Biomimetic Oxidation of 2-Methylimidazole Derivative with a Chemical Model System for Cytochrome P-450

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A chemical model system for cytochrome P-450, consisting of tetraphenylporphyrin manganese chloride (TPPMnCl) and iodosylbenzene, efficiently oxidized 2-methylimidazole to 2-methylimidazolone. This system was next applied to 4-(2-methyl-1-imidazolyl)-2,2-diphenylbutyramide, a muscarinic acetylcholine receptor antagonist under clinical trial, affording the previously unisolated imidazole ring 5-mono-oxidized derivative that is considered to be the precursor of the main metabolites. This system, which is superior to the copper-ascorbate system, should be applicable to *in vitro* studies of various drugs containing the 2-methylimidazole moiety.

Key words 2-methylimidazole derivative; 2-methylimidazolone derivative; metabolic intermediate; drug metabolism; P-450 chemical model; metalloporphyrin

Cytochrome P-450 (designated as P-450) is a family of heme-containing metallo-enzymes named after the absorption band at 450 nm of their carbon monoxide form. P-450 metabolizes various kinds of xenobiotics, including drugs, oxidatively or reductively, playing an important role in drug metabolism.[1] Many chemical models for P-450 have been developed to elucidate and/or mimic the function of the enzymes. Several models function as efficient oxidation systems for simple substrates, but there have been only a few reports on the application of these models to the *in vitro* metabolic study of heterocycles, which are often used as an important structural backbone for drugs and candidate drugs. For example, in the case of the oxidation of 3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine (Ibudilast; IBPP), which has been developed as an antiasthma drug and cerebral vasodilator, ring hydroxylation of the pyrazolo[1,5-a]pyridine nucleus occurred smoothly to give the monoepoxide derivative along with the diepoxide derivative.[2] Those compounds are unstable precursors of the final metabolites of IBPP.

2-Methylimidazole is an important pharmacophore, *e.g.*, in thromboxane A_2 synthase inhibitors, aromatase inhibitors, 5-hydroxytryptamine (5HT) inhibitors, muscarinic acetylcholine receptor antagonists, and so on.[3] In general, the imidazole ring is rather metabolically labile, affording ring polyoxygenated and related metabolites.[4] However, there has been no report on the isolation and structural determination of imidazole ring mono-oxygenated metabolites (designated as imidazolones), which are thought to be precursors of the final metabolites of various drugs and candidate drugs.

It has been reported for several imidazole-containing drugs that trace amounts of drug equivalents are retained in connective tissue after administration to laboratory animals.[5—7] Although the mechanism(s) of the retention in connective tissue is not yet known, it may involve the reaction of a reactive intermediate (such as imidazolone), formed by metabolism of the imidazole moiety, with tissue macromolecules.[8] Therefore, it is important to develop an efficient method for the preparation of imidazolone derivatives under biomimetic reaction conditions. Direct transformation of 2-methylimidazole to 2-methylimidazolone using a cupro-ascorbate system (cupric chloride and L-ascorbic acid) was reported, but the yield was very poor.[9] We retested this system, but found that reproducibility was poor (data not shown).

In this paper, we present a novel mono-oxidation of 2-methylimidazole by using metalloporphyrin–iodosylbenzene as a chemical model system for cytochrome P-450.[10—12] Application of this system to a 2-methylimidazole-containing candidate drug is also described.

Experimental

General Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured in $CDCl_3$ with tetramethylsilane (TMS) and the solvent peak as internal standards, on a JEOL JMN-A400 spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-HX110 spectrometer. Column chromatography was carried out on Merck silica gel 60. Analytical thin-layer chromatography (TLC) was performed on Merck precoated silica gel $60F_{254}$ plates, and the compounds were visualized by UV illumination (254 nm) or by heating after spraying with phosphomolybdic acid in ethanol. HPLC analysis was performed on a TOSOH ODS-$80T_M$ column (4.6×250 mm). Chromatographic conditions were as follows: samples were eluted at a flow-rate of 0.5 ml/min with 0.05 mol/l ammonium acetate–methanol solution (25 : 1 v/v). The samples were monitored by measuring the absorbance at 220 nm. The areas of the chromatographic peaks were calculated by use of a Hitachi chromato-integrator (D-2000). Elemental analysis was performed in the microanalytical laboratory of Kyorin Pharmaceutical Co., Ltd.

