is preponderant component accompanied by smaller amounts of α-furanose (Va) and α-pyranose (Vc) (Table II). The CMR spectra of D-*gluco*-heptulose (VI) and D-*manno*-heptulose (VII), whose GC–MS data of TMS ether exhibit pyranose only, and whose stabler conformation (α-pyranose, Cl) has no *cis* correlation of axial substituents, indicate that both of them form almost solely α-pyranose (VIc and VIIc), in accord with the observation by Perlin, *et al.*[16] The preponderance of furanose in V would then be attributable to the interaction between 1,3-diaxial hydroxyl groups in pyranose, and the high ratio of β-furanose (Vb) to α-furanose (Va) is consistent in the structural features with the Ia formation as the main component in equilibrated I. Analogous correlations of the equilibrium data of anomers with structures of 3-hexuloses are also observed (Table II).

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17) A.S. Perlin, P.Herve du Penhoat, and H.S. Isbell, "Carbohydrates in Solution," American Chemical Society, Washington, D. C., 1973, pp. 39—50.

[Chem. Pharm. Bull. 24(12)3229—3231(1976)]

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Formation of 4-Formylaminoantipyrine as a New Metabolite of Aminopyrine. II.[1] Enzymatic Demethylation and Oxidation of Aminopyrine and 4-Monomethylaminoantipyrine

4-Formylaminoantipyrine, a new metabolite of aminopyrine, was formed on the incubation with rat or rabbit liver slices, or with the microsomal fraction of rabbit liver.

In the previous papers,[2–4] we reported the detection of 4-formylaminoantipyrine (FAA) as a new metabolite of aminopyrine (AM) in the urine after the oral administration to man, rabbit, guinea pig or rat. Moreover, the formation route of FAA was clarified with ^{13}C-labeled AM by gas chromatography–mass spectrometry (GC–MS) and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) as shown in Chart 1. The observation mentioned above is very valuable, since neither report concerning the metabolite FAA itself nor such type of oxidative metabolite with formylamino group has been published.

The present communication describes further studies on the formation of FAA from AM or 4-monomethylaminoantipyrine (MAA) in liver system. The liver removed from 24 hr fasted animal immediately after stunning and slaughtering by exsanguination was used to obtain liver slices and microsomal fraction.

1) Part I: A. Noda, T. Goromaru, N. Tsubone, K. Matsuyama, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1502 (1976).
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 (1976).

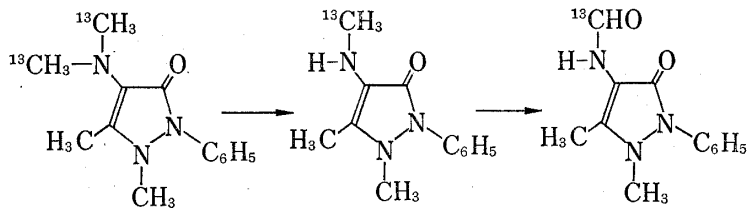

Chart 1

Initially, AM or MAA (30 μmoles) dissolved in 90 ml of 0.1M Krebs-Ringer buffer (pH 7.4) was incubated at 38° under the stream of oxygen containing 5% CO_2 with liver slices prepared mannually from a male albino rabbit (3 kg). The reaction was terminated after 60 min by the addition of 10N HCl (9 ml) under ice cooling. The reaction mixture was centrifuged at 3000 ***g*** for 10 min. The supernatant was adjusted to pH 6.0 with 20% NaOH and extracted twice with an equal volume of $CHCl_3$. The residue obtained after evaporation of the solvent from the combined extracts was trimethylsilylated in the standard manner, and analyzed by GC–MS reported in the previous paper.[4] Consequently, the formation of FAA from AM or MAA was observed in the incubation mixture with rabbit liver slices as shown in Fig. 1.

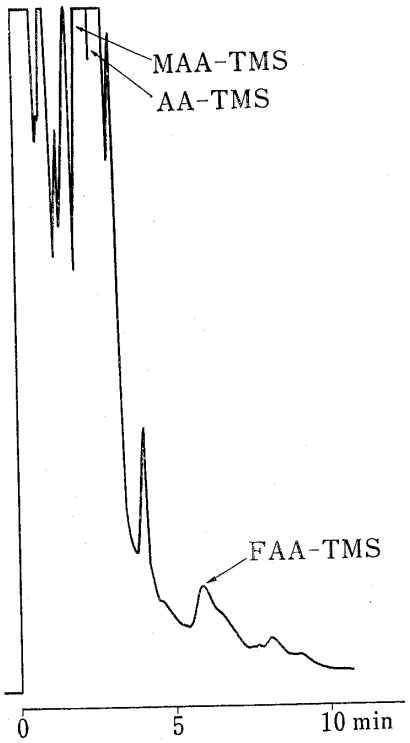

Fig. 1. Gas Chromatogram of Trimethylsilylated Derivatives of Chloroform Extract from the Incubation Mixture of MAA with Rabbit Liver Slices

