CHAPTER IV

RESULT AND DISCUSSION

To determine the effect of exposure time and type of organic solvent on photolysis of ketoconazole , the solution of the drug in four organic solvents were irradiated under UV lamp at 254 nm . The progress of reaction was followed by TLC ,using system 1 and 2 as a developing solvent.The spots size of degradation products which showed on chromatogram indicating the amount of degradation products occured . After process , the appearance of ketoconazole in each other solvent was changed in the same feature, from clear solution to brown solution, except in methanol that change to red-brown solution when increasing the time of irradiation (Table 2). The TLC pattern of the drug in ethylacetate, chloroform, acetone were similar to that in methanol, but the photodegradation product was observed at difference rates. In acetone, ethylacetate, chloroform and methanol, degradation product could be detected after 15, 10, 5 and 5 hours, respectively. In methanol it was found that the spot size of degraded product was largest after 15 hours irradiation comparing with other solvents (Figure 3) . Comparison of the spot size of degradation product occured in different solvents and to the spot size of ketoconazole on chromatogram indicated that the degradation product occured. In the process of using methanol

Table 2 : The results of the effect of exposure time and solvents on photolysis of ketoconazole

Solvent	Ethyl acetate		Chloroform		Acetone		Methanol	
Time (hrs)	Appearance	Degraded Spot Size	Appearance	Degraded Spot Size	Appearance	Degraded Spot Size	Appearance	Degraded Spot size
0	clear	-	clear	-	clear	-	clear	-
5	clear	-	pale brown	+	clear	-	pink	+
10	pale brown	+	pale brown	++	clear	-	pink brown	++
15	pale brown	+	pale brown	++	pale brown	+	red brown	+++
20	brown	++	brown	+++	pale brown	+	red brown	+++

- no degraded spot
+ small degraded spot
++ medium degraded spot
+++ large degraded spot

as solvent and irradiation time of 15 hours was the most effective. Therefore , the process using methanol as solvent and 15 hours of irradiation time was used in order to identify the photodegradation product of ketoconazole.

Structure elucidation of compound A (degradation product)

The solution of ketoconazole in methanol was irradiated at room temperature for 15 hours under UV lamp at 254 nm wavelength. After irradiation, the solvent was evaporated to dryness under reduced pressure and the residue was dissolved in chloroform and then partition with water. From chlorofom part, compound A was obtained by column chromatography. The yield of compound A was 0.66 % .

To elucidate the structure of compound A, the structure elucidation of ketoconazole by all spectroscopic data must be considered. The UV spectrum, IR spectrum, MS spectrum, ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of ketoconazole were shown in Figure 4 -10 .

Compound A was white powder that melt at 194-195 °C and the UV absorption spectrum (Figure 11) was shown charateristic K Band (λmax = 242.5 nm.) and R Band (λmax = 294 nm.) which spectrum similar to ketoconazole (Figure 3). The IR spectrum of compound A was shown the pattern of functional group in Table 3. The peaks in region of 3,000 - 2,840 cm^{-1} showed the aliphatic C-H stretching vibration. The strong peak at 1644 cm^{-1} represented the C=O stretching vibration of tertiary amide. The aromatic C-C stretching vibration together with the aliphatic C-H bending vibration absorbed at 1511 , 1444 cm^{-1} . The

peak at 1243 cm^{-1} represented the C-N stretching vibration of tertiary amine. The aromatic C-H out-of-plane bending vibration showed the band at 761 cm^{-1} whereas the peak at 1042 cm^{-1} represented aromatic C-H in plane bending vibration. The cyclic C-O-C stretching vibration absorbed at 995 cm^{-1} (Figure 12). These were similar to IR spectrum of ketoconazole (Figure 5) .

Table 3 : The IR spectrum assignment of compound A

Range of absorption (cm^{-1})	Assignment
3,000 -2840	ν aliphatic C - H
1644 (strong)	ν C = O
1511 , 1444 (strong)	δ C - H mixed with ν aromatic C - C ring
1243	ν C - N amine
1042	δ in plane aromatic C - H
995	ν cyclic C - O - C
761	δ out of plane aromatic C - H

Comparision with ketoconazole , the electron impact mass spectrum of compound A (Figure 13) was shown molecular ion peak at m/z 494 $[M-1]^+$ whereas the ketoconazole was 530 $[M-1]^+$ (Figure 6) which 36.5 a.m.u. heaveir than compound A . The

molecular ion peak at m/z 494 $[M-1]^+$ establishing the tentative molecular of $C_{26}H_{27}ClN_4O_4$.

