the chemical structure of the substrate but also the physical state. And it was suggested that the mixture of a series of single triglyceride was available for the investigation of substrate specificity of lipase for the chemical structure.

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New Sulfur-containing Metabolites of Caffeine

A new metabolite of caffeine in the urine of the horse, rabbit, rat, and mouse was isolated and identified as α-[7-(1,3-dimethylxanthinyl)] methyl methyl sulfoxide (II). Other two new metabolites were isolated from the urine of the mouse administered caffeine and identified as α-[7-(1,3-dimethylxanthinyl)] methyl methyl sulfide (I) and α-[7-(1,3-dimethylxanthinyl)] methyl methyl sulfone (III), respectively. The structural elucidation of these metabolites is described.

In general, it has been known that caffeine (1,3,7-trimethylxanthine) in man and experimental animals is metabolized to some methylated xanthines and methylated uric acids *via* two common metabolic reactions, the N-demethylation and the oxygenation of the C-8 carbon on the purine ring. Most of caffeine metabolites based on the reactions have already been detected and identified by many workers; however, some unidentified metabolites of caffeine have also been reported.[1] During studies on the metabolism of caffeine in the horse and other experimental animals,[2] we found and identified three new sulfur-containing metabolites of caffeine. This communication deals with the structural elucidation of these metabolites.

Caffeine was administered orally to horses and three species of experimental animals (mouse, rat, and rabbit) with each designed amount. Fourty-eight hour urine samples were collected and extracted with chloroform–methanol (9: 1) at pH 8.5. In the cases of horse and rabbit urine, a preliminary purification using XAD-2 resin columns were carried out prior to the solvent extraction. Unknown metabolites of caffeine in the urine extracts were isolated by means of thin-layer chromatography (TLC) and silica gel column chromatography, and then their structures were elucidated by using various analytical techniques.

A typical thin-layer chromatogram of the urinary extracts from mice after caffeine administration is shown in Fig. 1. Among seven zones on the chromatogram, four of them were identified as caffeine (*Rf* 0.71), theobromine (*Rf* 0.46), paraxanthine (*Rf* 0.23), and the mixture (*Rf* 0.12) of 3-methylxanthine and 7-methylxanthine by mass spectrometry (MS)[3]

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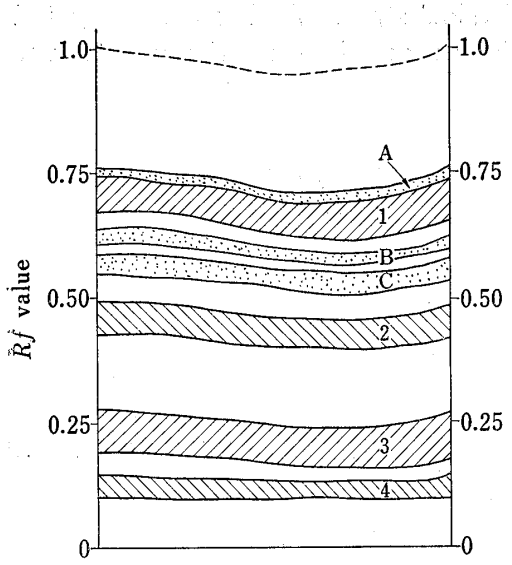

Fig. 1. A Typical Thin-Layer Chromatogram of the Urine Extracts from Mice after Caffeine Administration

development: successive runs in two solvent systems, first run: $CHCl_3$–MeOH–conc. NH_4OH (95:5:0.5), 5 cm; second run: $CHCl_3$–MeOH (95:5) –NH_3 vapor, 10 cm
zones: 1, caffeine; 2, theobromine; 3, paraxanthine; 4, the mixture of 3-methylxanthine and 7-methylxanthine; A, B, and C, unknown metabolites

and ultraviolet (UV) spectroscopy. The other zones corresponding to unknown metabolites (A: *Rf* 0.75, B: *Rf* 0.62, and C: *Rf* 0.57) gave also mass- and UV spectra (A: 275 mμ, B: 277 mμ, and C: 276 mμ in λ_{max}^{MeOH}) like methylated xanthines.

Metabolite C was found from the urine samples of horse, rabbit, and rat administered caffeine, respectively. It was isolated as crystals (mp 185—186°) from the horse and rabbit urine. The mass spectrum of the metabolite is shown in Fig. 2. In the spectrum, the molecular ion peak (M^+ 256) with a weak intensity and other characteristic peaks (*m/e* 240, 225, 193, *etc.*) were observed. The spectrum shows that the metabolite has a larger molecule than caffeine. The nuclear magnetic resonance spectrum revealed two new signals for methyl- and methylene groups (δ 2.54 and 5.48, in $CDCl_3$) instead of the 7-methyl group (δ 4.00) in the caffeine molecule. The infrared spectrum gave an absorption band at 1060 cm^{-1} (in $CHCl_3$) suggesting the presence of $>S\rightarrow O$, and this was also expected from the mass spectrum of the metabolite (see Fig. 2). From these analytical data, the metabolite C was considered to be α-[7-(1,3-dimethylxanthinyl)]-methyl methyl sulfoxide (II). The structure was confirmed by the synthesis of the compound II followed by its comparison with the isolated metabolite C.

