Communications to the Editor

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A New Metabolite of Aminopyrine (Aminophenazone) in Man, 4-Formylaminoantipyrine

The metabolism and excretion of aminopyrine in man were examined after oral administration. On the gas chromatogram, the extract from urine showed an unknown peak sometimes which seems to be a new metabolite. This new metabolite was identified as 4-formylaminoantipyrine by gas chromatography–mass spectrometry comparing with the synthesized authentic sample.

It has been about eight decades since aminopyrine was introduced to clinical use and it is still inserted in the present Japanese Pharmacopoeia and being used frequently as an analgesic-antipyretic drug. However, it is surprising that only a few studies on its metabolism and excretion in man have been reported.[1] As for the quantitative analysis of metabolites, the noticeable study by thin-layer chromatography (TLC) was performed by Fleischmann, *et al.*[2]

It has been clarified from the reports mentioned above that three main metabolites, 4-methylaminoantipyrine (MAA), 4-aminoantipyrine (AA) and 4-acetylaminoantipyrine (AcAA), are found in man's urine with some other metabolites, such as 4-hydroxyantipyrine, rubazonic acid and methylrubazonic acid, but it is generally pointed out that the ratio of total amounts of metabolites including unchanged aminopyrine to the given dose is not so

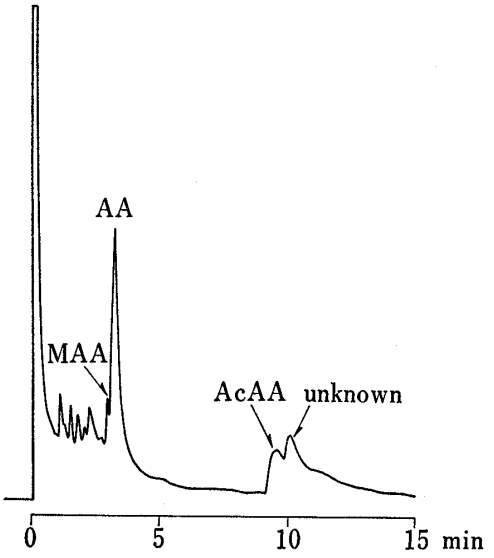

Fig. 1. Gas Chromatogram of Urine Extract after Oral Administration of Aminopyrine in Aqueous Solution (Subject: N.T., 12 hr)

Conditions: 1.5% OV-17 on Chromosorb W, AW, DMCS (80—100 mesh), 3mm × 2m, glass column, column temp: 225°, injection port temp: 250°, N_2: 20 ml/min, HFID, instrument: GC-4BM-PF

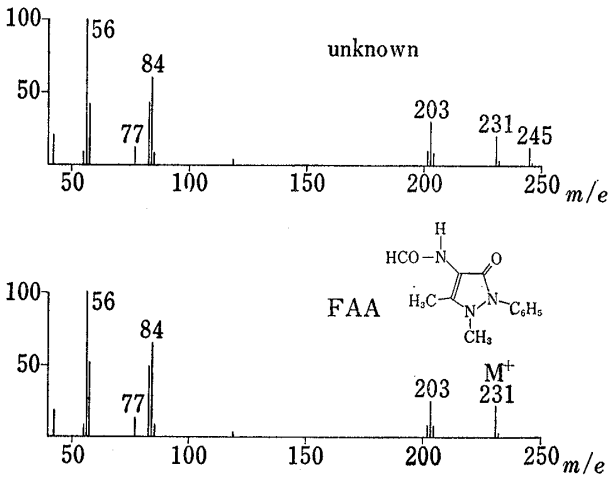

Fig. 2. Mass Spectra of Unknown Metabolite and FAA (Authentic Sample)

Mass spectra were measured by GC-MS.
GC: 4% OV-17 on Gaschrom Q (100—120 mesh) 2 mm × 1 m, 220°
MS: ionizing energy: 25 eV, ionizing current: 300 μA accelerated volt: 3 kV, separator temp: 240°, instrument: JMS-D100

1) B.B. Brodie and J. Axelrod, *J. Pharmacol. Exper. Therap.*, **99**, 171 (1950); J. Halberkann and F. Fretwurst, *Z. Physiol. Chem.*, **285**, 97 (1950); M. Jaffe, *Ber.*, **34**, 2737 (1901); J. Večerkovă, B. Kakáč, B. Večerek and M. Ledvina, *Pharmazie*, **22**, 30 (1967).
2) R. Gradnik and L. Fleischmann, *Pharm. Acta Helv.*, **48**, 181 (1973).

high as expected. Thus, it is suggested that there are many problems to be solved about the metabolism and the excretion of aminopyrine in man.