Chemicals 2-Methylimidazole, tetraphenylporphyrin manganese chloride (TPPMnCl), tetraphenylporphyrin iron chloride (TPPFeCl), manganese chloride ($MnCl_2$), iodosylbenzene (PhIO), dichloromethane, and acetonitrile were of reagent grade and were used without further purification. 4-(2-Methyl-1-imidazolyl)-2,2-diphenylbutyramide[13—16] (KRP-197) and its tetradeuterio derivative (D_4-KRP-197) were synthesized by the Discovery Research Laboratory of Kyorin Pharmaceutical Co. Ltd.

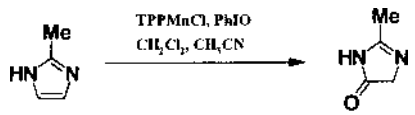

Chart 1. Reaction of 2-Methylimidazole with the TPPMnCl–PhIO System

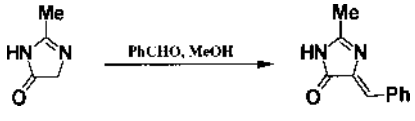

Chart 2. Reaction of 2-Methylimidazolone with Benzaldehyde

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Reaction with the Chemical Model In accordance with the method reported by Cook *et al.*,[17] 1 equivalent of substrate, 0.01—0.05 molar equivalent(s) of metalloporphyrin and 1—3 molar equivalent(s) of oxidant were dissolved in 10 ml of mixed solvent of dichloromethane and acetonitrile (1 : 1 v/v), and the mixture was stirred at room temperature (see Chart 1). The reaction was monitored by HPLC and/or TLC.

Results and Discussion

Figure 1 shows HPLC chromatograms indicating the formation of 2-methylimidazolone from 2-methylimidazole in the TPPMnCl–PhIO system. When an equimolar amount of PhIO was used as the oxidant, the reaction was incomplete, and a considerable amount of the starting material remained, even after 5 h. However, the reaction went to completion when 2 molar-equivalents of PhIO was used, and 2-methylimidazolone was obtained almost quantitatively. Without metalloporphyrin manganese chloride, little reaction took place. Furthermore, little reaction took place when $MnCl_2$ was used instead of TPPMnCl. These results clearly indicated that 2-methylimidazole was oxidized not by PhIO itself, but by an oxidant formed by the reaction between TPPMnCl and PhIO, presumably a manganese-oxenoid species.[18] The effect of the central metal is small, because similar efficiency was obtained by the use of TPPFeCl as a catalyst. The structural determination of 2-methylimidazolone was done by ^{1}H-NMR, and high-resolution mass spectrometry.[19] Furthermore, the structure was confirmed by derivatization of 2-methylimidazolone to the aldol adduct with benzaldehyde.

2-Methylimidazolone reacted smoothly with benzaldehyde at room temperature in the absence of base to afford the (*Z*)-α,β-unsaturated ketone derivative[20] in good yield, indicating that the acidity of the methylene moiety of 2-methylimidazolone is high.

Based on these observations, we applied this biomimetic oxidation reaction system to 4-(2-methyl-1-imidazolyl)-2,2-diphenyl butyramide[13—16] (KRP-197; Figure 2), which was developed as a dual antagonist of muscarinic acetylcholine receptors M_1 and M_3. KRP-197 is a candidate drug for the treatment of urinary incontinence associated with bladder muscle instability, and is currently under clinical study. The imidazole ring mono-oxidation product of KRP-197 (designated as KRP-197-imidazolone) is thought to be an unstable precursor of the final metabolites of KRP-197, but has not yet been isolated.[21] To make structural analysis easier, a mixture of KRP-197 and its tetradeuterio analog (designated as D_4-KRP-197 (Chart 3)) was used as a substrate (designated as (H_4, D_4-KRP-197)).