The 500 MHz ^{1}H-NMR spectra of compound A (Figure 14-16) showed fourteen signals of twenty seven protons. The protons were divided into nine aromatic protons , and eighteen SP^3 protons. In nine aromatic protons, the low field region (δ 6.89 - 8.08 ppm) enables one to quickly assign the protons of the two benzene rings. The signal at δ 6.89 ppm, δ 6.91 ppm showed a classical A_2B_2 splitting pattern and thus must arise from the H-21, H-25 and H-22, H-24. The signal at δ 8.08 ppm, δ 7.48 ppm δ 7.34 ppm could readily be assigned to the H -3, H-6 and H-5. The 1H signal at δ 7.34 ppm (dd, J = 2.13, 8.24 Hz) could be assigned as H-5 that ortho coupled with H-6 (δ 7.48 ppm,d, J = 8.24 Hz) and meta coupled with H-3 (δ 8.08 ppm, d, J = 2.13 Hz). This leaves the signal at δ 7.16 ppm (d, J = 1.22 Hz) and δ 6.94 ppm (d, J = 1.22 Hz) could be assigned with H-12, H-13 (or reversed) on the imidazole ring, which lossing one proton at H-10 from imidazole ring of ketoconazole when their NMR spectra were compared. The eighteen SP^3 protons could be assigned by H-H COSY experiments (Figure 19-22). The three protons singlet signal at δ 2.14 ppm must be the methyl protons (H-33 , 3H). The eight protons at δ 3.04, 3.08, 3.62 and 3.77 ppm could be assigned as H-27, H-28, H-30, H-31 on piperazine ring which chemical shift similar to ketoconazole.The signal at δ 4.73ppm (m) could be assigned as H-15, confirmed by the fact that this multiplet correlates with all four other protons signals in this group in the H-H COSY experiment. The

signal at δ4.28 ppm (d, J = 13.13 Hz)and δ 4.25 (d, J = 13.13 Hz) could be assigned as two H-8 protons which were coupled with each other, their geminal coupling constant equaled to 13.13 Hz. The other signal at δ 4.17 ppm (dd, J = 6.86, 8.70 Hz), δ 4.24 ppm (dd, J = 6.86, 8.70 Hz) and δ 4.10 ppm (dd, J = 4.43, 10.22 Hz), δ 4.13 ppm (dd, J = 4.43 , 10.22 Hz) could be assigned to H-16 (2H) , H-18 (2H) , respectively and coupled each other in H-H COSY, it confirmed by comparision with ketoconazole that the signal at H-16 (2H) showed chemical shift downfield than H-18 (2H).

Compound A ^{13}C-NMR spectrum (Figure 17) showed twenty-four signals of twenty-six carbons. The carbon types could be classified by Distortionless Enhancement by Polarization Transfer (DEPT) experiment, the DEPT 135 spectrum (Figure 18) showed positive signals of methine and methyl carbons, and negative signals of methylene carbons. From this experiment , twenty-six carbons of compound A could be divided into eight quaternary carbons at δ 168.98 ppm, δ 152.99 ppm, δ 146.01 ppm, δ 141.67 ppm, δ 136.26 ppm, δ 131.40 ppm, δ 128.20 ppm and δ 105.21 ppm, Ten methine carbons at δ 129.76 ppm, δ 128.20 ppm, δ 125.60 ppm, δ 123.89 ppm, δ 120.17 ppm, δ 118.79 ppm (2-carbons) , δ 115.50 ppm (2-carbons) and δ 75.97 ppm, seven methylene carbons at δ 67.97 ppm, δ 66.95 ppm, δ 51.95 ppm, δ 50.99 ppm, δ 50.62 ppm, δ 46.34 ppm and δ 41.45 ppm, and one methyl carbon at δ 21.30 ppm.

