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Analytical Studies on Mepirizole and Its Metabolites. II.[1] Identification of the Human Urinary Metabolites of Mepirizole with Stable Isotope Labeling[2]

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The metabolism of mepirizole (I) in human urine was studied by labeling the drug with a stable isotope, deuterium. An equimolar mixture of the deuterium labeled mepirizole (II) and its protium counterpart (I) was administered orally to a man. The metabolites were extracted from the pooled urine with CH_2Cl_2, treated with diazomethane and applied to GC-MS. The ion clusters in the mass spectra were used for detection of the urinary metabolites of I in man. By this technique, three metabolites, 1-(4-methoxy-6-methyl-2-pyrimidinyl)-5-methoxy-3-hydroxymethylpyrazole(III), 1-(4-methoxy-6-methyl-2-pyrimidinyl)-5-methoxypyrazole-3-carboxylic acid (IV) and 1-(4-methoxy-6-carboxy-2-pyrimidinyl)-5-methoxy-3-methylpyrazole (V) were identified in human urine.

The metabolism of mepirizole (I, 1-(4-methoxy-6-methyl-2-pyrimidinyl)-5-methoxy-3-methylpyrazole), an analgesic and anti-inflammatory agent, has already been studied in rats by using the radioisotope labeled compound.[4,5] Radioactive tracers are widely used for investigations of drug metabolism in experimental animals, but its utility for clinical studies is limited. Recently, much attention has been focussed on stable isotope tracers which are detectable by a combination of gas chromatograph (GC) and mass spectrometer (MS). This paper presents a qualitative method to identify the human urinary metabolites of mepirizole by using the stable isotope labeled compound. The metabolic pathways of mepirizole are also presented.

Materials and Methods

Materials——Mepirizole (I)[6] and its deuterium labeled compound (labeled at the methoxy group of the pyrimidine ring) (II)[1] were prepared as described previously.

Instruments and Conditions——GC-MS analyses were carried out on Japan Electron & Optics Laboratory JGC-20K gas chromatograph-JMS Ol-SG 2 mass spectrometer connected with JEC-6 spectrum computer. A column packed with 2% OV-17 on 80/100 mesh Gas Chrom Q in a silicated glass tube (2 m × 3 mm i.d.) was used under the following GC conditions; inlet pressure of carrier gas (He): 0.5 kg/cm², column temperature: 200→280° (programmed rate at 5°/min), injection temperature: 280°. Mass spectra were measured under the following conditions; ionizing energy: 75 eV, emission current: 200 μA, accelerating voltage: 10 kV, ion multiplier voltage: 2.5 kV, main slit: 100 μ.

Preparative thin-layer chromatography (preparative TLC) was carried out by using silica-gel plates (Merck Kieselgel HF_{254} containing fluorescent indicators, 0.5 mm thick, activated at 120° for 2 hr). The developing solvent used was benzene–acetone (8: 2). The metabolites were visualized as fluorescent spots by ultraviolet lamp.

Administration of the Drugs and Preparation of the GC-MS Samples of the Urine Extracts——An equimolar mixture (471 mg) of I and II was administered orally to a male volunteer, and for the following 24 hr, urine was collected. The urine (total volume, 1050 ml) was adjusted to pH 2 with conc. HCl and shaken

1) Part I: M. Sano and Y. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **23**, 209 (1975).
2) This work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan (1974), Sendai.
3) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
4) T. Akimoto, *Tokyo Jikeikai Medical J.*, **86**, 638 (1971).
5) E. Takabatake, R. Kodama, Y. Tanaka, R. Dohmori, H. Tachizawa, and T. Naito, *Chem. Pharm. Bull.* (Tokyo), **18**, 1900 (1970).
6) T. Naito, T. Yoshikawa, S. Kitahara, and N. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1467 (1969).

with *n*-hexane (300 ml). The separated aqueous layer was shaken three times with CH_2Cl_2 (each 400 ml) to extract the metabolites, and the combined CH_2Cl_2 solution was dried with Na_2SO_4 and evaporated to dryness. To the oily brown residue (470 mg), was added EtOH (2 ml) and the colorless crystalline powder thus formed was isolated by filtration. The crystalline metabolite was identified to be 1-(4-methoxy-6-methyl-2-pyrimidinyl)-5-methoxypyrazole-3-carboxylic acid (IV) (180 g, 34.1% of the administration), mp 190—194° (lit.,[5] mp 193—195°). NMR (5% in CD_3OD) δ ppm: 2.43 (3H, CH_3 on the pyrimidine ring), 3.97 (4.5H, OCH_3 on the pyrazole and the pyrimidine rings), 6.24 and 6.68 (each 1H, aromatic protons on the pyrazole and the pyrimidine rings, respectively).

