FACILE PREPARATION OF UNSTABLE METABOLIC INTERMEDIATES; EPOXIDE(S) OF PYRAZOLO[1,5-a]PYRIDINE DERIVATIVES BY THE CYTOCHROME P-450 CHEMICAL MODEL

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Biomimetic oxidation of bioactive pyrazolo[1,5-a]pyridine derivatives carried out with the chemical model for cytochrome P-450, afforded 6,7-epoxides in relatively high yields, these appear to be chemically unstable precursors of the main metabolites, i.e., 6,7-dihydro-6,7-diols.

KEYWORDS pyrazolo[1,5-a]pyridine derivative; epoxidation; metabolic intermediate; drug metabolism; P-450 chemical model; metalloporphyrin

Cytochrome P-450 plays a very important role in drug metabolism. A wide variety of chemical models for P-450 have been developed to elucidate the functions of the enzymes. However, there have been only a few reports on the application of these model systems to the study of the metabolism of practical drugs having many functional groups.[1] From this point of view, we chose the 3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine (IBPP), an antiasthma[2] and cerebral vasodilator,[3] as the substrate to study the reaction by P-450 chemical models. We found that IBPP was oxidized by the TPPMnCl-NaOCl system[4] to preferentially afford 6,7-epoxide which appeared to be an intermediate of the main metabolite, 6,7-dihydro-6,7-diol, in vivo[5] and in vitro, which had not yet been isolated.

We report a novel epoxidation of the pyrazolo[1,5-a]pyridine ring by the chemical model system, metalloporphyrin-NaOCl. The derivatives of pyrazolo[1,5-a]pyridine were synthesized by the method of K. T. Potts et al.[6] Fig. 1 shows the time course of formation of the 6,7-epoxide and 4,5;6,7-diepoxide of IBPP. Based on these results, the monoepoxide was selectively prepared using TPPMnCl[7] and the

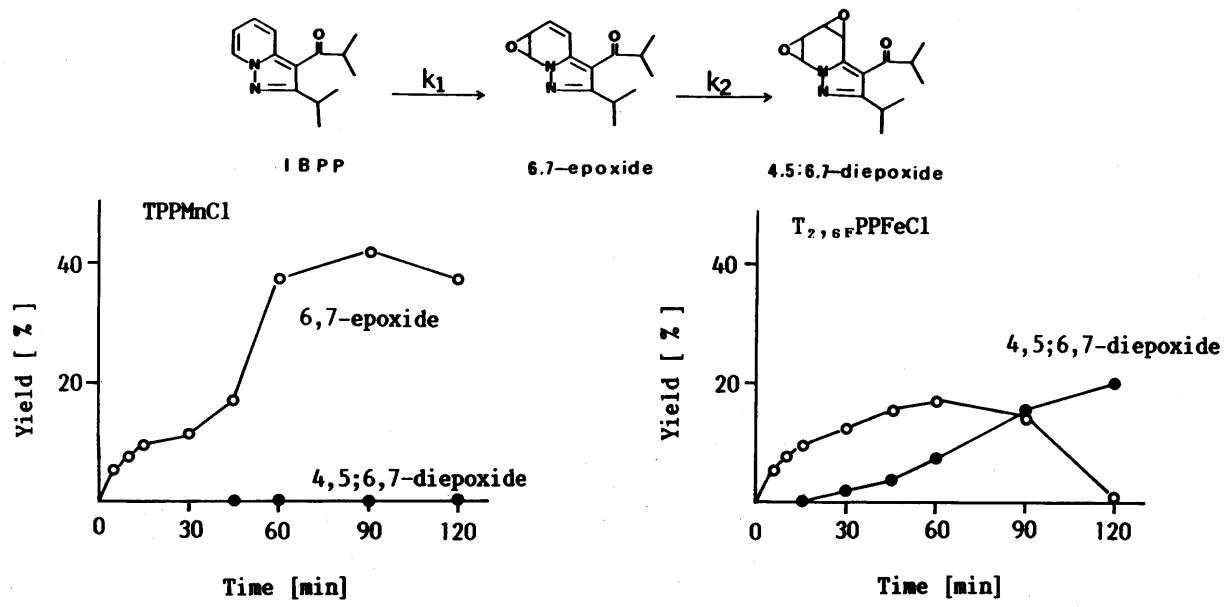
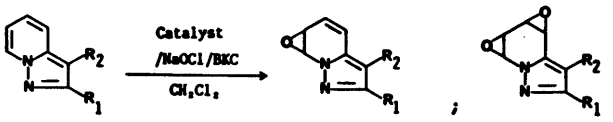


Fig. 1. Time Course of Epoxidation of IBPP by the Catalyst-NaOCl Chemical Model
The yields of 6,7-epoxide and 4,5;6,7-diepoxide were determined by HPLC.

diepoxide using mainly $T_{2,6F}$PPFeCl[8] at 120 min. The rate of formation of 6,7-epoxide (k_1 = 0.42 mmol/min) was about three times higher than that of 4,5;6,7-diepoxide ($k_1 \times k_2$ = 0.16 mmol/min). In a typical experiment, substrate, 1 mmol; catalyst, 0.01 mmol; benzalkonium chloride (BKC), 0.1 mmol; 4′-imidazolylacetophenone (4′-ImAP), 0.12 mmol; NaOCl, 7 mmol and CH_2Cl_2, 10 ml were stirred for 2 h at 0°C under Ar atmosphere. When the catalyst was $T_{2,6F}$PPFeCl, 4′-ImAP was not used. The epoxidation of the pyrazolo [1,5-a]pyridine derivatives is summarized in Table I.[8]

Table I. Epoxidation of Pyrazolo[1,5-a]pyridine Derivatives by Cytochrome P-450 Chemical Model