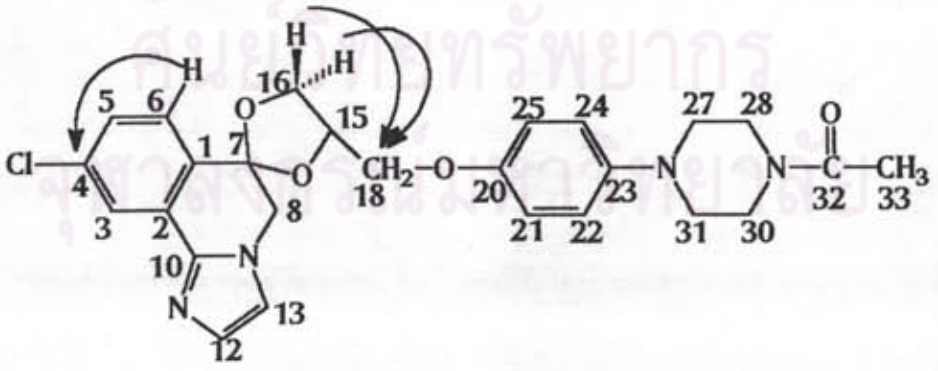
The structure of compound A was finally determined via several techniques in two dimensional NMR experiments, including H-H COSY,

C-H COSY, COLOC. From C-H COSY (Figure 23-25) the signal at δ 168.98 ppm and δ 21.30 ppm could be assigned to acetyl carbon at C-32, C-33, repectively which chemical shifts similar to ketoconazole. The next four high field signals at δ 50.99 ppm, δ 50.61 ppm, δ 46.34 ppm, δ 41.45 ppm arise from piperazine ring carbons , that could be assigned to C-31 , C-27 , C-30 and C-28 , respectively . The quaternary carbons at δ 152.99 ppm and δ 146.02 ppm , that were correlated with proton H-21 , H-22 , H-24 , H-25 , belonged to C-20 and C-23 , which in agreement with that one would expected using electronegativity arguments (e.g. carbons bonded to oxygen are at lower field than those bonded to nitrogen). The methine carbons at δ 118.50 ppm and δ 115.50 ppm had high intensity thus it contained each two aqual carbons that assigned to C-22 , C-24 and C-21 , C-25 , respectively . The aromatic carbons at C-1 , C-2 , C-3 , C-4 , C-5 , and C-6 showed signal at δ 131.46 ppm , δ 128.20 ppm , δ 123.89 ppm , δ 136.26 ppm , δ 128.20 ppm and δ 125.60 ppm confirmed by C-H COSY and COLOC (Figure 23-36). The signal at δ 141.67 ppm was assigned to C-10 , correlated with H-12 , H-13 that chemical shift was lower than ketoconazole and changed type of carbon from methine carbon to quaternary carbon in this. The other carbon on imidazole ring at C-12 and C-13 showed signal at δ 129.76 ppm and δ 120.168 ppm (or reversed). The quaternary carbon at δ105.21 ppm could be assigned to C-7 on grounds of chemical shift alone , as well as by its correlation to H-8 . The signal at δ 75.97 ppm could be assigned to C-15 , correlated with H-16

and H-18. The methylene carbon at C-8 , C-16 and C-18 showed signal at δ 51.95 ppm , δ 66.95 ppm and δ 67.97 ppm , respectively.

