

Analytical Studies on Mepirizole and Its Metabolites. I. Mass Spectra of Mepirizole and Its Some Metabolites¹⁾

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The ionic structures of mass fragments of mepirizole and its derivatives were studied with use of deuterium labelling technique, and the fragmentation processes were discussed. The structural elucidation of the metabolites based on their mass spectra is also described.

Mepirizole (I),³⁾ 1-(4-methoxy-6-methyl-2-pyrimidinyl)-5-methoxy-3-methylpyrazole, is an analgesic and anti-inflammatory agent which has currently wide clinical use. Its main metabolic pathway in rats and rabbits has been reported by Takabatake, *et al.*⁴⁾ In the course of further studies of its metabolites it was required to use highly sensitive analytical methods for detection of the metabolites. Recent progress in the application of a gas chromatograph-mass spectrometer system (GC-MS) has provided a new tool for identification of small quantities of metabolites of drugs in biological samples. This series of papers are concerned with investigations on the metabolism of I by use of the GC-MS method, and first this paper deals with the mass fragmentation of I and mass spectral characteristics of its metabolites.

Materials and Methods

Mepirizole Deuterated at 4-Position of the Pyrazole Ring (II)—Aromatic hydrogen atoms, which are much more susceptible to electrophilic attack, can be exchanged with deuterium under acidic conditions.⁵⁾ A solution of I (500 mg) in D₂O (20 ml) was adjusted to pH 3.0 with 10% DCl and allowed to stand at room temperature overnight. After disappearance of the proton signal at 5.5 ppm in the nuclear magnetic resonance (NMR) spectrum of the reaction mixture, it was neutralized with 10% NaOD and concentrated to dryness *in vacuo*. The product was extracted with CHCl₃ and recrystallized from isopropyl ether to give colorless prisms, mp 89–90°. Mass Spectrum *m/e*: M⁺ 235, isotopic purity was minimum 95%. NMR (6% in CDCl₃) δ ppm: 2.30 and 2.48 (each 3H, CH₃ of pyrazole and pyrimidine, respectively), 3.90 and 3.98 (each 3H, OCH₃ of pyrazole and pyrimidine, respectively) and 6.40 (1H, aromatic proton of pyrimidine).

Mepirizole Deuterated at the Methyl Group of the Pyrazole Ring (III)—III was synthesized according to the procedure of Naito,³⁾ by treating 2-hydrazino-4-methoxy-6-methylpyrimidine with ethyl γ,γ,γ -trideuteroacetoacetate in place of ethyl acetoacetate. An ethanolic solution (10 ml) of ethyl γ,γ,γ -trideuteroacetoacetate (700 mg, 5.25 mmoles) and 2-hydrazino-4-methoxy-6-methylpyrimidine (820 mg, 5.30 mmoles) was refluxed for 1 hr. To the reaction mixture was added 20% NaOH (1.0 ml) and the alkaline solution was heated at 60° for further 0.5 hr. After evaporation of the solvent, the residue was dissolved in small amount of water and acidified with AcOH. The precipitate, 1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-trideutero-methyl-3-pyrazolin-5-one, was collected by filtration. The yield of the crude product was quantitative. The crude product was dissolved in MeOH (5 ml) and treated with large excess of diazomethane in ether. After 3 hr, the solvent was removed by evaporation and the residue was chromatographed over neutral alumina by elution with benzene. The aimed compound thus obtained was further purified by recrystallization from isopropyl ether to give colorless prisms, 800 mg (64%), mp 88–90°. Mass Spectrum *m/e*: M⁺ 237, isotopic purity was above 98%. NMR (10% in CDCl₃) δ ppm: 2.50 (3H, CH₃ of pyrimidine), 3.93 and 4.01 (each 3H, OCH₃ of pyrazole and pyrimidine, respectively), 5.50 and 6.42 (each 1H, aromatic proton of pyrazole and pyrimidine, respectively).

- 1) This work was presented at the 5th Symposium of Organic Mass Spectrometry, June (1970), Sendai, Japan.
- 2) Location: Minamifunabori-cho, Edogawa-ku, Tokyo.
- 3) T. Naito, T. Yoshikawa, S. Kitahara and N. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1467 (1969).
- 4) E. Takabatake, R. Kodama, Y. Tanaka, R. Dohmori, H. Tachizawa and T. Naito, *Chem. Pharm. Bull.* (Tokyo), **18**, 1900 (1970).
- 5) Y. Kawazoe, *Chem. Pharm. Bull.* (Tokyo), **15**, 826 (1967).

