CHAPTER III

EXPERIMENT

I. Chemicals

Ketoconazole was obtained from Biolab Company (Bangkok , Thailand ,Lot No.KZL-30/93).

All solvents used were either B.P. or laboratory grade.

II. General Technique

2.1 Thin-layer chromatography (TLC)

Technique : One way ascending

Absorbent : Silica gel GF_{254} (E.Merck) 30 g in 60 ml distilled water

Plate sizes : 5 x 20 mm.

Layer Thickness : 0.25 mm.

Activation : Air-dried for 15 minutes and then warm in hot air oven at 110 ^{0}C for 1 hour.

Solvent system : system 1 , chloroform : acetone (1:1)

system 2 , 5 % methanol in chlorform

Distance : 15 cm.

Laboratory Temperature : 28-35 ^{0}C

Detection : 1. Visual detection under ultraviolet light at the wavelength of 254 nm and 366 nm.

2. Iodine vapour generated from iodine crystal that bind with unsaturated organic compound and present as colour spot.

2.2 Column chromatography (CC)

Column Size : The glass column 3/4 - 2 inches in diameter were used depending on the quantity of sample to be seperated

Absorbent : Silica gel 60 (E . Merck) particle size 0.063 - 0.2 μm (70-230 mesh ASTM)

Packing method : Wet packing

Solvent : various solvent systems depending on materials

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) Absorption Spectra

The spectra were obtained by Milton Roy Spectronic 3000 array (Department of Pharmaceutical Sciences ,Chulalongkorn University). Chloroform was used as solvent.

2.3.2 Infrared (IR) Absorption Spectra

The spectra were obtained by a Magna - IR 750 infrared Spectrometer (Department of Pharmaceutical Sciences, Silapakorn University)

2.3.3 Mass Spectra (MS)

The Electron Impact Mass Spectra (EIMS) generated by a Fisios VG Trio 2000 quadrupole mass spectrometer (Department of Sciences , Mahidol University).

2.3.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

The 500 MHz ^{1}H-NMR and 125 MHz ^{13}C spectra were obtained by a JEOL JMN-A 500 spectrometer (The Scientific and Technological Research Equipment Centre , Chulalongkorn University).

Chloroform-*d* were used as operating solvent. Chemical shifts were reported in ppm (δ) scale and tetramethylsilane (TMS) was used as standard reference.

2.4 Crystallization Technique

The impure crystalline compounds were purified by crystallization from suitable solvent. The crystallization process consisted of :

1. Dissolved impure compounds with suitable small amount of solvent and warmed until all crystal were dissolved.

2. The hot solution was filtrated.

3. Allowed the solution cool down to room temperature.

4. If it did not crystallize out , add the second solvent that the compound was less dissolved into the solution.

5. Separated the crystal by vacuum filtration.

Checked the purify of crystal by TLC and melting point. Recrystallization could be used if the compound was not pure.

2.5 Melting point

Melting point was obtained by a Buchi glass capillary apparatus (Department of Pharmaceutical Chemistry , Faculty of Pharmaceutical Sciences , Chulalongkorn University)

Effect of exposure time and solvents on photolysis of ketoconazole.

Solution of ketconazole in ethylacetate, chloroform, acetone and methanol were prepared by adding 5 mg of the compound to 10 ml of each solvents in glass test tube and were shaken until the solution were clear. The solutions were irradiated by UV lamp (15 W, 45 cm long) of 254 nm wavelength placed 20 cm vertically from the samples in a darken cabinet at room temperature .The sample was shaken every 1 hour and the solvent was added to maintain original volume. Reaction was monitored at 5 hours interval over a 20 hours period. The progress of reaction was followed by thin layer chromatography (TLC) with system 1 and 2 and detected spot, reacting with iodine vapour in iodine tank. The result was shown in Table 2 and Figure 3.

Photolysis of ketoconazole

Ketoconazole in methanol (5 g , 9.41 mmol in 100 ml)was irradiated at room temperature for 15 hours under UV lamp (15 W, 45 cm long , wavelength 254 nm) which was placed 20 cm vertically from the sample in a darken cabinet . After irradiation, the solvent was concentrated and evaporated to dryness under reduced pressure at room temperature to yield 5.47 g of red-brown syrupy residue .

Isolation and Purification of Degradation product.

The residue was dissolved in 30 ml. of chloroform and then was partitioned with each 30 ml water for two times. The chloroform extract was concentrated and evaporated to dryness under reduced pressure at room temperature, thus giving the 4.76 g of brown syrupy residue. The brown syrupy residue was chromatographed over silica gel 60 (column 37.5 cm high and diameter 3 cm). The column was eluted with 300 ml. ethylacetate and 550 ml. chloroform : acetone (1 : 1),respectively. The eluates were collected 25 ml per fraction and examined by TLC , using 5% methanol in chloroform as a developing solvent.The fraction which showed the same pattern on TLC were combined and test for degradation products.The combined fractions 25-34 were concentrated under reduced pressure. Compound A, as a white powder , was crystallized from these fractions and recrystallzed in chloroform which gave 33 mg yield .

Characterization of Degradation product

Degradation product was crystallized in chloroform as white powder compound. It was soluble in chloroform, methanol, less soluble in ethylacetate.

EIMS : m/z (relative intensity) ; Figure 13

494 (16.55, $[M-1]^+$), 435 (24.64) , 422 (44.26) , 396 (11.42), 275(18.14) , 231(34.25) , 219(53.19) , 217 (41.31) , 191(16.99) , 177(23.29) , 148(28.67) , 120 (39.47) , 70 (15.15) , 56(100)

UV : λmax nm (log ε), in chloroform ; Figure 11

242.5 (4.09) , 294 (4.04)

IR : cm^{-1}, KBr (disc) ; Figure 12

2996 , 2923 , 2822 , 1644 , 1511 , 1444 , 1243 , 1042 , 995

^{1}H - NMR : ppm , 500 MHz , in $CDCl_3$; Figure 14 - 16

8.08 (1H,d,J = 2.13 Hz) , 7.48 (1H,d,J = 8.24 Hz) , 7.34 (1H,dd,J = 2.13 , 8.24 Hz) , 7.16 (1H,d,J = 1.23Hz) , 6.94 (1H,d,J = 1.22 Hz) , 6.91 (2H,m) , 6.89 (2H,m) , 4.73 (1H,m) , 4.34 (1H,dd,J = 6.56 , 8.70 Hz), 4.28 (1H,d,J = 10.13 Hz), 4.25 (1H,d,J = 10.13 Hz), 4.17 (1H,dd , J = 6.56, 8.70Hz), 4.13 (1H,dd,J = 4.43,10.22Hz) , 4.10 (1H,dd,J = 4.43,10.22 Hz) , 3.71 (2H,t,J = 5.19 Hz), 3.62 (2H,t,J = 5.19 Hz) , 3.08 (2H,t,J = 5.19 Hz), 3.04 (2H,t,J = 5.19 Hz) , 2.14 (3H,s)

^{13}C - NMR : ppm , 125 MHz in $CDCl_3$; Figure 17

168.98 , 152.99 , 146.01 , 141.67 , 136.26 , 131.40 , 129.76 , 128.33 , 128.20 ,125.60 , 123.89 ,120.17 , 118.795 , 50. 99 , 50.61 , 46.33 , 41.45 , 21.30