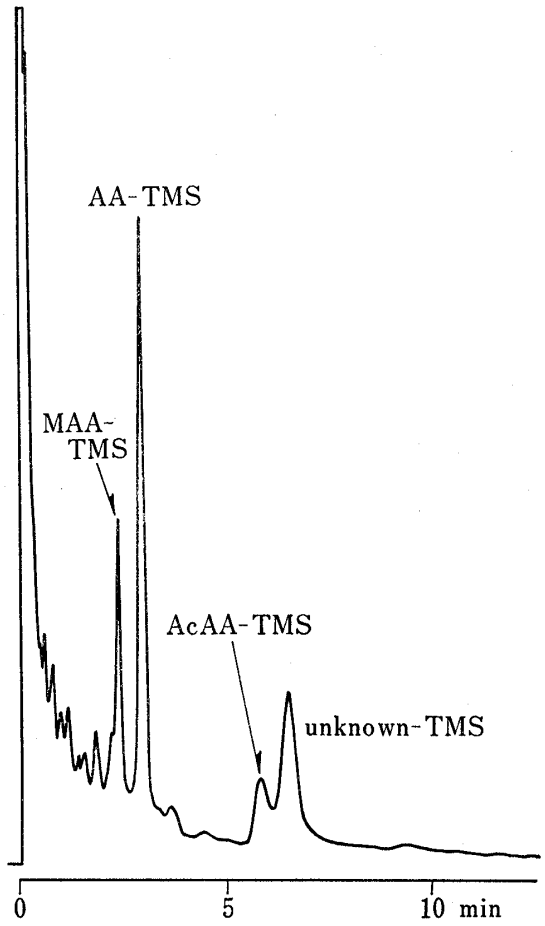

Fig. 3. Gas Chromatogram of TMS Derivatives of Urine Extract

GC conditions were the same as those in Fig. 1. The sample was trimethylsilylated (TMS) using N, O-bis (trimethylsilyl) acetamide (BSA) in pyridine at 60°

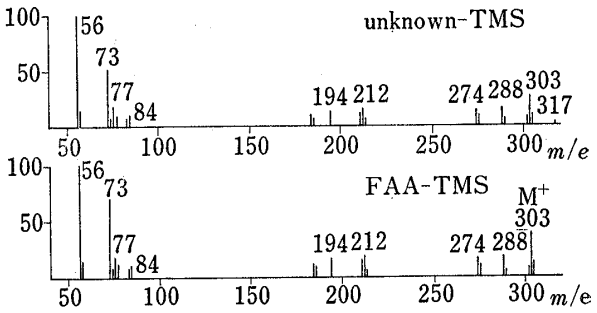

Fig. 4. Mass Spectra of TMS Derivatives of Unknown Metabolite and FAA (Authentic Sample)

GC-MS conditions were the same as those shown in Fig. 2.

This time, quantitative analysis on the metabolites in man's urine were undertaken with both of the high sensitive gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), in order to clarify the state of metabolism and excretion of aminopyrine in more detail. As a result of the experiment, a new interesting metabolite, 4-formylaminoantipyrine, was found in man's urine. In this communication, these results are described briefly.

From the urine of twelve healthy adult man subjects following oral administration of 100 mg of aminopyrine on fasting, the metabolites were extracted with chloroform, separated and estimated quantitatively by GC. The extract from four men's urine showed an unknown peak which seems to be a new metabolite just after the peak of AcAA on the gas chromatogram (Fig. 1). As shown in Fig. 1, these two peaks could not be separated completely by GC. Therefore, GC–MS was adopted to measure the mass spectrum of the new metabolite (Fig. 2). The ion peak at *m/e* 245 was based on AcAA as a contaminant, while the ion peak at *m/e* 231 was considered to be the molecular ion of the new metabolite, since AcAA did not have the fragment ion at 231. Thus, a plausible derivative of aminopyrine with molecular weight 231, 4-formylaminoantipyrine (FAA), was synthesized according to the method reported by Kondo, *et al.*[3] in order to compare the gas chromatogram and the mass spectrum with those of the unknown metabolite. As a result, the metabolite was proved to be FAA itself, since the retention time on GC and the mass spectrum of FAA were coincident with those of the unknown metabolite completely. Further identification performed with their trimethylsilylated derivatives by GC and GC–MS was also successful (Fig. 3 and Fig. 4).