Mepirizole Deuterated at the Methoxy Group of the Pyrazole Ring (IV)—To a mixture of sodium methoxide (346 mg, 6.4 mmoles) and 1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methyl-3-pyrazolin-5-one⁶⁾ (880 mg, 4.0 mmoles) in dimethylacetamide (10 ml), was added dropwise hexadeuterodimethyl sulfate (792 mg, 6.0 mmoles) in dimethylacetamide (2 ml) at room temperature, and the reaction mixture was stirred for 2 hr. After evaporation of the solvent below 40° under reduced pressure, the residue was dissolved in water (20 ml) and adjusted to pH 12–13 with dil. NaOH. The solution was allowed to stand at room temperature for 2 hr and the product was extracted three times with dichloroethane. The extracts were combined and washed with dil. (NH₄)₂SO₄–H₂SO₄ solution to remove by-products, and the organic layer was dried over Na₂SO₄. By evaporation of the solvent followed by crystallization of the residue from isopropyl ether, IV (520 mg, 54%) was obtained as colorless prisms, mp 89–90°. Mass Spectrum *m/e*: M⁺ 237, isotopic purity was above 98%. NMR (10% in CDCl₃) δ ppm: 2.30 and 2.49 (each 3H, CH₃ of pyrazole and pyrimidine, respectively), 3.99 (3H, OCH₃ of pyrimidine), 5.47 and 6.40 (each 1H, aromatic proton of pyrazole and pyrimidine, respectively).

Mepirizole Deuterated at the Methyl Group of the Pyrimidine Ring (V)—1-(4-Methoxy-6-methyl-2-pyrimidinyl)-3-methyl-3-pyrazolin-5-one⁶⁾ (500 mg, 2.27 mmoles) was dissolved in 2% NaOD (5.0 ml) and heated at 80° for 4 hr. The precipitate formed by acidifying the solution with AcOH was dissolved in MeOH (2 ml) and methylated with large excess of diazomethane in ether. The solvent was removed and the residual crude product was purified by alumina column chromatography. Elution with benzene gave the fraction containing V, from which pure V was obtained as colorless prisms, 230 mg (43%), mp 88–90°. Mass Spectrum *m/e*: M⁺ 237, isotopic purity was minimum 90%. NMR (10% in CDCl₃) δ ppm: 2.30 (3H, CH₃ of pyrazole), 3.93 and 4.01 (each 3H, OCH₃ of pyrazole and pyrimidine, respectively), 5.51 and 6.42 (each 1H, aromatic proton of pyrazole and pyrimidine, respectively).

Mepirizole Deuterated at the Methoxy Group of the Pyrimidine Ring (VI)—VI was prepared in a similar way as IV by using 1-(4-hydroxy-6-methyl-2-pyrimidinyl)-5-methoxy-3-methylpyrazole as a starting material, which was obtained by heating mepirizole in 1N NaOH. Yield 76%, mp 89–90°. Mass Spectrum *m/e*: M⁺ 237, isotopic purity was above 99%. NMR (10% in CDCl₃) δ ppm: 2.31 and 2.51 (each 3H, CH₃ of pyrazole and pyrimidine, respectively), 3.94 (3H, OCH₃ of pyrazole), 5.51 and 6.41 (each 1H, aromatic proton of pyrazole and pyrimidine, respectively).

Mepirizole Deuterated at Both of the Methoxy Groups (VII)—VII was prepared in a similar way as IV by using 1-(4-hydroxy-6-methyl-2-pyrimidinyl)-3-methyl-3-pyrazolin-5-one⁶⁾ as a starting material in a 54% yield, mp 88–90°. Mass Spectrum *m/e*: M⁺ 240, isotopic purity was above 99%. NMR (10% in CDCl₃) δ ppm: 2.28 and 2.49 (each 3H, CH₃ of pyrazole and pyrimidine, respectively), 5.50 and 6.41 (each 1H, aromatic proton of pyrazole and pyrimidine, respectively).

4-Methoxy-6-methylpyrimidine (IX)—IX was prepared according to the method of Marshall.⁷⁾

4-Trideuteromethoxy-6-methylpyrimidine (X)—Sodium (27 mg, 1.2 mmoles) was dissolved in 0.5 ml of methanol-*d*₄, and the solution was completely evaporated to dryness in an oil bath. The sodium methoxide-*d*₃ thus obtained was suspended in a solution of 4-chloro-6-methylpyrimidine (128 mg, 1.0 mmole) in tetrahydrofuran (2 ml). The mixture was stirred for a few minutes at room temperature. After evaporation of the solvent from the reaction mixture, the product was purified by preparative thin-layer chromatography to give light yellow oil. Mass Spectrum *m/e*: M⁺ 127, isotopic purity was above 98%. NMR (10% in CDCl₃) δ ppm: 2.45 (3H, CH₃), 6.58 and 8.70 (each 1H, aromatic proton).