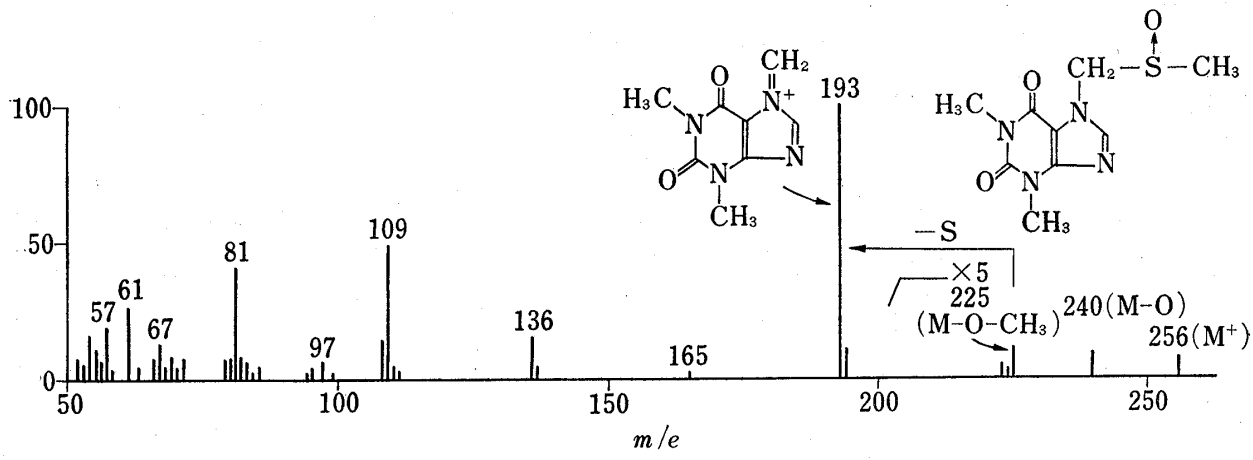

Fig. 2. Mass Spectrum of the Metabolite C

From the above results, the presence of the other metabolites related to the metabolite C was suggested; namely, two metabolites, a sulfide (I) and a sulfone (III). The mass spectrum of the metabolite A (M^+ 240) and the metabolite B (M^+ 272) showed that they corresponded to the compound I and the compound III, respectively. Then, we synthesized the compounds[4] and analyzed them by means of the MS, UV spectroscopy, TLC, and gas chromatography. All the analytical data of the synthetic compounds I and III were entirely identical with those of metabolites A and B, respectively.

4) These compounds (I and III) were synthesized by the method reported in a following literature. K. Onodera, S. Hirano, N. Kashimura, and T. Yajima, *Tetrahedron Letters*, **48**, 4327 (1965).

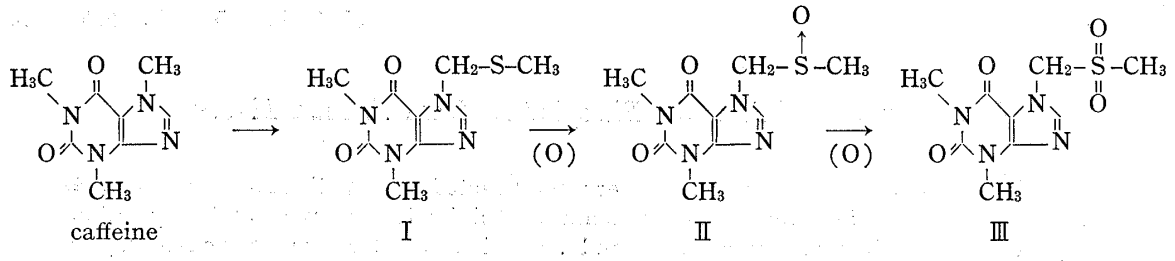

Fig. 3. A Proposed Metabolic Pathway of Caffeine

As described above, we found three new metabolites of caffeine and elucidated their structures. Recently, some metabolites with a thiomethyl group in their molecules have been found as the metabolites of carcinogenic agents, such as N-methyl-4-aminoazobenzene,[5] N-hydroxy-2-acetylaminofluorene,[6] and N-hydroxy-4-acetylaminobiphenyl,[7] and phenacetin.[8] The thiomethyl group in these metabolites is directly substituted to a ring proton of the aromatic amines, and that of the new metabolites of caffeine, however, is placed on the side chain of the purine ring. The substitution of thiomethyl group in the latter case could not be found in the literature. Therefore, it is very interesting to investigate the metabolism on the formation of the sulfur-containing metabolites of caffeine. A proposed metabolic pathway for caffeine is shown in Fig. 3.

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