as a New Metabolite of Aminopyrine. I

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The route of *in vivo* formation of 4-formylaminoantipyrine which is a new metabolite of aminopyrine was examined. As a result of examination by gas chromatography–mass spectrometry and ^{13}C-NMR after oral administration of ^{13}C-labeled aminopyrine to man and experimental animals, formylamino group was formed by oxidation of N-methyl side chain of aminopyrine.

4-Formylaminoantipyrine (FAA) has been detected in the urine of men, rats, guinea pigs and rabbits as a new type of metabolite after oral administration of aminopyrine (AM) solution.[2–4] Except for our reports, formamido compounds like FAA do not appear to have been described as metabolites of drugs with N-methyl group in man's urine. But, only one report about the formyl conjugation of aromatic amines was published by Boyland, *et al.*[5] According to the report, 2-formylamido-1-naphthyl hydrogen sulfate was excreted by dogs and rats dosed with 2-naphthylamine or with 2-amino-1-naphthyl hydrogen sulfate.

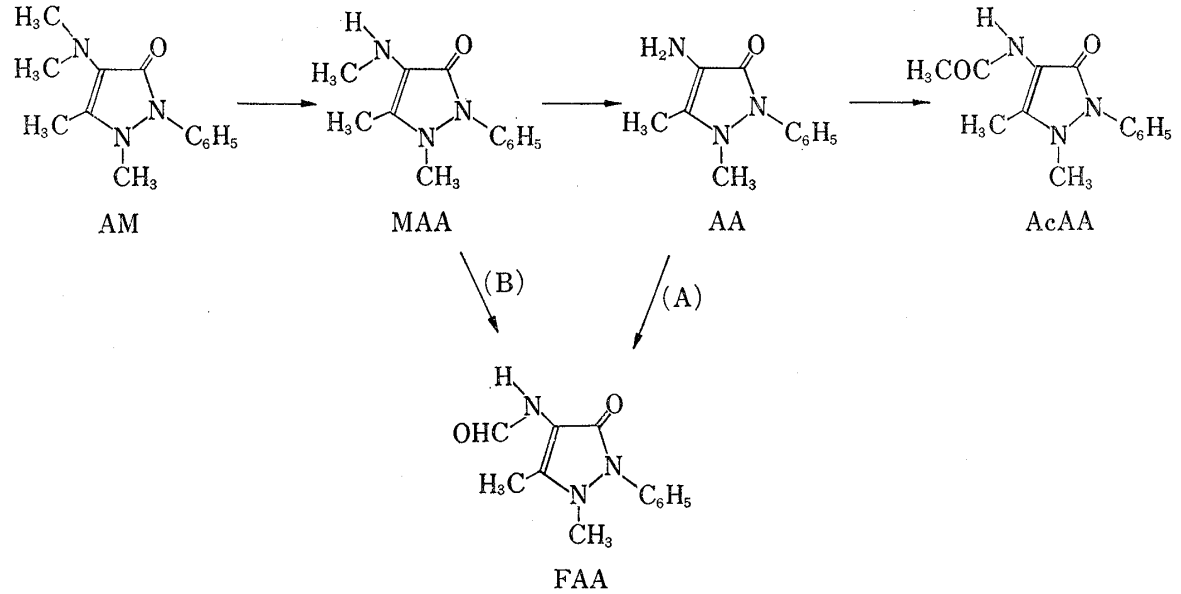

Chart 1. *In Vivo* Formation Route of FAA

Thus, in order to examine the mechanism of FAA formation in mammal, especially in man, we assumed two kinds of metabolic pathways, route A and B, as shown in Chart 1. At first, route A which is formyl conjugation of 4-aminoantipyrine (AA) was considered

1) Location: *Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan*; *a*) To whom communications should be directed.
2) S. Iguchi, T. Goromaru, and A. Noda, *Chem. Pharm. Bull.* (Tokyo), **23**, 932 (1975).
3) S. Iguchi, T. Goromaru, A. Noda, and N. Tsubone, *Chem. Pharm. Bull.* (Tokyo), **23**, 1889 (1975).
4) T. Goromaru, A. Noda, K. Matsuyama, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1376 (1979).
5) E. Boyland and D. Manson, *Biochem. J.*, **99**, 189 (1966).

to be the probable mechanism of FAA formation. On the contrary, it is also possible that AM is N-demethylated first and the formylamino group is formed by oxidation of remaining N-methyl group *via* route B.