When a mixture of (H_4, D_4)-KRP-197, 0.01 molar-equivalent of TPPMnCl, and 1.5 molar-equivalent of PhIO in a mixed solvent of dichloromethane–acetonitrile (1 : 1 v/v; 10 ml) was stirred for 6 h at room temperature, a product which showed molecular-ion peaks at 336 $(M+H)^+$ and 340 $(M+H)^+$ was isolated in about 30% yield, together with considerable recovery of the starting material (Chart 4).[22] In the mass spectrum (see Fig. 3), $(M+H)^+=336$ was 16 *m/z* larger than KRP-197, and $(M+H)^+=340$ was 16 *m/z* larger than D_4-KRP-197. These mass unit gains correspond to one oxygen atom. These results indicated that the product is a mono-oxygenated derivative of (H_4, D_4)-KRP-197. The mass fragments of 238 $(M+H)^+$ and 242 $(M+H)^+$ corresponded to the $(C_6H_5)_2C(CONH_2)CX_2CX_2$ (X=H, or D) moiety, and no fragment with a mass 16 units higher was seen in the spectrum. These results indicated that mono-oxygenation had occurred not on the phenyl ring, but on the imidazole ring of KRP-197. The oxygenated position of the imidazole nucleus was determined by heteronuclear multiple bond connectivity (HMBC) analysis to be the 5-position (see Chart 4). Furthermore, the 5-imidazolone structure was confirmed by comparison of the NMR spectra of the isolated product and an authentic sample, which was prepared from 2,2-diphenylacetonitrile.[23] These compounds exhibited identical ^{1}H- and

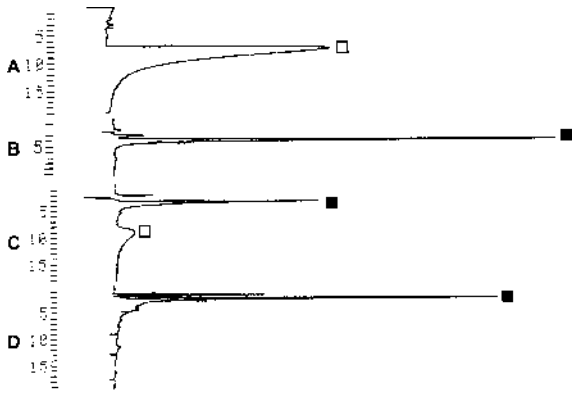


Fig. 1. HPLC Chromatograms of the Reaction Products of 2-Methylimidazole with the TPPMnCl-PhIO System

(A) 2-methylimidazole. (B) Synthetic 2-methylimidazolone.[29] (C) After 5 h reaction (1 eq of PhIO was used). (D) After 4 h reaction (2 eq of PhIO was used). □ and ■ indicate 2-methylimidazole and 2-methylimidazolone respectively.

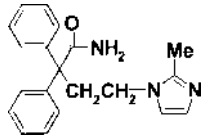
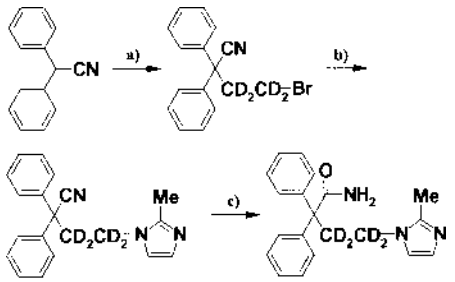


Fig. 2. Chemical Structure of KRP-197



a) $BrCD_2CD_2Br$, DB-18-crown-6, 50% NaOH; b) 2-methylimidazole, TEA, DMF; c) KOH, 2-PrOH.