Table 4 : The ^{1}H (δ ppm) correlated with ^{13}C (δ ppm) at 4 Hz

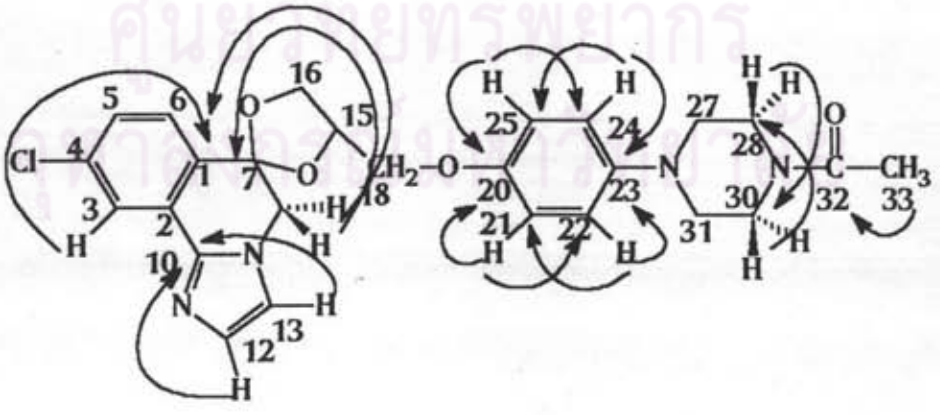
^{1}H (δ ppm)	^{13}C (δ ppm)
H-6 (δ 7.48)	C-4 (δ 136.257)
H-16 (δ 3.86,3.74)	C-18 (δ 67.986)



Scheme 12 : The correlation pattern (H to C) of compound A at 4 Hz

Table 5 : The ^{1}H (ppm) correlated with ^{13}C (ppm) at 8 Hz

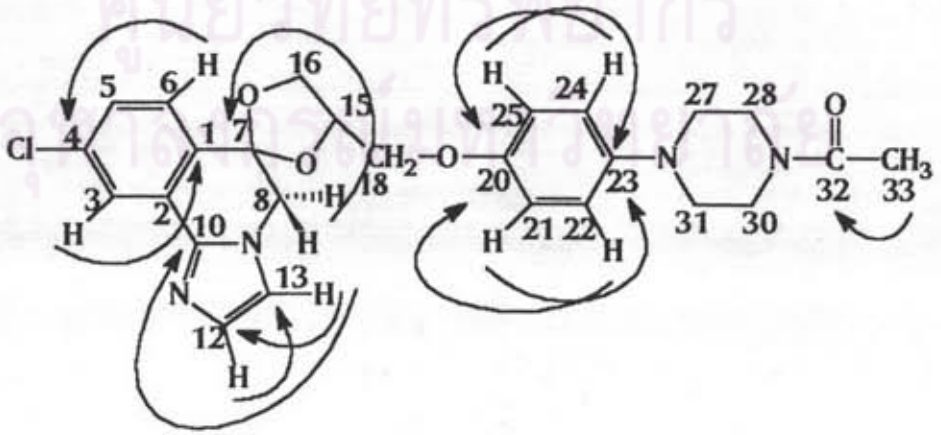
^{1}H (δ ppm)	^{13}C (δ ppm)
H-3 (δ 8.08)	C-1 (δ 131.404)
H-8 (δ 4.28 , 4.25)	C-1 (δ 131.404)
	C-7 (δ 105.214)
H-12 (δ 7.16) } (or reversed)	C-10 (δ 141.670)
H-13 (δ 6.94)	C-10 (δ 141.670)
H-21,H-25 (δ 6.89)	C-20 (δ 152.988)
	C-22 C-24 (δ118.786)
H-22,H-24 (δ 6.91)	C-21 , C-25 (δ 115.496)
	C-23 (δ 146.013)
H-33 (δ 2.14)	C-32 (δ 168.979)



Scheme 13 : The correlation pattern (H to C) of compound A at 8 Hz

Table 6 : The ^{1}H (ppm) correlated with ^{13}C (ppm) at 12 Hz

^{1}H (δ ppm)	^{13}C (δ ppm)
H-3 (δ 8.08)	C-1 (δ 131.404)
H-6 (δ 7.48)	C-4 (δ 136.257)
H-8 (δ 4.28 , 4.25)	C-7 (δ 105.214)
H-12 (δ 7.16) (or reversed)	C-10 (δ 141.670)
	C-13 (δ 120.168)
H-13 (δ 6.94)	C-12 (δ 129.759)
H-22 , H-24 (δ 6.91)	C-20 (δ 152.988)
H-21 , H-25 (δ 6.89)	C-23 (δ 146.013)
H-28 (δ 3.77 , 3.62)	C-30 (δ 46.335)
H-30 (δ 3.77 , 3.62)	C-28 (δ 46.449)
H-33 (δ 2.14)	C-32 (δ 168.979)