The Metabolites of I in Rat Urine—All of the authentic samples of the metabolites which were found in rat urine were prepared by the same method as described in a previous paper.⁸⁾

5-Bromo-mepirizole (VIII)—VIII was synthesized according to the method of Kitahara by reaction of 5-bromo-2-hydrazino-4-methoxy-6-methylpyrimidine with ethyl acetoacetate, followed by methylation with diazomethane.⁹⁾

Instruments and Conditions—The mass spectrometric analyses were carried out on Hitachi RMS-4 mass spectrometer. The samples were introduced through the all glass heated direct inlet system. High resolution mass spectrometry was carried out by using JEOL JMS 01-SG 2 mass spectrometer and JMD-2C microphotometer connected with JEC-6 spectrum computer.

JEOL 4H-100 NMR system was used for the measurements of nuclear magnetic resonance spectra. All spectra were taken on standard operation. Internal standard for measuring chemical shifts was tetramethylsilane (TMS) in CDCl₃.

6) T. Hirayama, S. Asai and K. Nakagawa, The 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, 1973, Abstr. Paper, No. 2, p. 4.

7) J.R. Marshall and J. Walker, *J. Chem. Soc.*, 1951, 1004.

8) R. Dohmori, R. Yoshimura, S. Kitahara, Y. Tanaka and T. Naito, *Chem. Pharm. Bull.* (Tokyo), 18, 1908 (1970).

9) S. Kitahara, private communication.

Results and Discussion

Mass Fragmentation of Mepirizole

A prominent feature of the structure of mepirizole is the presence of the methoxy and the methyl groups at the carbon atoms adjacent to nitrogen atoms in both of the pyrimidine and pyrazole rings. It is therefore presumed that the presence of such two analogous structural groups in the molecule may complicate the interpretation of the mass spectrum. Then, in order to have definite information on the fragmentation, several deuterated derivatives of mepirizole (Chart 1) were synthesized.

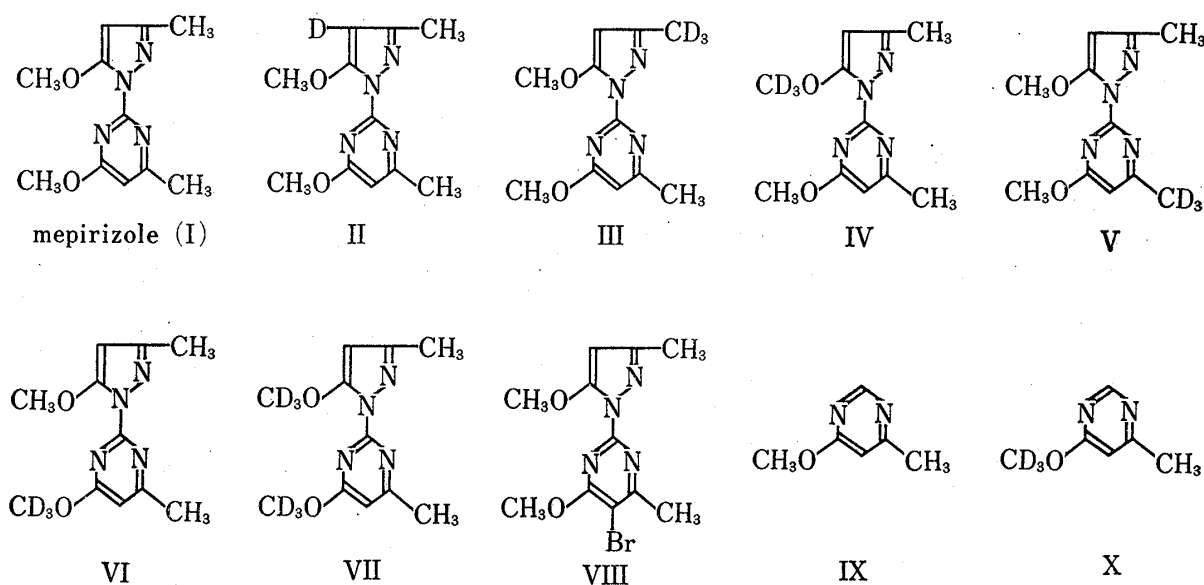


Chart 1. Mepirizole (I) and Its Derivatives

Analysis by mass spectrometric shift technique revealed the most remarkable spectral feature of I. The derivative VIII with a bromine at the 5-position of the pyrimidine ring was used for comparison. As shown in Fig. 1 and 2, the peaks in the region from the molecular ion peak (m/e 234) to the base peak (m/e 123 and 124) in the spectrum of I are shifted by 79 and 81 mass units to give the corresponding peaks of VIII, and the shifted peaks have characteristic double isotope peaks. Therefore, those peaks correspond to fragments which contain the bromine atom and hence the pyrimidine ring. Comparison of the spectrum of IV with that of VII also confirmed this view, showing that most peaks in the above range are shifted by 3 mass units by replacing the OCH_3 group of the pyrimidine ring with the OCD_3 group. The base peak (m/e 124) of I accompanies a neighbouring intense peak (m/e 123), and this is also a spectral feature of the series of the compounds.