Recently, it was clarified that FAA was formed *via* route B as a result of examination by gas chromatography–mass spectrometry (GC–MS) and ^{13}C-NMR after oral administration of ^{13}C-labeled AM to men and experimental animals. The details are described in this paper.

Materials and Methods

Chemicals——AM, FAA, 4-methylaminoantipyrine (MAA) and 4-acetylaminoantipyrine (AcAA) were obtained by the same methods in the previous papers.[2-4] Synthesis of AM with ^{13}C-labeled dimethylamino group (^{13}C-AM): 4-Aminoantipyrine (1.4 g), ^{13}C-labeled methyl iodide (2.5 g, purchased from BOC Ltd., 92%), potassium hydroxide (0.9 g) and methanol (2 ml) were mixed in a sealed tube under cooling. The mixture was heated in a boiling bath for 16 hr. After cooling, the tube was opened and the mixture was diluted with methanol. The solution was evaporated *in vacuo* to obtain solid residue. Then column chromatography on Al_2O_3 was performed. The compound separated from benzene eluate was identified as ^{13}C-AM on the basis of elementary analysis, mass spectrum and ^{1}H-NMR spectrum (Fig. 2 and 3). White needles from ligroin, mp 106—108°. *Anal.* Calcd. for $^{13}C_2{}^{12}C_{11}H_{17}ON_3$: C, 67.50; H, 7.41; N, 18.17. Found: C, 67.90; H, 7.44; N, 18.04. Mass Spectrum *m/e*: 233 (M^+).

Administration of 4-Aminoantipyrine (AA)——An aqueous solution of AA was administered orally in the morning after 12 hrs' fast as follows; 44 mg/body weight to man, 44 mg/kg to rabbit, 53 mg/kg to rat and 44 mg/kg to guinea pig, respectively.

Administration of ^{13}C-AM——^{13}C-AM was administered orally with an equivalent mole of non-labeled AM in aqueous solution. The amount of AM mixture (labeled: non-labeled = 1 : 1) administered was as follows; 100 mg/body weight to man, 25 mg/kg to rabbit and 50 mg/kg to guinea pig, respectively

Sample Collection and Assay Procedure——The experiments about men[4] and animals[3] were performed under the same conditions already described. The aliquots of the 0 to 24 hr urine samples from men and animals were adjusted to pH 6.0 with hydrochloric acid or sodium hydroxide to extract with chloroform, respectively. Each extract was dehydrated with anhydrous sodium sulfate, and evaporated to dryness. Just before GC–MS, trimethylsilylation was performed by the same method in the previous paper.[3]

Gas Chromatography–Mass Spectrometry (GC–MS)——JMS-D100 mass spectrometer was equipped with JGC-20 K gas chromatograph. A glass column (1 m × 2 mm inner diameter) containing 1.5% OV-17 on Shimalite W (80—100 mesh) was used. Temperature of the injection port was 250°, while the column oven was 220°. Mass spectrometer conditions were as follows; accelerating voltage, 3 kV; ionizing current, 300 μA; ionizing energy, 23 *e*V; separator temperature, 260°; ion multiplier gain, 1.3 kV.

Mass Fragmentography——^{13}C-FAA was detected by mass fragmentography as shown in Fig. 6. Two fragment ion peaks at *m/e* 303 (M^+, non-labeled FAA-TMS) and 304 (M^+, ^{13}C-FAA-TMS) were utilized for analysis.

Results and Discussion

In order to discuss route A, AA was administered orally to men, rabbits, guinea pigs and rats, respectively. But the unchanged AA and/or AcAA were observed as main metabolites, while any amount of FAA could not be detected by GC (Fig. 1). From this result, route A was denied, and route B was considered to be the probable route of FAA formation.