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

In the case of liver slices of male Donryu rat (250 g), only MAA was used as a substrate, since Brodie, *et al.* has reported that the demethylation of MAA proceeds about four times as rapidly as AM.[5] The experiment was performed under the same condition in the case of rabbit liver slices. The amount of FAA was too small to detect by GC. Therefore, the formation of FAA was proved by mass fragmentography to compare the peak heights at *m/e* 303 (FAA—TMS, M^+), 288 (M^+–CH_3) and 274 (M^+–CHO) with those of a standard sample as shown in Fig. 2.

Secondly, it was discussed whether FAA was formed from MAA on incubation with liver microsomal fraction or not. Microsomal fraction was prepared from the liver of male albino rabbit according to the usual procedure.[6] MAA (10 μmoles) was incubated at 37° with liver microsomes (from 2 g liver), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (30 μmoles), nicotinamide (100 μmoles) and $MgCl_2$ (100 μmoles) in 1.15% KCl solution (20 ml). The reaction was terminated after 10 min by the addition of 10N HCl (2 ml) under ice cooling. Thereafter, the sample for GC was prepared by the same way described in the case of liver slices. Although detection of FAA by GC was impossible, it was clarified by mass fragmentography that FAA was aerobically formed with rabbit liver microsomes in the presence of NADPH.

In 1955, Brodie, *et al.* reported that the demethylation of AM or MAA into 4-aminoantipyrine (AA) took place in rabbit liver homogenate, but about twice as much AM or MAA disappeared as could be accounted for by the appearance of AA or formaldehyde, indicating that another metabolic pathway for these substrates is present in liver system.[5] FAA forma-

5) B.N. La Du, L. Gaudette, N. Trousof, and B.B. Brodie, *J. Biol. Chem.*, 214, 741 (1955).
6) Y. Imai and R. Sato, "Seitaimaku Zikken Gijutsu," editted by T. Onishi, Nankodo, Tokyo, 1967, p. 151.

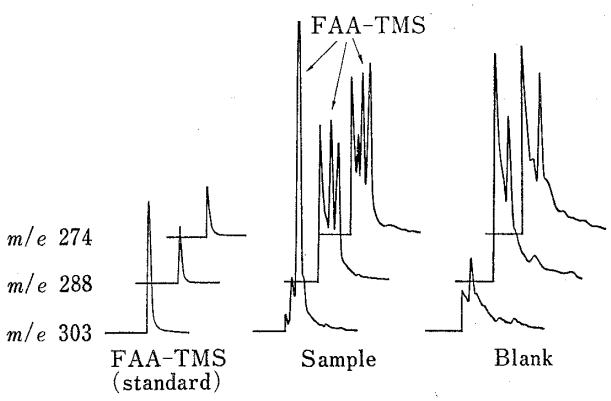

Fig. 2. Mass Fragmentogram of Trimethylsilylated Derivatives of Chloroform Extract from the Incubation Mixture of MAA with Rat Liver Slices

GC conditions were the same as those shown in Fig. 1.

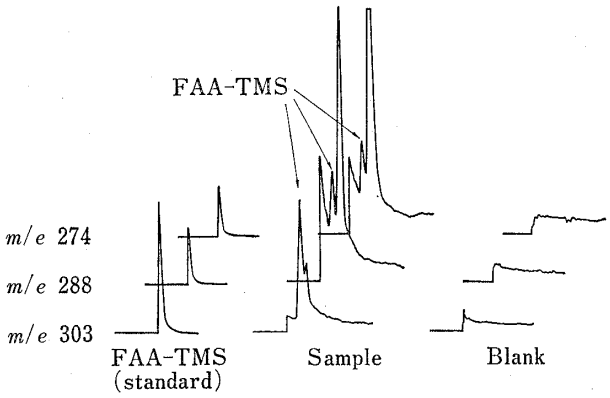

Fig. 3. Mass Fragmentogram of Trimethylsilylated Derivatives of Chloroform Extract from the Incubation Mixture of MAA with Rabbit Liver Microsomes

GC conditions were the same as those in Fig. 1.

tion might be one of the alternative pathways suggested by Brodie, *et al.* In order to solve the problem, the quantitative studies are in progress.

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