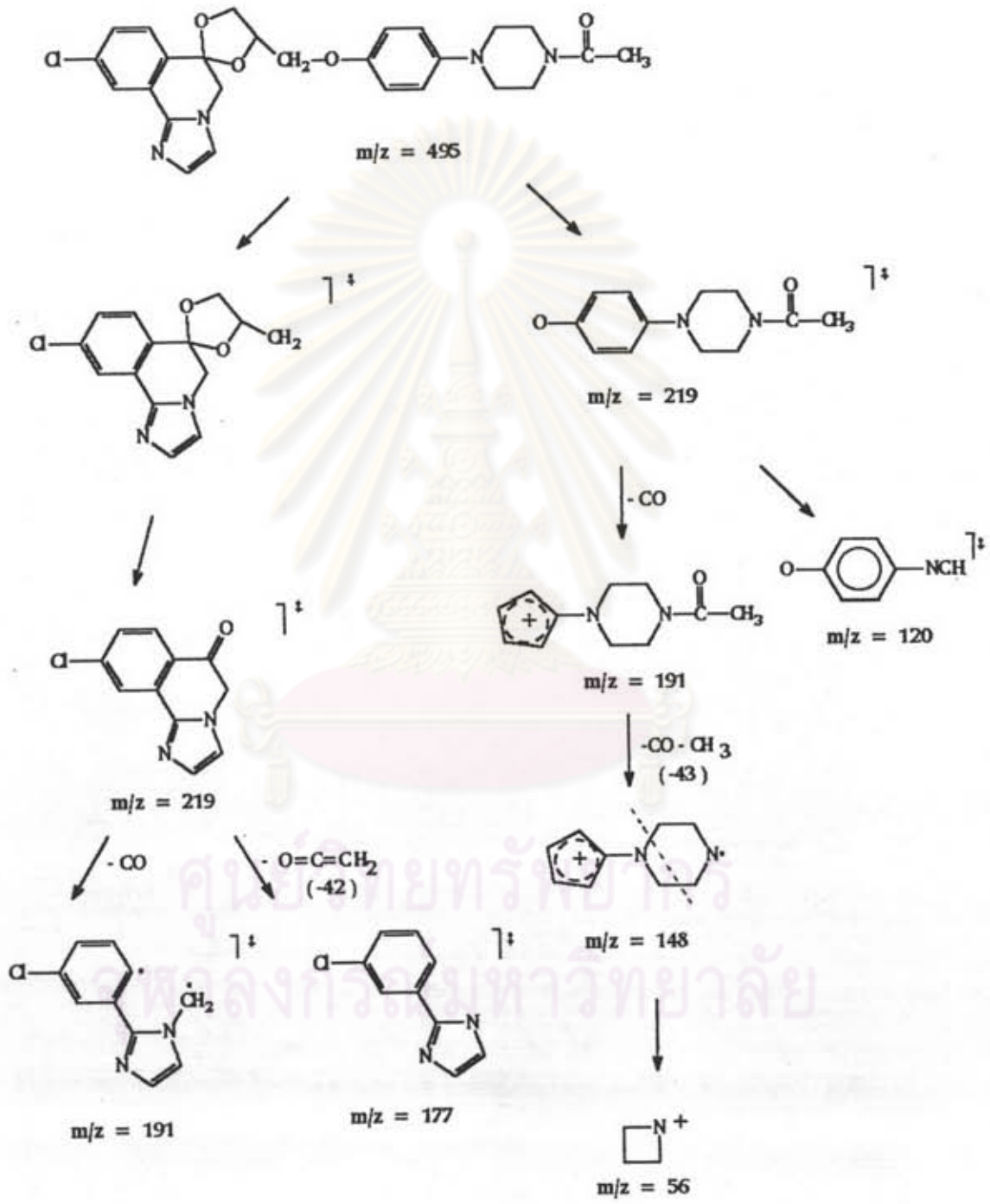


Scheme 14 : the correlation pattern (H to C) of compound A at 12 Hz

Position	(ppm) of C	(ppm) of H (multiplicity, J Hz)	long range correlation from H to C in COLOC		
			J = 4Hz	J = 8Hz	J = 12Hz
1	131.404				
2	128.196				
3	123.886	8.08 (d,2.13)		C-1	C-1
4	136.257				
5	128.328	7.34 (dd,2.13,8.24)			
6	125.597	7.48 (d,8.24)	C-4		C-4
7	105.214				
8	51.945	4.28 (d,13.13),4.25 (d,13.13)		C-1,C-7	C-7
10	141.67				
12 •	129.759	7.16 (d,1.22)		C-10	C-10,C-13
13 •	120.168	6.94(d,1.22)		C-10	C-12
15	75.964	4.73(m)			
16	66.948	4.34 (dd,6.56,8.70) 4.17 (dd,6.56,8.70)	C-18		
18	67.968	4.13 (dd,4.43,10.18) 4.10 (dd)4.43,10.18)			
20	152.988				