Secondly, in order to prove route B, the oxidation of N-methyl group in the side chain of AM, there is only one method in which ^{13}C- or ^{14}C-labeled compound is used as a tracer. It is the best way to utilize the stable isotope (^{13}C) which is acknowledged to be safe for animals[6,7] and men[8] unless high dose. Especially in the case of ^{13}C-AM, the administration to men had been reported.[9] Therefore, ^{13}C-AM was synthesized to examine whether ^{13}C-FAA is excreted

6) C.T. Gregg, Annual Report of the Biological and Medical Research Group, 1971, Los Alamos Scientific Labo. Report LA-4923-PR Los Alamos, New Mexico, 1973.
7) D. Sammuel, Proceedings of the First International Conference on Stable Isotope inChemistry, Biology and Medicine, editted by P.D. Klein and S.V. Peterson, U.S.A. E.C., 1973, pp. 203—209.
8) N.A. Matwiyoff and D.G. Otto, *Science*, **181**, 1125 (1973).
9) Reported by W.W. Shreeve in the Symposium of Stable Isotope, Oct. 9, 1974, Tokyo.

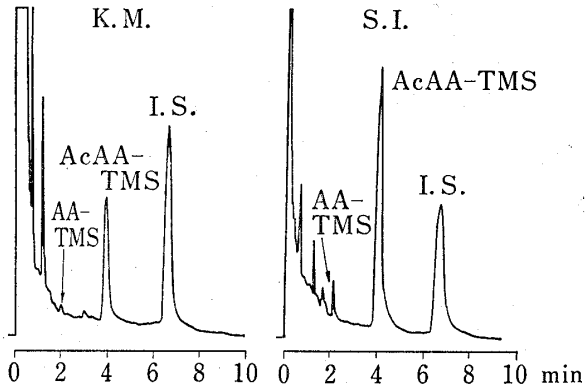

Fig. 1. Gas Chromatogram of Urine Extract after Administration of 4-Aminoantipyrine