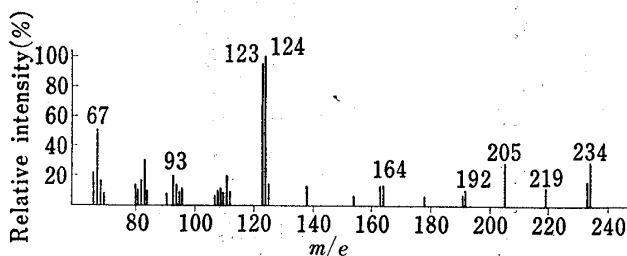


Fig. 1. Mass Spectrum of Mepirizole (I)

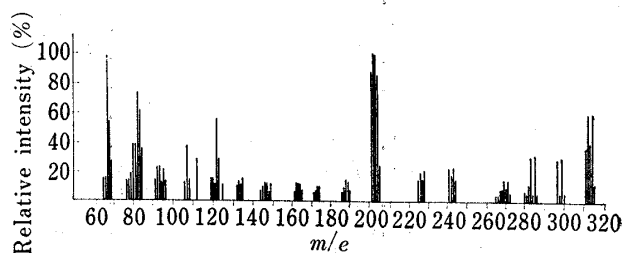


Fig. 2. Mass Spectrum of 5-Bromo-Mepirizole (VIII)

These preliminary results suggested that the principal scission of I first occurs at the pyrazole ring and that the base peak consists of the pyrimidine moiety.

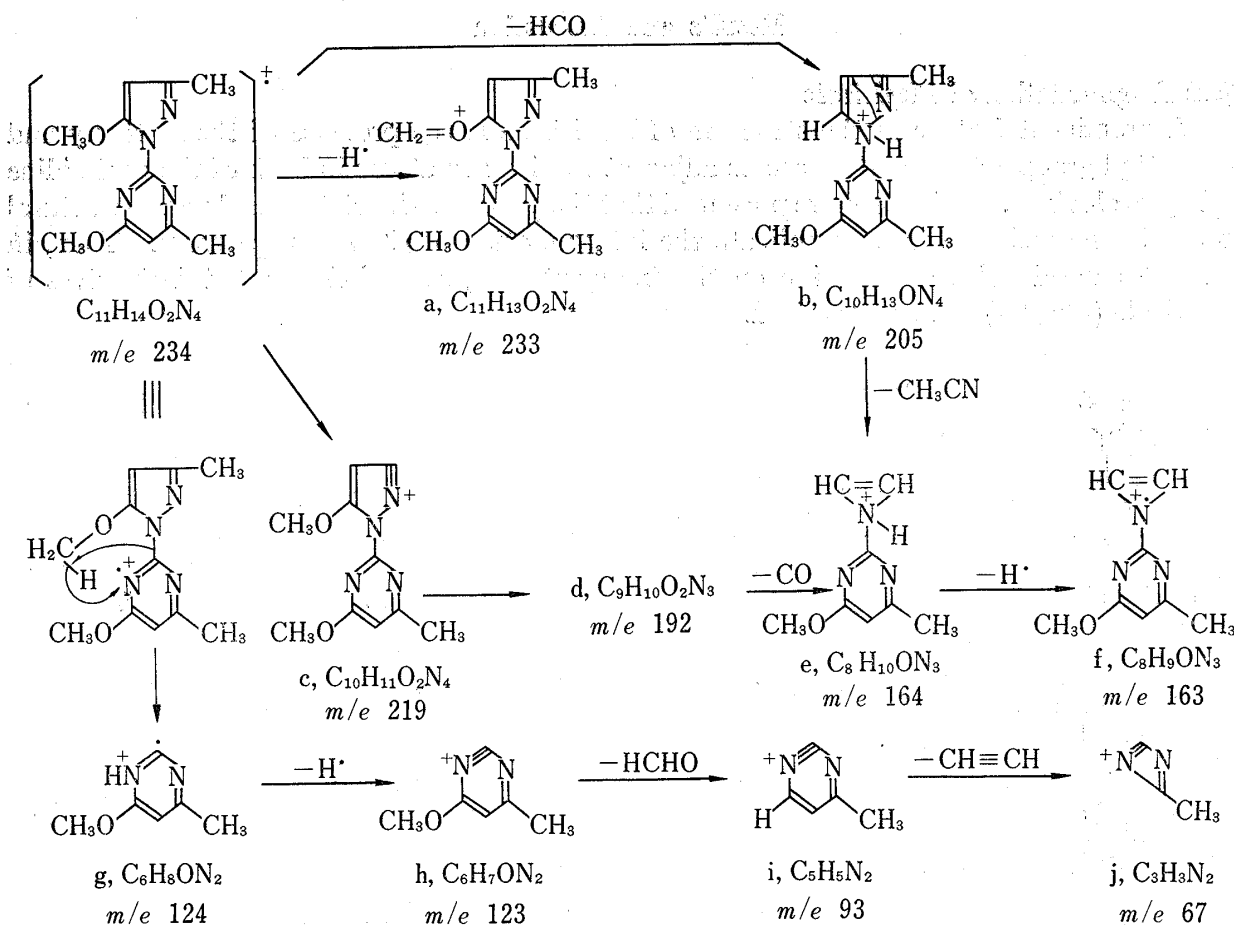


Chart 2. Fragmentation Process of Mepirizole (I)