21,25	115.496	6.89 (dd)		C-20 C-22,C-24	C-23
22,24	118.786	6.91 (dd)		C-21,C-23 C-25	C-20
23	146.013				
27	50.612	3.08 (t,5.19),3.04(t,5.19)		C-31	
28	41.449	3.71 (t,5.19),3.62(t,5.19)		C-30	C-30
30	46.335	3.71 (t,5.19),3.62(t,5.19)		C-28	C-28
31	50.991	3.08 (t,5.19),3.04(t,5.19)		C-27	
32	168.979				
33	21.296	2.14 (s)		C-32	C-32

• = the position 12 & 13 assignment may be reversible

Table 7 : ^{1}H and ^{13}C assignment of compound A

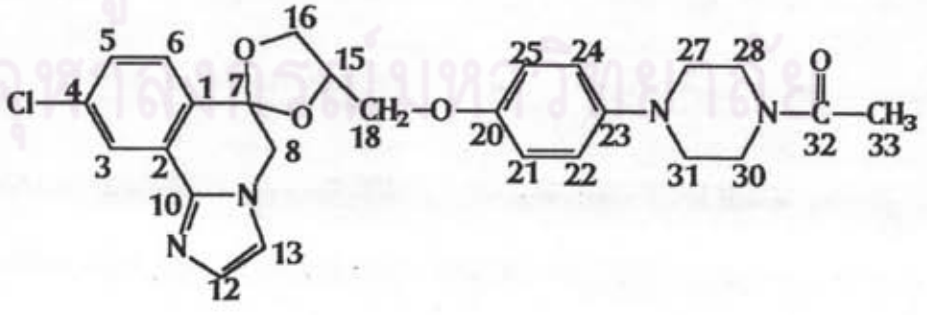


Scheme 15 : The proposed MS - Fragmentation of compound A

From 4 Hz , 8Hz , and 12Hz COLOC spectrum (Figure 26-26 , at confirmed the carbon position and defined the quaternary carbons as followed (see Scheme 12-14) .