To the filtrate was added large excess of diazomethane in ether, and the mixture was allowed to stand at room temperature for 3 hr. The solvent was removed under reduced pressure and the residue was dissolved in a small amount of MeOH. The methanolic solution was submitted to the GC-MS analysis and the preparative TLC separation. The spots on the TLC detected by ultraviolet lamp were scratched and the metabolites in these scratched silica-gel were extracted with $CHCl_3$–acetone (9: 1). Each extract was concentrated to dryness and the residue was dissolved in a small amount of MeOH. The methanolic solution was used as the purified sample for the GC-MS analysis.

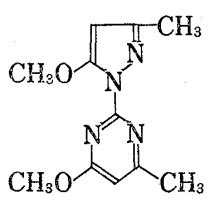
Results and Discussion

Stable Isotope Labeling

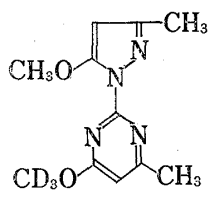
It is well known in the studies of drug metabolism that a stable isotope labeled drug and its metabolites can be recognized in their mass spectra by the occurrence of their characteristic patterns. Especially, the equimolar mixture of a labeled and an unlabeled compounds can be used to creat a visually conspicuous isotope cluster in the mass spectrum.[7]

The tracer used in this study was the deuterio compound (II) with labeling at the methoxy group of the pyrimidine ring. The position of the labeling, which is important to the detection by GC–MS, was chosen in consideration of the following points; first, the site should be metabolically stable so that most metabolites might retain the isotope in their structures. In this regard, it seems that the labeling with deuterium at aromatic nucleus is not suitable because deuterium on this position is easily biotransformed by oxidative elimination or rearrangement by NIH shift. Secondly, in the analysis by MS, the labeled atom must also be retained in fragment ions of relatively high intensity so that the mass spectra of the metabolites might be distinguished from those of endogeneous components. Thirdly, the M, M+2 or M, M+3 doublets created by di- or trideuterium-labeled drug are preferable to M, M+1 doublets because naturally occurring isotope ^{13}C could effect the intensity of the M+1 peak significantly.

Of the various deuterium labeled analogs of I reported previously,[1] the compound II was most satisfactory to the requirements mentioned above. The methoxy group of the pyrimidine moiety of I appears to be biologically stable, since no metabolite biotransformed at this site was isolated from the experimental animals administered this compound. In the study of mass spectra of I and its metabolites, the base peaks were attributed to the fragments of pyrimidine moiety.[1]



mepirizole (I)



deuterium-labeled mepirizole (II)

Metabolites of I in Human Urine

After an equimolar mixture of I and II was administered to a man, the urine was collected for the following 24 hr. From the CH_2Cl_2 extract of the urine, an EtOH-insoluble crystalline

7) A. Prox, *Xenobiotica*, 3, 473 (1973).

metabolite was isolated by filtration and identified as 1-(4-methoxy-6-methyl-2-pyrimidinyl)-5-methoxypyrazole-3-carboxylic acid (IV) by comparing with the authentic sample.[5] The filtrate from IV was treated with diazomethane. The gas chromatogram of the methylated mixture, obtained by using a total ion monitor as a detector, is shown in Fig. 1.

The fraction corresponding to the GC peaks were analyzed mass spectrometrically. The spectra which exhibited isotope ion clusters were assigned to those of the metabolites. Since the mass spectrum of the peak X fraction was not well resolved, it was necessary to purify the methylated mixture by preparative TLC. The purified peak X fraction which exhibited the same GC retention time as the non-purified, was subjected to GC-MS. The mass spectrum of this fraction showed distinct clusters of isotope ions.

