CHAPTER II

HISTORICAL

Chemistry of Ketoconazole

1. Chemical name : The chemical name of ketoconazole is *cis* -1- Acetyl -4- [4-[[2- (2 , 4 - dichlorophenyl) -2- (1*H*- imidazol -1- yl methyl)-1,3-dioxolan-4-yl]-methoxy] phenyl] piperazine. Its structure and numbering scheme are shown in figure 2 .
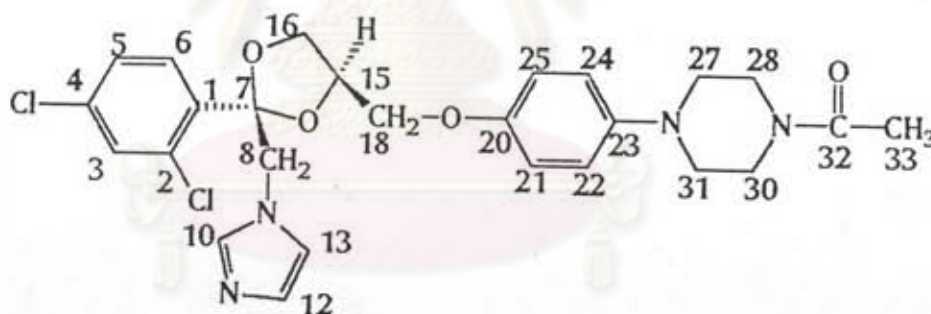

Figure 2 : Chemical structure and numbering of ketoconazole

Empirical formula : $C_{26} H_{28} Cl_2 N_4 O_4$

Molecular weight : 531.44

pKa : 2.94 , 6.51

2. Description : white to slightly beige powder , melting range 147 - 150 °C

3. <u>Solubility</u> : Practically insoluble in water , soluble 1 in 54 of ethanol , 1 in 2 of chloroform , 1 in 9 of methanol , very slightly soluble in ether.

4. <u>Synthesis</u> (Heeres et al ., 1979)

4.1 The synthesis , starting from 2,4-dichloroacetophenone is outlined in Scheme 2 . Ketalization of 1 with glycerine was performed in a benzene , *n*-butanol medium with azeotropic removal of water in the presence of a catalytic amount of *p* - toluenesulfonic acid. Without isolation , the ketal 2 was brominated at 30 °C to bromo ketal 3.

4.2 Benzoylation of 3 in pyridine afforded the ester as a cis/trans mixture , from which the cis form 4 could be isolated by crystallization from EtOH. The pure trans isomer could be obtained by liquid chromatography of the mother liquor.

4.3 Coupling of bromo ketal 4 in dry DMA with imidazole gave the imidazole derivative 5. The ester 5 was saponified at reflux with NaOH in dioxane-water medium to the alcohol 6. This alcohol was converted to methanesulfonate 7, which was coupled with the sodium salt of 8 to give ketoconazole .

5. <u>Determination of Ketoconazole</u> : Many method have been used to assay ketoconazole in pharmaceutical preparation , raw material and human serum. Some of them are described below ;
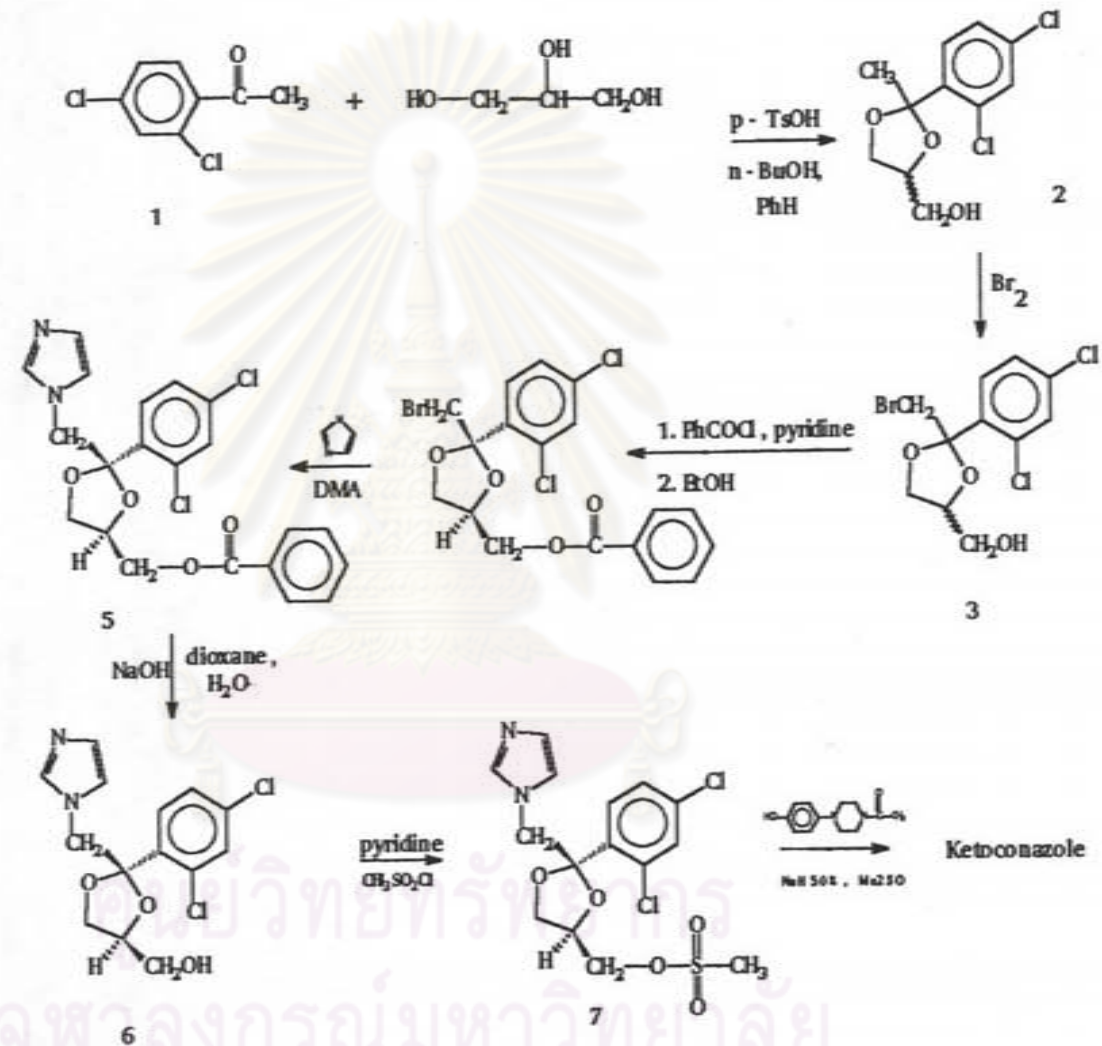
Scheme : 2   Syntheses of Ketoconazole