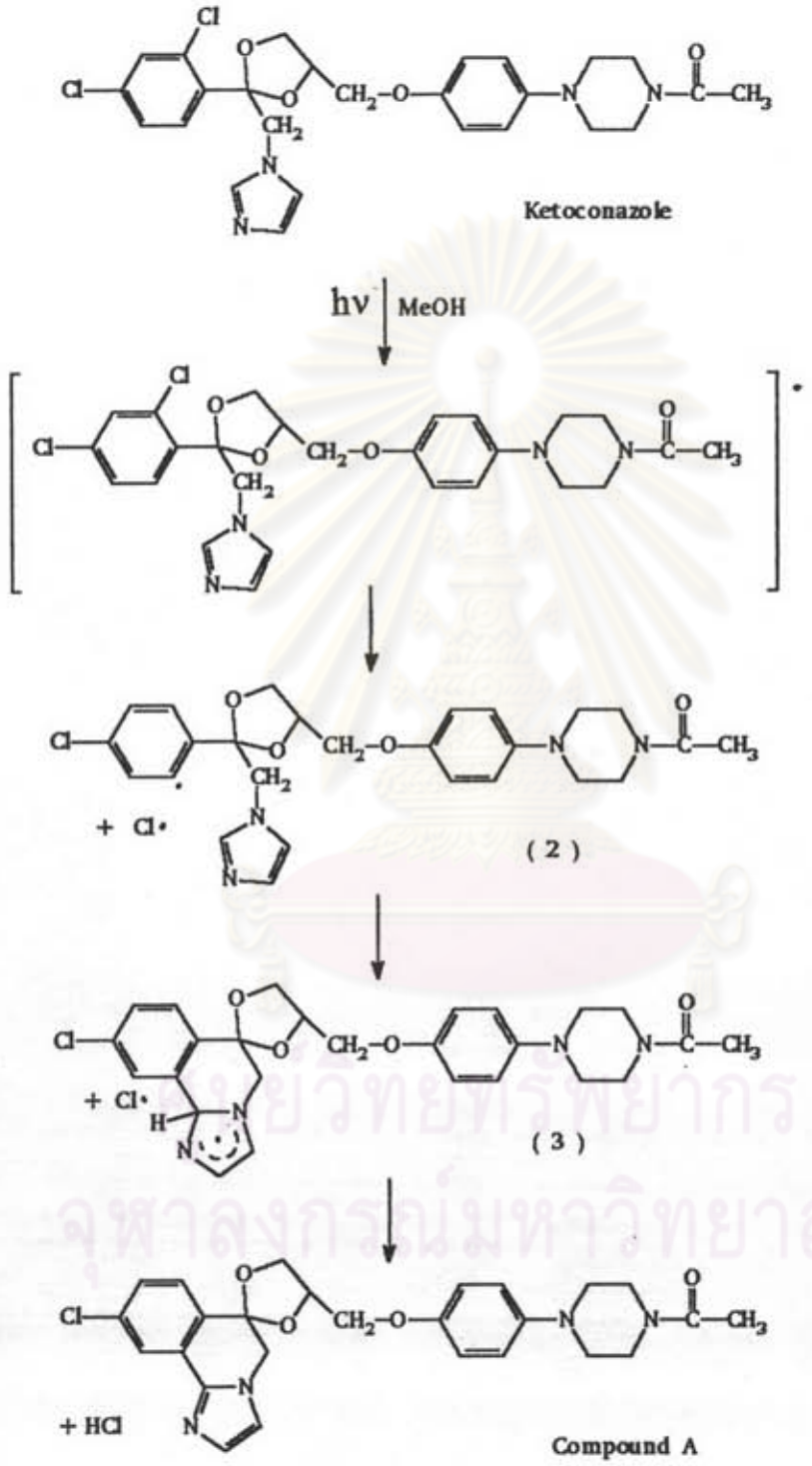
All protons and carbons assignment of compound A were shown in Table 7 . This structure was confirmed by analysis of the mass fragmentation (Scheme 15). The molecular ion showed at m/z 219 (M-275) which this fragment be produced by cleavage of ether linkage in molecule , that continued lossing carbon monoxide (- CO) giving m/z 191 and gave m/z 148 , when it loss -acetyl group (-CO-CH_3) and continued cleavaging on piperazine ring to give base peak at m/z 56 that represented azoetidine cation. Some important ions in the mass fragmentations were peak at m/z 70 , 120 , 435 . The fragmentation patterns described aboved were shown in Scheme 15.

All data support the structure of compound A was 1-acetyl-4-[-4-[[(1*H*-imidazo2,1-a]3,4-dihydro-7-chloro-isoquinolyl)-6-spiro-2′-(1,3-dioxolan-4-yl)]methoxy]phenyl]piperazine.