The fragmentation of I (Chart 2) follows four distinct routes. All the formulae of the fragment ions of I were established by high resolution mass spectrometry (Table I). The molecular ion (m/e 234) of I loses a hydrogen atom to yield a relative intense M-1 peak (m/e 233) which is observed as M-2 peak (m/e 235) in IV, and (m/e 238) in VII. This ion was formulated as a fragment structure a. Appearance of M-1 ion peak by losing the hydrogen from a methoxy group is usually observed in heterocyclic compounds.¹⁰⁾

TABLE I. High Resolution Mass Spectral Data of Mepirizole

Observed (m/e)	Calcd. (m/e)	Error (m mass)	Component
234.1119	234.1116	0.3	$C_{11}H_{14}O_2N_4$
233.1046	233.1038	0.8	$C_{11}H_{13}O_2N_4$
219.0883	219.0881	0.1	$C_{10}H_{11}O_2N_4$
205.1072	205.1089	-1.6	$C_{10}H_{13}ON_4$
192.0778	192.0772	0.5	$C_9H_{10}O_2N_3$
191.0925	191.0932	-0.7	$C_9H_{11}ON_4$
164.0823	164.0823	0.0	$C_8H_{10}ON_3$
163.0772	163.0745	2.7	$C_8H_9ON_3$
124.0632	124.0636	-0.4	$C_6H_8ON_2$
123.0554	123.0558	-0.3	$C_6H_7ON_2$
93.0446	93.0452	-0.6	$C_5H_5N_2$
67.0290	67.0296	-0.5	$C_3H_3N_2$

10) T. Nishiwaki, *Tetrahedron*, **22**, 3117 (1966).

The ring contraction of the pyrazole moiety follows two distinct routes. The predominant feature is expulsion of HCO radical from M^+ to lead to ion b, $C_{10}H_{13}ON_4$ (m/e 205), followed by elimination of CH_3CN to form ion e (m/e 164). The second process is loss of a methyl radical from M^+ giving ion c, $C_{10}H_{11}O_2N_4$ (m/e 219). In the deuterated compounds II, IV and VII, the corresponding fragment ions are observed at m/e 220, 222 and 225, respectively, without loss of the deuterium atom. This suggests that the ejection of methyl group results from one of two C- CH_3 in I. Additional information, which shows the presence of $M-15$ ion peak, was obtained from the compound, 1-(4-methoxy-2-pyrimidinyl)-5-methoxy-3-methylpyrazole,³⁾ which lacks the 6-methyl group of the pyrimidine ring of I. On the other hand, III, as well as I, shows $M-CD_3$ peak at m/e 219. These results support the view that the fragment ion c (m/e 219) was yielded by loss of the methyl group at the pyrazole ring of I. Subsequent elimination of HCN and CO from c gives also ion e (m/e 164), which is further converted to a stable azirinium ion f,¹¹⁾ $C_8H_9ON_3$ (m/e 163) by the ejection of a hydrogen.

It is shown that the base peak (m/e 124) and the second prominent peak (m/e 123) arise from the pyrimidine moiety in I as explained above. The base ion fragment is produced by the rearrangement of a hydrogen atom of the methoxy group on the pyrazole ring, since in the spectra of IV and VII, the base peaks are found at m/e 125 and m/e 128, respectively. If the hydrogen atom would be replaced at the 2-position of the pyrimidine, the spectral pattern of I in the lower mass region should be expected to be almost identical with that of 4-methoxy-6-methylpyrimidine (IX) (Fig. 3). The fragmentation path of IX is elucidated by the deuterium labelled compound (X), and by high resolution mass spectrum as shown in Chart 3. Compound IX itself yields a molecular ion M^+ (m/e 124), which loses a hydrogen radical from the methoxy group to produce a prominent $M-1$ fragment 1 (m/e 123). In another pathway, the molecular ion radical loses a formyl radical HCO, and then a hydrogen, giving the ions m (m/e 95) and n (m/e 94). By successive elimination of HCN, the fragment ion n