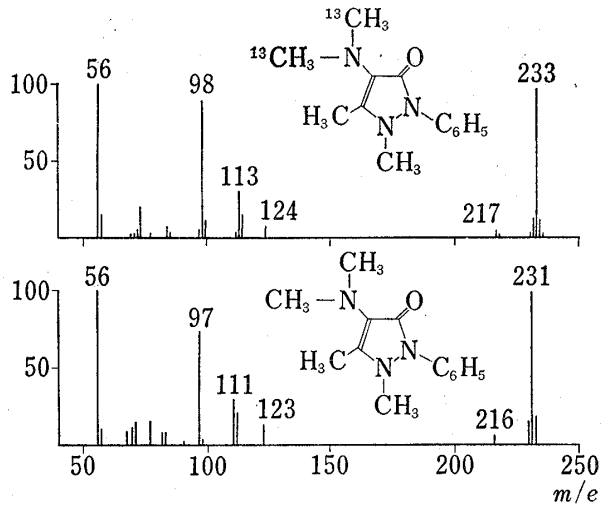

Fig. 2. Mass Spectra of ^{12}C-Aminopyrine and ^{13}C-Aminopyrine

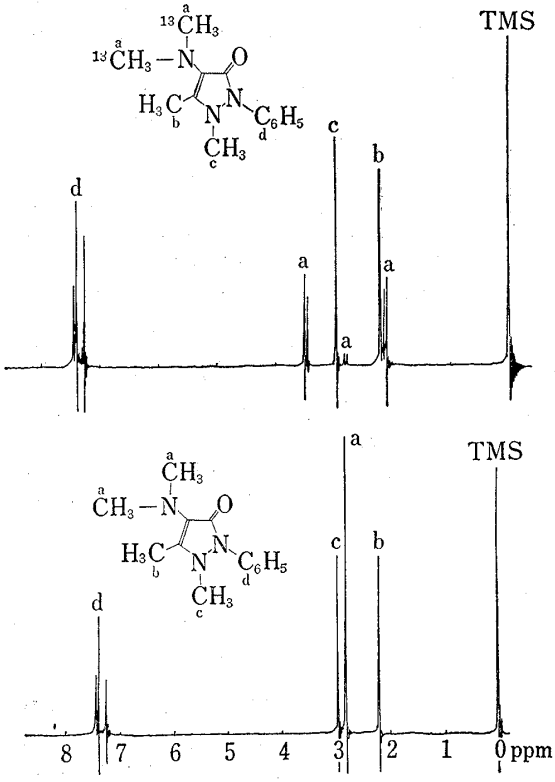

Fig. 3. ^{1}H-NMR Spectra of ^{12}C-Aminopyrine and ^{13}C-Aminopyrine

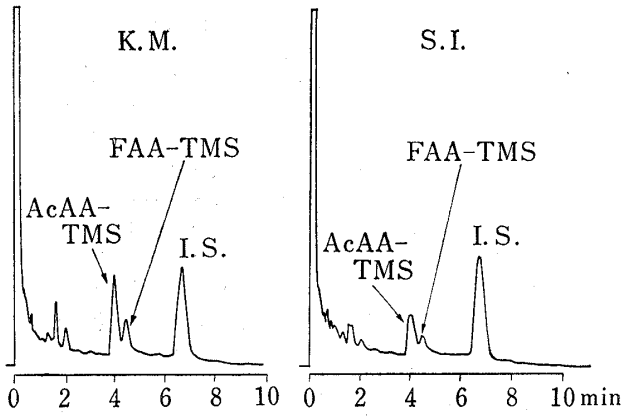

Fig. 4. Gas Chromatogram of Urine Extract after Administration of ^{12}C-Aminopyrine and ^{13}C-Aminopyrine Mixture

or not after administration of ^{13}C-AM. The structure of synthesized ^{13}C-AM was determined by the elementary analysis, mass spectrum and ^{1}H-NMR spectrum (Fig. 2 and Fig. 3). The purity of ^{13}C-AM was about 93% from the peak area of it's NMR chart.

Then the mixture of ^{13}C-AM[10] and non-labeled AM (1:1) was administered orally to men (S.I., K.M.[11]), rabbits and guinea pigs in an aqueous solution. The metabolites during 24 hr were extracted with chloroform from the urine adjusted to pH 6. Just before GC–MS, trimethylsilylation was performed with whole samples. The relative amount of FAA was detected from the extract of man's urine on gas chromatogram as shown in Fig. 4.

According to the mass spectra in Fig. 5, the urinary extract by guinea pigs gave a stronger ion peak at *m/e* 304 which corresponds to M^+ ion peak of ^{13}C-FAA-TMS than that of non-labeled

10) A half amount of the dose throughout the series of this experiment was used to minimize the amount of stable isotope.

11) Two of authors participated in this experiment.

FAA-TMS (authentic sample). The same results were obtained from the urinary extracts of men and rabbits.

The fragmentography at *m/e* 303 and 304 was also performed. If it is assumed that ^{13}C-AM shows the same metabolic behavior as non-labeled AM *in vivo*, *i.e.* ^{13}C isotope effect is not observed, ^{13}C-FAA should be excreted with an equivalent mole of non-labeled FAA after administration of the mixture of ^{13}C-AM and non-labeled AM (1: 1). The results in expectation were obtained. It was demonstrated obviously in Fig. 6 that ^{13}C-FAA was formed and excreted as one of the oxidative metabolites of AM.