It seems that FAA is a noticeable metabolite of aminopyrine, since the detection of formyl type of compound in man's urine as a metabolite of drug is the first time, and moreover the ratio of the metabolite to the given dose of aminopyrine reached around 20% sometimes.

3) R. Kondo and N. Kikuchi, *Eisei Shikensho Hokoku*, **44**, 22 (1934).

The studies about the mechanism of FAA formation and its biological activity are going on now. The details of this study will be reported near future.

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Arnottinin: Structural Establishment by Chemical Correlation with Osthenol

The structure of arnottinin (**1**) was established by the transformation of osthenol (**2**) to it.

In the previous paper,[1] we reported the isolation of arnottinin (**1**), a new phenolic coumarin from the xylem of *Xanthoxylum arnottianum* Maxim. (Japanese name: Iwa-Zansho). We wish to report here its structural establishment by means of chemical correlation of arnottinin (**1**) with osthenol (**2**).

Arnottinin (**1**) is obtained as colorless needles, mp 191—193°, $C_{14}H_{14}O_4$. It shows the following spectral data. IR ν_{max}^{Nujol} cm^{-1}: 3290, 3100 (OH), 1693 (C=O); NMR (CD_3OD) δ: 1.74 (3H, s, vinyl CH_3), 3.55 (2H, d, J=7.0 Hz, $ArC\underline{H}_2CH{=}C$), 4.34 (2H, s, $C{=}C(C)C\underline{H}_2OH$), 5.35 (1H, dif. t, J=7.0 Hz, $CH_2C\underline{H}{=}C$), 6.14 (1H, d, J=9.6 Hz, C_3–H), 6.77 (1H, d, J=8.6 Hz, C_6–H), 7.29 (1H, d, J=8.6 Hz, C_5–H), 7.80 (1H, d, J=9.6 Hz, C_4–H); $[\alpha]_D^{20}$ ±0° (c=0.65, EtOH). Inspection of these spectral data with regard to the general biogenetic pathway of coumarins allowed to depict the structure of arnottinin with the structure **1**, its geometrical isomer (**3**), or the structural isomer (**4**). As arnottinin, however, showed no optical rotation we could exclude the structure **4** from the possible structures. Unfortunately, the yield of arnottinin from the plant (*ca* 0.0018%) is so scarce that we could not establish its structure by chemical means. Therefore, we attempted to chemically correlate this coumarin with osthenol (**2**).

In 1973, Steck[2] reported that hydrolysis of macrocarpin (**5**) gave a coumarin (**6**), the structure of which was supposed to correspond to the methyl ether of arnottinin. Therefore, we first aimed at synthesizing this coumarin (**6**) as a model experiment.

In 1972, for allylic oxidation of olefins by SeO_2, Sharpless, *et al.*[3] proposed a mechanism which suggested an initial ene addition[4] of Se^+–O^- moiety followed by dehydration and [2,3]-sigmatropic shift of the resulting allylseleninic acid. This mechanism suggests that oxidation of osthol (**7**) with SeO_2 should give the coumarin (**8**) having an E-configuration, because the ene reaction takes place at the least substituted allylic position preferentially and [2,3]-sigmatropic rearrangement of the resulting allylseleninic acid derivative (**9**) should proceed through the process giving an E-configuration product.

1) H. Ishii, K. Hosoya, T. Ishikawa, and J. Haginiwa, *Yakugaku Zasshi*, **94**, 309 (1974); H. Ishii, K. Hosoya, T. Ishikawa, E. Ueda, and J. Haginiwa, *ibid.*, **94**, 322 (1974).
2) W. Steck, *Phytochemistry*, **12**, 2283 (1973).
3) K.B. Sharpless and R.F. Lauer, *J. Am. Chem. Soc.*, **94**, 7154 (1972); D. Arigoni, A. Vasella, K.B. Sharpless, and H.P. Jensen, *ibid.*, **95**, 7917 (1973).
4) H.M.R. Hoffmann, *Angew. Chem. Intern. Ed. Engl.*, **8**, 556 (1969).