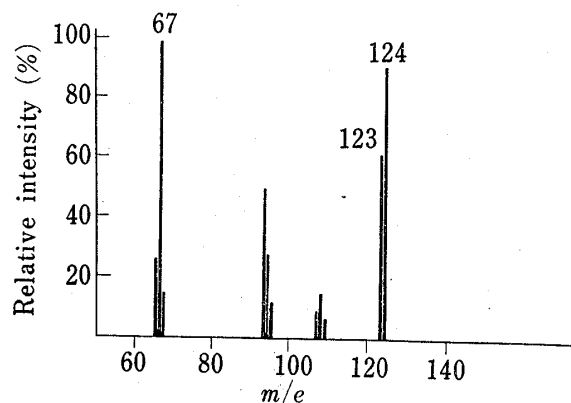


Fig. 3. Mass Spectrum of 4-Methoxy-6-methylpyrimidine (IX)

is converted to C_4H_5N (m/e 67). Although the corresponding mass numbers (m/e 124, 123, 95, 94, 67 etc.) of IX are also detected in the spectrum of I, the fragmentation of I is obviously different from that of IX in two points. One of them concerns the structure of the fragment ion at m/e 123. In the case of IX, ion 1 at m/e 123 is evidently produced by elimination of a hydrogen from the methoxy group¹⁰⁾ as shown by the $M-2$ peak in the spectrum of the deuterium labelled compound X. In the case of I, on the contrary, the ion peak at m/e 123 is resulted from loss of a hydrogen atom which is rearranged from the methoxy group of the pyrazole ring, as confirmed by the deuterium labelled compound IV. The another point concerns the difference in the compositions of the fragment ion of I and of IX at m/e 67, that is, $C_3H_3N_2$ in the former and C_4H_5N in the latter. These facts indicate that the fragment ion of I at m/e 124 does not correspond to the molecular radical ion of IX. Alternatively, in a mechanism leading to the base peak, rearrangement of a hydrogen atom from the methoxy group of the pyrazole ring is considered to transfer the hydrogen to the nitrogen atom of the pyrimidine rather than to the carbon atom at position 2, as shown in ion g in Chart 2.

11) T. Nishiwaki, *J. Chem. Soc. (B)*, 1967, 885.

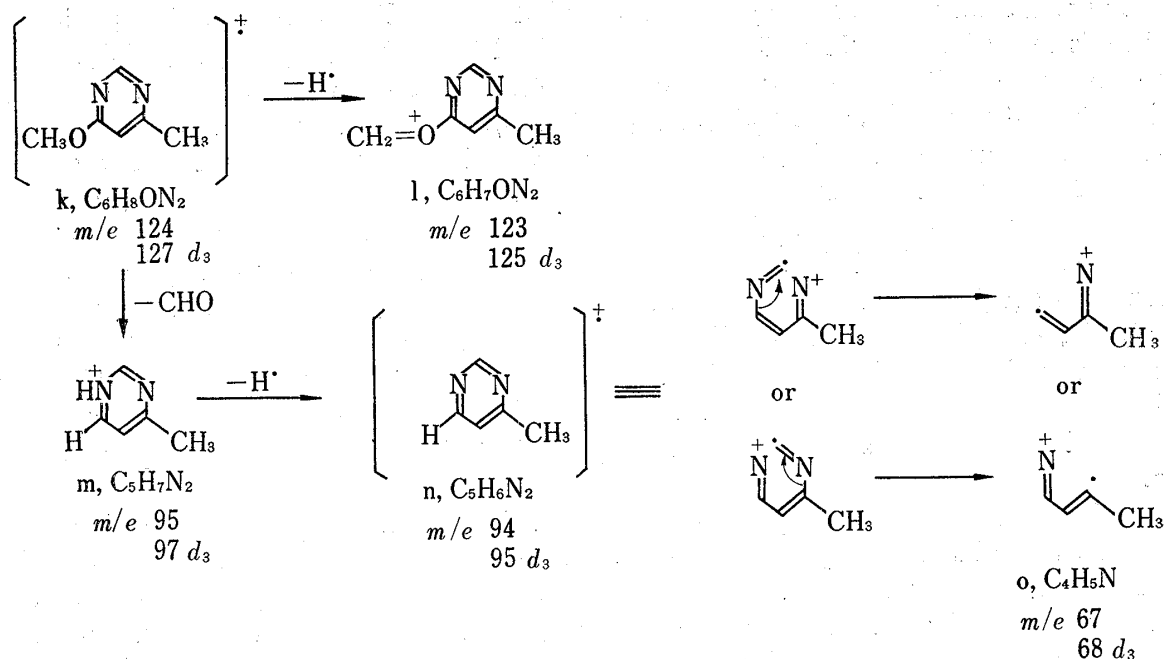


Chart 3. Fragmentation Process of 4-Methoxy-6-methylpyrimidine (IX)