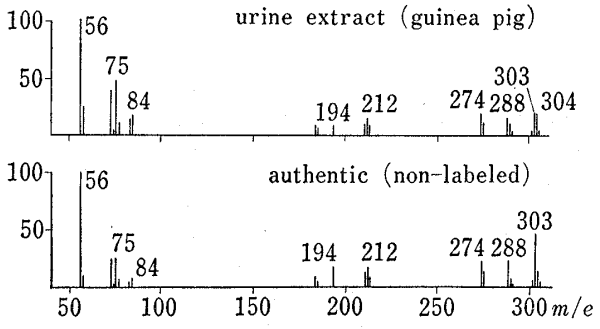

Fig. 5. Mass Spectra of FAA-TMS

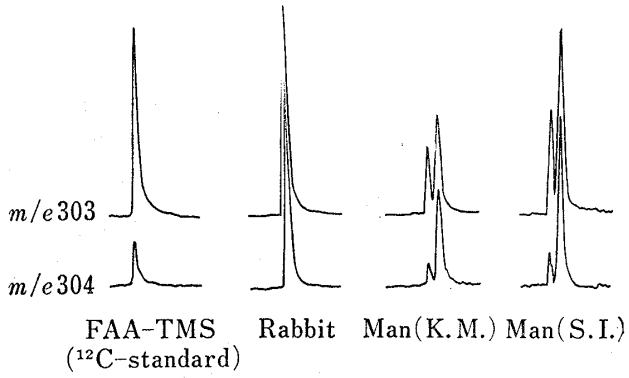

Fig. 6. Mass Fragmentogram of Urine Extract after Administration of ^{12}C-Aminopyrine and ^{13}C-Aminopyrine Mixture

Furthermore, the measurement of ^{13}C-NMR was performed with man's urinary extract. Comparing the spectrum of the extract with that of non-labeled FAA (authentic sample), only one peak which depends on $-NH^{13}CHO$ at 4-position of pyrazole ring appeared markedly at 161.3 ppm after 5643 times scanning in the case of the extract (Fig. 7 and 8).

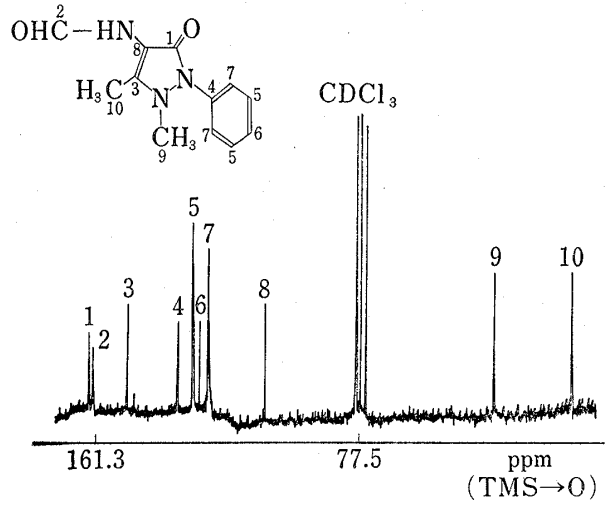

Fig. 7. ^{13}C-NMR Spectrum of 4-Formylamino-antipyrine in $CDCl_3$

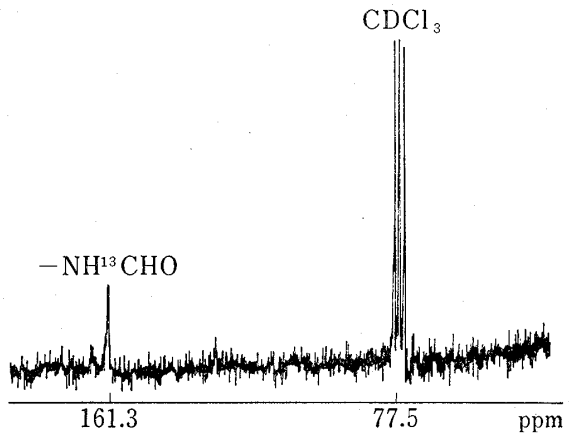

Fig. 8. ^{13}C-NMR Spectrum of Urine Extract after Administration of ^{12}C-Aminopyrine and ^{13}C-Aminopyrine Mixture in $CDCl_3$

From the results mentioned above, it was proven this time that formylamino group in FAA was not formed by formyl conjugation of AA, but by the oxidation of N-methyl group in the side chain of AM *via* route B. It was also noticed that a new type of metabolite, FAA, was a very stable compound which was relatively soluble in water as AcAA. However, the physical and chemical properties of FAA and its biological activities will be reported soon.

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