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5.1 Colorimetric Method

In 1988 , Sane and workers had reported the determination of ketoconazole in pharmaceutical preparation. The method was based on the formation of ion-pair complexes of the drug with reagents like bromocresol green (BCG) , bromocresol purple (BCP) , bromophenol blue (BPB) and bromopheol red (BPR) in acidic medium . The ion-pair complexes formed were quantitatively extracted in chloroform and its absorbance was measured at 420nm. The method was statistically validated and was found to be precise and accurate.

5.2 High Performance Liquid Chromotographic Methods (HPLC)

Many methods for the determination of ketoconazole by HPLC have been reported by Alton (1980) , Swezey et at. (1982) , Badcock (1984) , Column , mobile phase , wavelength of UV - detector and conditions used were different in order to be applicable for each method. For example , in 1982 , Swezey and workers assayed ketoconazole in human plasma by using μ-Bonapack C-18 reversed phase column , acetonitrile : 0.05 M Sorensen ' s phosphate buffer (pH 6.6 with hydrochloric acid) (60 : 40) as mobile phase and 370 nm UV-detector.

5.3 Potentiometric Method

Potentiometric method for the determination of ketoconazole in raw material was recommended by the USP XXII(1990). Ketoconazole was dissolved in glacial acetic acid and titrated with perchloric acid.

6. Identification of the chemical structure

      6.1 Ultraviolet spectrum (Moffat et al ., 1986)

In aqueous acid it has absorption maxima at 269 nm ($A_1^1 = 26$ a)

In aqueous alkali it has absorption maxima at 287 nm ($A_1^1 = 29$ b)

In methanol it has two absorption maxima at 244 nm ($A_1^1 = 280$ b)
and 296 nm($A_1^1 = 32$ b)

      6.2 Infrared spectrum(Moffat et al ., 1986)

      Principle peaks at wavenumber 1640 , 1507, 1258 , 1240 ,
1211, 1200 , cm^{-1} (KBr disk)

      6.3 NMR proton and ^{13}C spectrum (Dawson , 1990)

      A Burker AM -400 was used to measure the proton and
^{13}C spectrum. The spectrum was measured with the base dissolved
in $CDCl_3$, using TMS as the internal standard. Table 1 gives the valves
of δ (ppm) and J (Hz) corresponding to their structural assignation.

Pharmacology

Antifungal activity : Ketoconazole is active against most
pathogenic fungi, including dermatophytes and yeasts so it used to treat a
wide variety of superficial or systemic fungal infection. Ketoconazole is
effective after oral administration against superficial mycoses , such
as dermatophyte or yeast skin infection , *Pityriasis versicolor*,
oncomycosis, oral or vaginal candidosis and systemic mycoses , such as
systemic candidosis , paracoccidioidomycoses , histoplasmosis (Heel et
al.,1982 ;Borger and Bossche ,1982).