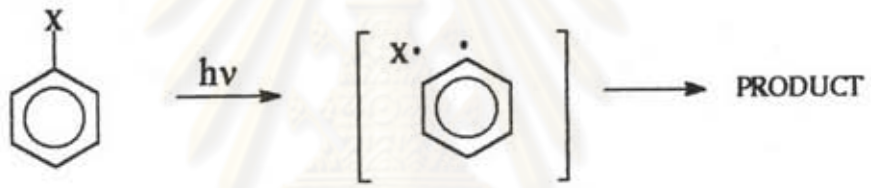


A possible pathway for the photodegradation of ketoconazole was shown in Scheme 16 . There were many reports involved the mechanism of photolysis which were similar to that described in this report. (Nijhoff and Havinga , 1965 ; Henderson and Zweig , 1967 ; Kharasch and Sharma , 1968 ; Pinhey and Rigby ,1969 ; Robinson and Vernon , 1970 ; Hey , Jones , and Perkins , 1971) . In 1969 , Pinhey and Rigby reported that the photolysis of haloaromatic compounds resulted in homolytic cleavage to give phenyl radical as shown in Table 8 and Scheme 17. In 1967 , Henderson and Zweig reported that the photodegradation products of 1-*o*-chlorophenylnaphthalene (1) were compound (3), (4) , (5) (Sheme 18). This reaction was believed to proceed via free radical intermediate (2) derived by homolytic cleavage C-Cl bond .

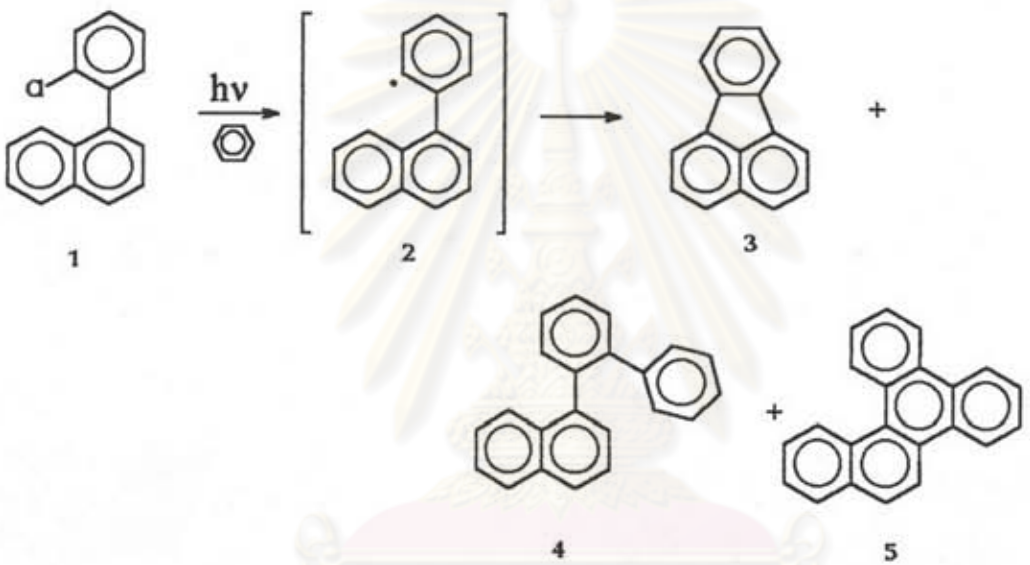
The photocyclization of ketoconazole, involving a novel bond formation between the ortho-position of phenyl ring and 2-position of the imidazole ring was also considered to be feasible. This reaction should undergo cyclization of the initial aryl radical (2), derived by loss of a chlorine atom to give isoquinoline intermediate (3) . The intermediate could gave product by reaction with some unspecified hydrogen acceptor such as chlorine atom within the reaction system, to give hydrochloric acid.



Scheme 16 : The proposed mechanism photodegradation of ketoconazole



Scheme 17 : The photolysis mechanism haloaromatic compounds



Scheme 18 : The degradation products of 1 - o - chlorophenylnaphthalene on photolysis

Table 8 : Results of the photolysis of haloaromatic compounds.

Compounds	Solvents	Time of irradiation (hrs)	Degradation products
p-Chlorophenol	Ethanol	5.3	Phenol
Chlorobenzene	Isopropanol	12	Benzene
Bromobenzene	Isopropanol	6	Benzene
Iodobenzene	Isopropanol	12	Benzene
p-Chlorophenol	Isopropanol	3.5	Phenol
p-Bromphenol	Isopropanol	6	Phenol
p-Iodophenol	Isopropanol	5.5	Phenol
o-Chlorophenol	Isopropanol	6.5	Phenol
o-Bromphenol	Isopropanol	8	Phenol
o-Iodophenol	Isopropanol	3.5	Phenol