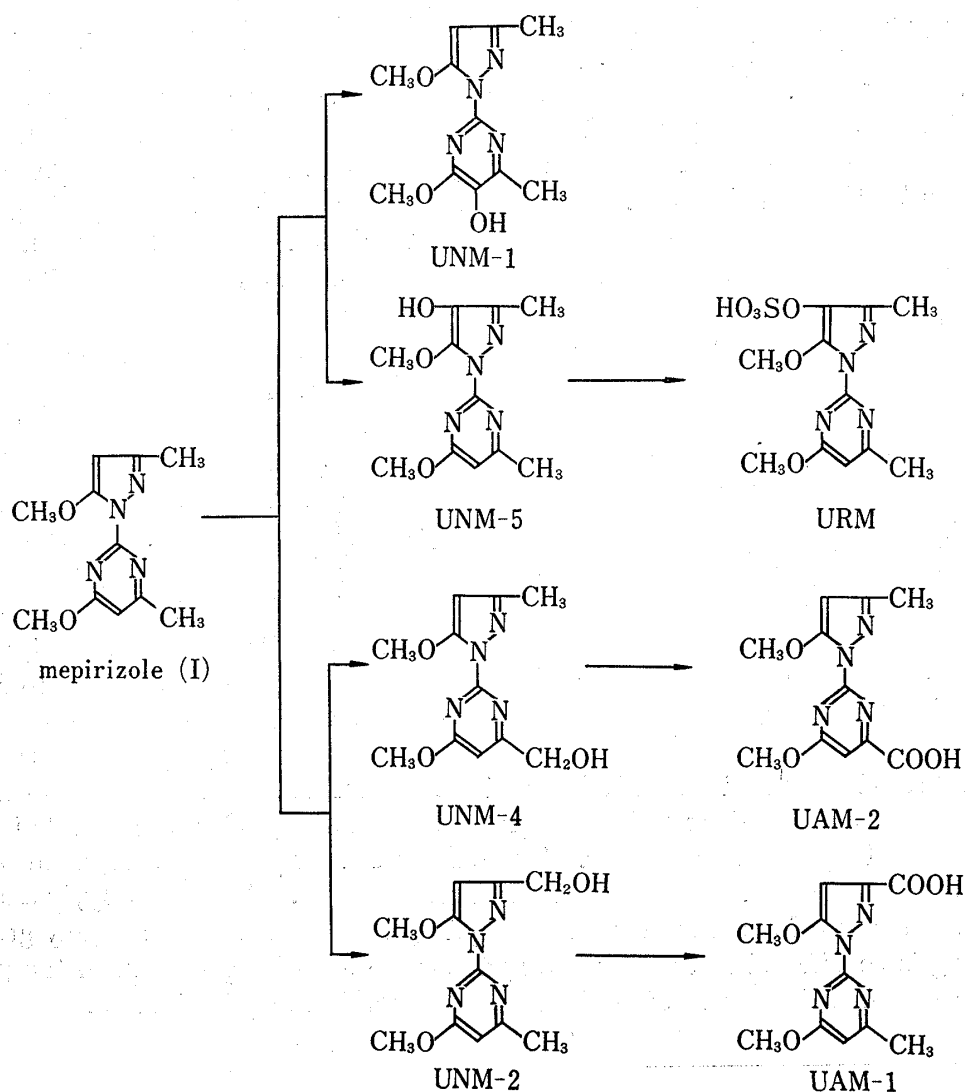


Chart 4. Metabolic Pathways of Mepirizole (I)

The base peak (m/e 124) of I undergoes the fragmentation as shown in Chart 2, and gives the ion h (m/e 123) which expels HCHO (30 mass units) to produce the peak i (m/e 93). Finally, the peak i loses an acetylene to give the ion j (m/e 67).

The mass spectral data of mepirizole and its deuterium labelled compounds are summarized in Table II.

TABLE II. Mass Spectral Data of Mepirizole and Its Deuterium Labelled Compounds

Symbol	—	a	c	b	e	f	g	h	i	j
	M ⁺	M -H	M -CH ₃	M -CHO	b -CH ₃ CN	e -H	—	g -H	h -HCHO	i -CH=CH
Pattern coeff.	29.8	17.3	12.4	27.4	14.4	14.3	100.0	97.0	19.0	50.0
Mepirizole (I)	234	233	219	205	164	163	124	123	93	67
II	235(1)	234(1)	220(1)	206(1)	165(1)	164(1)	124	123	93	67
III	237(3)	236(3)	219	208(3)	164	163	124	123	93	67
IV	237(3)	235(2)	222(3)	207(2)	166(2)	164(1)	125(1)	123	93	67
V	237(3)	236(3)	222(3)	208(3)	167(3)	166(3)	127(3)	126(3)	96(3)	70(3)
VI	237(3)	236(3)	222(3)	208(3)	167(3)	166(3)	127(3)	126(3)	94(1)	67
VII	240(6)	238(5)	225(6)	210(5)	169(5)	167(4)	128(4)	126(3)	94(1)	67

Shifts in mass units from the corresponding fragments of mepirizole are shown in parentheses.

Application of GC-MS Method to Structural Elucidation of the Metabolites

The metabolic pathway of I in rats after oral administration was deduced from the urinary metabolites and is summarized in Chart 4. The mass spectra of major urinary metabolites are shown in Fig. 4.