I II ; III

Compound I		II	III
R_1	R_2	TPPMnCl	$T_{2,6F}$PPFeCl
$CH(CH_3)_2$	$COCH(CH_3)_2$	16.2 % (16)	56.4 % (113)
$CH(CH_3)_2$	SCN	7.7 (8)	
C_6H_5	H	38.2[a] (19)	27.7[a] (28)
C_6H_5	COC_6H_5	9.5 (16)	
p-$SO_2CH_3C_6H_4$	I	12.6 (6)	27.9 (28)
p-$SO_2CH_3C_6H_4$	H	10.1[b]	
C_6H_5	NO	21.3[c]	

a) Position 3 of each epoxide was chlorinated; b) Epoxide was not isolated, and gave 3-Cl of compound I; c) Epoxide was not obtained, and gave 3-NO_2 of compound I.
Each value was an isolated yield based on the substrate.
Values in parentheses are turnover numbers based on catalyst.

The 2-C_6H_5-3-H derivative was chlorinated at position 3 simultaneously, forming epoxides in high yields. The 2-p-$SO_2CH_3C_6H_4$-3-H derivative was chlorinated at position 3 and the 2-C_6H_5-3-NO derivative was oxidized to the 3-NO_2 form without epoxidation.

The ring protons of position 6 and 7 in the 6,7-epoxide shifted to 2~2.5 ppm higher magnetic field from IBPP.[10] In the mass spectrum, M^+ = 246 m/z of epoxide was 16 m/z higher than IBPP. This mass unit corresponded to one oxygen atom and (M^+-43) = 203 m/z was the base peak for which the fragment of 43 m/z agreed with $\cdot CH(CH_3)_2$. These results indicated the 6,7-epoxide structure of IBPP. The diepoxide was a single product which was of the syn or anti form from the ^{1}H-NMR spectrum.[11] The coupling constants were $J_{8,7}$= $J_{7,8}$= 4.0 Hz, $J_{4,5}$= $J_{5,4}$= 5.0 Hz, $J_{5,6}$= $J_{8,5}$= 2.5 Hz. E. Vogel et al.[12] showed that the coupling constants of position 2 and 3 of the syn and anti forms of the 1,2;3,4-diepoxide of naphthalene were 2.97 Hz and 1.75 Hz, respectively. From the coupling constant of position 5 and 6 in the diepoxide of pyrazolo[1,5-a]pyridine ring, diepoxide was indicated to be the syn form. This point must be studied further. M^+ = 262 m/z was the molecular peak in the mass spectrum of diepoxide and 32 m/z higher than IBPP. The base peak of the diepoxide was (M^+-43) = 219 m/z.[13] Other epoxides of pyrazolo[1,5-a]pyridines were identified by ^{1}H-NMR and MS in a similar manner. The main

metabolite of IBPP in humans and various animals is the 6,7-diol form.[5] Its precursor was expected to be 6,7-epoxide, but has not been isolated and could not be synthesized from IBPP by by common oxidizing reagents such as m-chloroperbenzoic acid, hydrogen peroxide or hypochlorite. We prepared for the first time, the 6,7-epoxide by the cytochrome P-450 chemical model in a one-step procedure. Because of instability of the 6,7-epoxide in buffer solution ($t_{1/2}$ = 1.8 h), it was difficult to obtain appreciable amounts by dehydration with triphenylphosphine of 6,7-dihydro-6,7-diol, which had been isolated from rabbit urine by Miura et al.[14] In the present reaction system, the active species was considered to be a metalloxenoid.[15] Consequently, position 6 and 7 of the pyrazolo[1,5-a]pyridine ring was primarily epoxidized in a manner similar to those of olefins,[4] followed by epoxidation of position 4 and 5. Therefore, the epoxide did not seem to be formed via the 4,5-monoepoxide. In this reaction system, epoxidation did not occur without a catalyst. We succeeded in synthesizing the relatively unstable 6,7-epoxide efficiently by direct oxidation of IBPP with a chemical P-450 model system TPPMnCl-NaOCl. Chemical P-450 models could be used to obtain the unstable metabolites and to study the metabolism of drugs.

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8) $T_{2,6F}$PPFeCl: meso-Tetrakis-2,6-difluorophenylporphyrinatoiron chloride was prepared according to the method in the following report; R. W. Wagner, D. S. Lawrence and J. S. Lindsey, Tetrahedron Lett., 28, 3069 (1987).
9) 6,7-Epoxide and 4,5;6,7-diepoxide were isolated by flash silica gel column chromatography. Some of the 6,7-epoxide was lost by decomposition on silica gel during column chromatography. The reaction condition in Table I was optimized while the condition in Fig. 1 was not. Therefore the yield of diepoxide in Table I was higher than that in Fig. 1.
10) ^{1}H-NMR (400MHz, $CDCl_3$) δ; 7.22 (1H, d, 4-H), 6.46 (1H, dd, 5-H), 5.18 (1H, d, 7-H), 4.11 (1H, t, 6-H), 3.46 (1H, m), 3.14 (1H, m), 1.34 (6H, d, 3-CH_3), 1.19 (6H, d, 2-CH_3).
11) ^{1}H-NMR (400MHz, $CDCl_3$) δ; 5.18 (1H, d, 7-H), 4.26 (1H, d, 4-H), 4.10 (1H, d, 5-H), 4.08 (1H, d, 6-H), 3.40 (1H, m), 3.15 (1H, m), 1.30 (6H, d, 3-CH_3), 1.20 (6H, d, 2-CH_3).
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13) Elemental analysis of diepoxide was as follows: Calcd. for $C_{14}H_{18}N_2O_3$: C, 64.11 ; H, 6.92; N, 10.68. Found: C, 64.30; H, 6.89; N, 10.56.
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