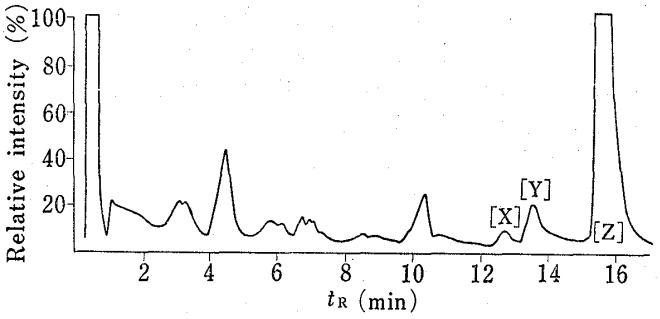


Fig. 1. Gas Chromatogram of Methylated Urine Extract

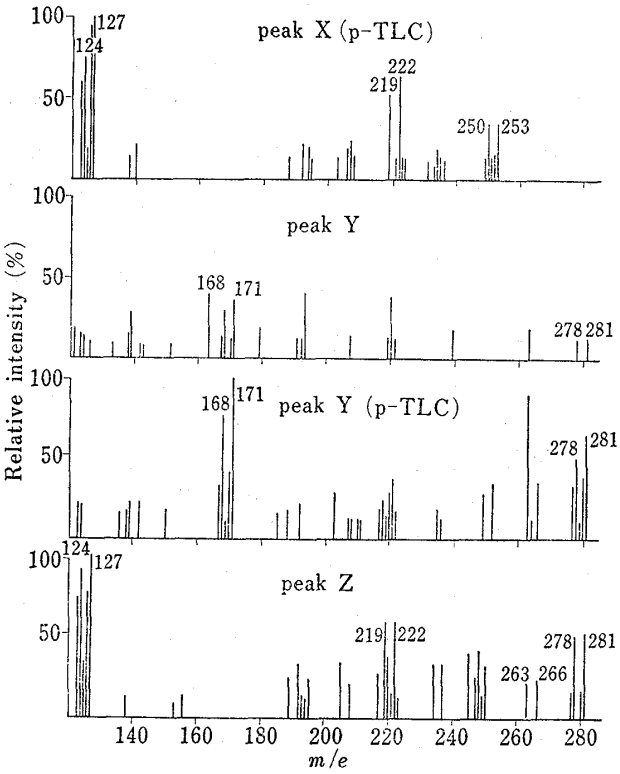


Fig. 2. Mass Spectra of the Metabolites
(p-TLC: mass spectrum of the sample purified by preparative TLC)

The presence of the 3 urinary metabolites corresponding to the peaks X, Y and Z was thus proved by these spectra which are shown in Fig. 2.

On the basis of the spectral data, the structures of the metabolites were clarified. The mass spectrum of I reveals the M^+ ion at *m/e* 234 and the base ion peak at *m/e* 124 which is ascribed to the fragment of the pyrimidine moiety. The fragmentation processes of I and its derivatives were reported previously.[1] The spectrum of the peak X metabolite, which exhibited the M^+ ion at *m/e* 250 (234+16) and the base ion peak at *m/e* 124, led to assignment of the structure III for the metabolite. The assignment was confirmed by comparison of the spectrum with that of an authentic sample. Similarly, the metabolite of the peak Y, whose spectrum exhibited the M^+ ion at *m/e* 278 and the base ion peak at *m/e* 168, was identified as 1-(4-methoxy-6-carboxy-2-pyrimidinyl)-5-methoxy-3-methylpyrazole (V). The metabolite of the peak Z, which revealed the M^+ ion at *m/e* 278 and the base ion peak at *m/e* 124, was identical with IV which was isolated as a crystalline form.

From these results, the metabolic routes of I in man were deduced as summarized in Chart 1. The metabolic pathways of I in man were distinct from those in experimental animals in the point that in man metabolic hydroxylation did not occur on each of the aromatic ring.

The method of identifying a drug and its metabolites in biological fluids by isotopic labeling is one of the useful means for the metabolic studies, since there is no need to isolate a metabolite as a pure form, and therefore, the identification can be accomplished in a short time.

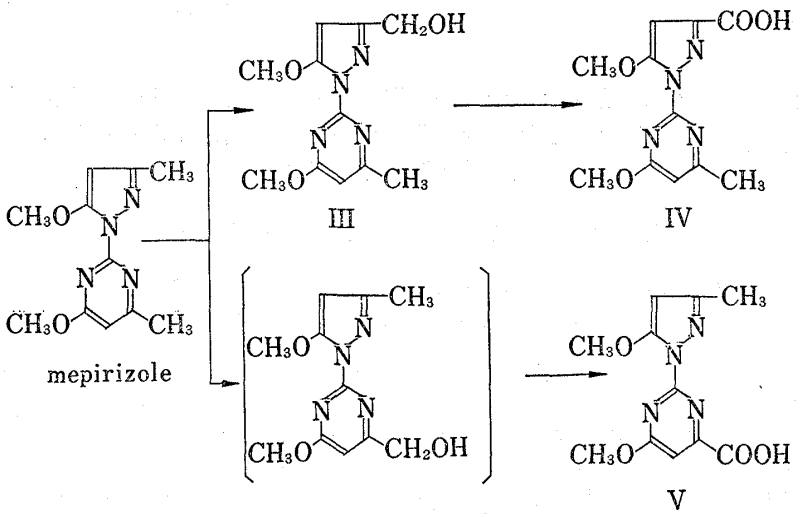


Chart 1. The Metabolic Pathways of Mepirizole in Man

As described in the present report, the metabolites of I in human urine were thus easily confirmed using this method. Although only one labeled compound was used in this study, independent utilization of several derivatives labeled in different sites of the drug will give more information about the structures of the metabolites.