The assignment of the fragment ion peaks of I enabled us to apply mass spectrometry to the structural elucidation of its metabolites in rat urine. The mass spectra of the metabolites were characterized by the following observations:

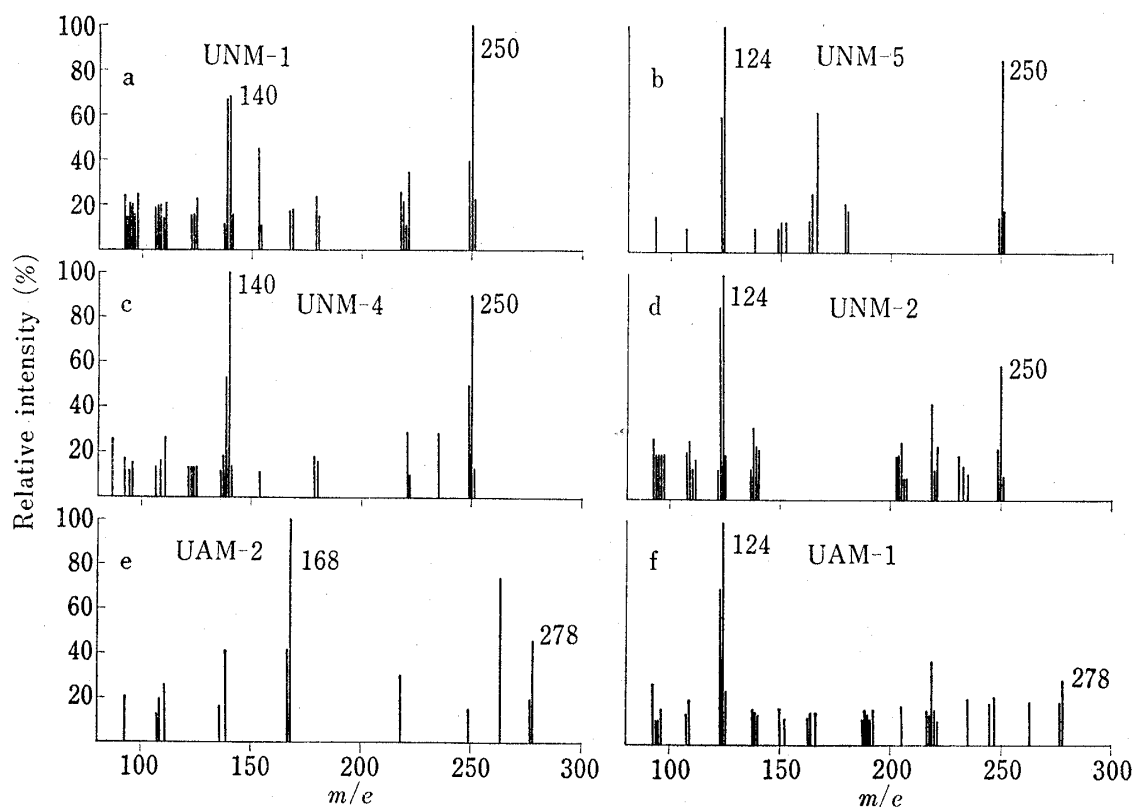


Fig. 4. Mass Spectra of the Major Metabolites of Mepirizole (I)

A) The presence of molecular ion peak at m/e 234+16 indicates the introduction of one oxygen atom into mepirizole. Therefore, the oxidation must have occurred on an aromatic ring to yield a phenolic hydroxy derivative, or on a methyl group to give a hydroxymethyl compound. These two metabolic pathways are distinguishable by observation of a M-18 peak (dehydration of the hydroxymethyl group) (Fig. 4-c, 4-d). As mentioned above, the strongest ion peaks of mepirizole appear at m/e 123 and 124 as an ion cluster which are attributable to the pyrimidine moiety. Therefore, the appearance of the base peak of the metabolite at this region suggests that oxidation has occurred on the pyrazole moiety (Fig. 4-b, 4-d). If it appeared at the position higher by 16 mass units than m/e 123 and 124, the pyrimidine part must have been oxidized (Fig. 4-a, 4-c).

B) If sample prepared by treating the metabolite with diazomethane shows the molecular ion peak at m/e 234+44, the metabolite must bear one carboxylate group arising from the oxidation of a methyl group. A position of the carboxylate group can be discriminated by observing the base peak which appears at m/e 123 and 124, or 44 mass units higher.

Conclusion

The mass fragmentation of mepirizole under electron impact was studied in detail with the aid of deuterium labelling. Several specific fragment ions proved to serve the structural elucidation; especially, the base peak revealed the structure of the pyrimidine moiety. The utility of mass spectral information derived from mepirizole is demonstrated by the structure elucidation of its metabolites isolated from rat urine. The results can be effectively utilized hereafter to the metabolic studies of mepirizole in man.

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