Chart 3. Synthesis of D_4-KRP-197

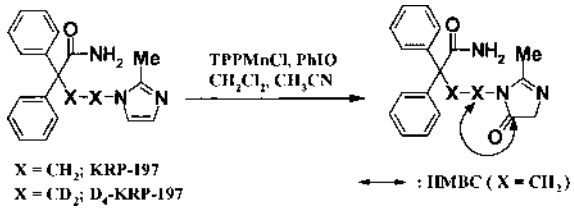


Chart 4. Oxydation of (H_4, D_4)-KRP-197 with the P-450 Chemical Model System

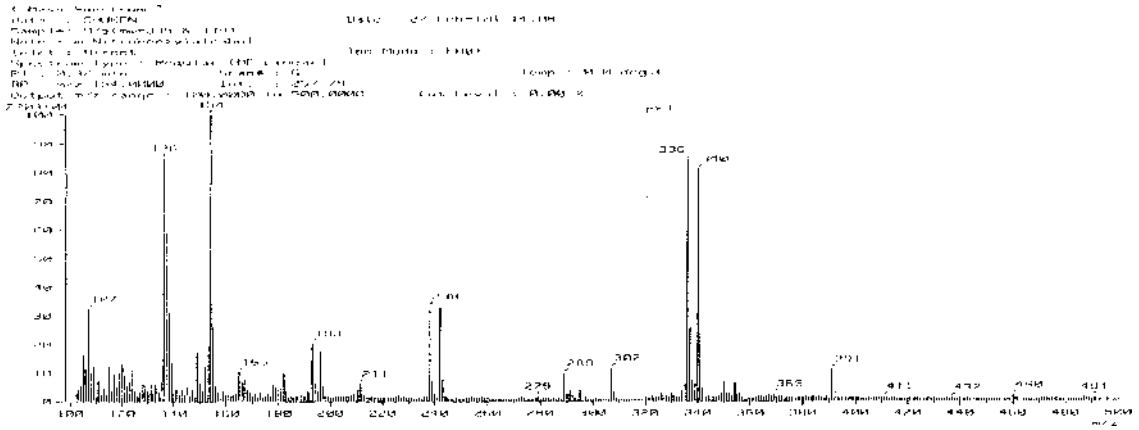


Fig. 3. Mass Spectrum of the Product of the Reaction of (H_4, D_4)-KRP-197 with the TPPMnCl-PhIO System

^{13}C-NMR spectra.

In this paper, we have shown that 2-methylimidazole and a derivative of it were oxidized effectively with a chemical model system for cytochrome P-450, TPPMnCl–PhIO, to afford the 2-methylimidazolone mono-oxidation product. The reaction system is superior to the previously described cuproascorbate system in respect of reproducibility and yield of the product. The formation of 2-methylimidazolone from 2-methylimidazole was reported to occur through a cytochrome P450-independent pathway,[24] but this suggestion was based on very limited findings. Considering our present results and a report showing that simple imidazole was oxidized through a P-450-dependent pathway to afford hydantoin and hydantoic acid,[24] we consider that the metabolic pathway to 2-methylimidazolone from 2-methylimidazole is at least partly P-450-dependent. Further study is under way.

Interestingly, various imidazoles, such as imidazole, 2-methylimidazole, *N*-methylimidazole and so on, have been used as ligands for metalloporphyrins in order to enhance the reactivity of the catalyst and/or to increase the yield of the product.[25—27] However, there is little information about the fate of the added imidazoles during the oxidation reaction, although they were used in excess molar amounts as compared to the metalloporphyrin catalyst.[28] Our present results indicate that in certain cases, not only the substrate itself, but also the added imidazole might be oxidized, affording both the desired product and the oxidized imidazole.

Conclusion

We showed that a chemical model system for cytochrome P-450 could oxidize 2-methylimidazole to 2-methylimidazolone. A 2-methylimidazole-containing drug was similarly mono-oxidized to afford a putative precursor of the main metabolites. The efficiency of the reaction was superior to that of a reported method using a copper-ascorbate system. Further application of this chemical model system for *in vitro* metabolic studies of various drugs and/or drug candidates containing a heterocyclic structure is in progress.

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19) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.99 (3H, s), 3.90 (2H, s), 10.76 (1H, br s). FAB-MS *m/z*: 98.0505 (Calcd for $C_4H_6N_2O$: 98.0480).
20) (*Z*)-5-Benzylidene-2-methyl-3,5-dihydro-imidazol-4-one; mp. 171.0—171.5 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.39 (3H, s), 7.08 (1H, s), 7.37—7.46 (3H, m), 8.13 (2H, dd, J=8.3, 1.7 Hz), 8.82 (1H, br s). FAB-MS *m/z*: 186.0772 (Calcd for $C_{11}H_{10}N_2O$: 186.0793). *Anal.* Calcd for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.89; H, 5.38; N, 14.99.
21) The metabolic study of KRP-197 will be published elsewhere.
22) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.14 (3H, t, J=2.0 Hz), 2.61—2.65 (2H, m), 3.44—3.47 (2H, m), 3.99 (2H, d, J=2.0 Hz), 5.51 (1H, br s), 5.57 (1H, br s), 7.30—7.40 (10H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 15.71, 37.77, 38.41, 58.23, 58.92, 128.44—128.80, 142.26, 163.57, 176.24, 181.42. FAB-MS *m/z*: 336.1728 (Calcd for $C_{20}H_{22}N_3O_2$: 336.1712), 340.1977 (Calcd for $C_{20}H_{18}D_4N_3O_2$: 340.1963).

23) Synthesis of authentic KRP-197-5-imidazolone will be published elsewhere.
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