Site	δH	δC
1		134.36
2		132.68
3	7.45 (d , $J_{3,5}$ = 2.0 Hz)	131.02
4		135.49
5	7.25 (dd)	126.93
6	7.58 (d, $J_{5,6}$ = 8.4 Hz)	129.25
7		107.70
8	4.49 , 4.39 (q)	50.92
10	7.50 (dd)	138.51
12	6.98 (dd)	128.22
13	6.96 (dd)	120.90
15	4.33 (m)	74.47
16	3.86 (dd) , 3.74 (m)	67.23
18	3.74 (dd) , 3.29 (dd)	67.34
20		152.56
21,25	6.77 (m)	114.95
22,24	6.88 (m)	118.43
23		145.41
27	3.05 (dd) , 3.01 (dd)	50.34
28	3.74 (m) , 3.60 (dd)	41.15
30	3.74 (m) , 3.60 (dd)	46.05
31	3.05 (dd) , 3.01 (dd)	50.69
32		168.61
33	2.12 (s)	21.07

Table 1 : ^{1}H and ^{13}C chemical shift of ketoconazole (δH and δC from TMS ± 0.01). , (Dawson ,1990)

Mechanism of action : Like other imidazole derivatives, ketoconazole presumably exerts its antifungal activity by altering cellular membrane, resulting in increased membrane permeability, secondary metabolic effects, and growth inhibition. (Heel et al.,1982 ; McEvoy, ed ., 1989). Although the exact mechanism of action of ketoconazole has not been fully determined, it has been suggest that the fungistatic activity of the drug may result from interference with ergosterol synthesis probably via inhibition of C-14 demethylation of sterol intermediate (e.g. lanosterol) (McEvoy , ed ., 1989).

Pharmacokinetic

Absorption : Ketoconazole is rapidly adsorbed from the GI tract. The bioavailability of oral ketoconazole depends on the pH of the gastric content in the stomach ; an increase in the pH results in decrease absorption of the drug. The effect of food on the rate and extent of GI absorption of ketoconazole has not been clearly determined (Heel et al., 1982).

Distribution : Ketoconazole has been detected in urine, bile, saliva , sebum , cerumen , and synovial fluid, following oral administration of a 200 mg dose of drug in adult . In human blood, only 1% of ketoconazole is presented as free drug in plasma , 83.7% is bound to plasma proteins ,primarily albumin and 15.3% in blood cell (Daneshmend and Wornock , 1988).

Elimination : Ketoconazole is partially metabolized in the liver, to several inactive metabolites by oxidation and degradation of the imidazole and piperazine rings, by oxidative O-dealkylation , and by aromatic hydroxylation. The major route of elimination of ketoconazole and the metabolites appears to be excretion into the feces via the bile (McEvoy , ed . , 1989).

Photolysis

Consideration of the decomposition of pharmaceutical compounds resulting from the absorption of radiant energy in the form of light has become more important in recent years because of the complex chemical structure of many new drugs (Lachman ,Lieberman and Kanig ,eds. , 1986).

Mechanism of photolysis

Two major mechanisms can be identified (Lin and Lachman , 1969 ; Stewart and Tucker , 1985 ; Conners , Amidon and Stella ,eds. , 1986)

(a) primary photochemical decomposition ;

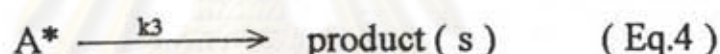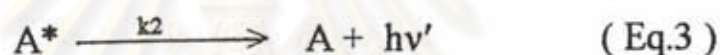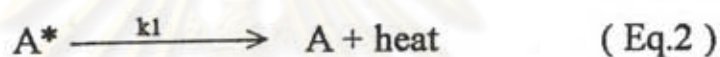(b) photosensitiser or secondary photochemical decomposition

Primary photochemical reactions occur when the drug molecule (A) itself absorbs energy from the radiation source , then an unstable excited state species (A*) is produced (Eq.1). The absorbed energy can be lost either by a radiative mechanism in which the energy is given off several ways :

(a) as thermal energy producing an increase in temperature in the surrounding medium (Eq.2) ;

(b) as fluorescence or phosphorescence where the absorbed energy is re-emitted as longer wavelength radiant energy (Eq.3) ; or

(c) as chemical energy initiating chemical decomposition (Eq.4).

The whole process can be defined by Equations 1 - 4

$$A \xrightarrow{\;h\nu\;} A^* \qquad\qquad (Eq.1)$$
$$A^* \xrightarrow{\;k1\;} A + heat \qquad (Eq.2)$$
$$A^* \xrightarrow{\;k2\;} A + h\nu' \qquad (Eq.3)$$
$$A^* \xrightarrow{\;k3\;} product (s) \qquad (Eq.4)$$

The potential for decomposition of a drug will be greater at shorter wavelengths since the energy of radiation is related to wavelength by :

$$E = h\nu \qquad\qquad (Eq.5)$$
$$\nu = c / \lambda \qquad\qquad (Eq.6)$$

Where E = energy absorbed

      h = Planck's constant (6.625×10^{-27} erg-s)

      ν = frequency of the radiation in Hz (s^{-1})

      c = velocity of light

      λ = wavelength

Thus the shorter the wavelength (λ) or the higher the frequency (v) , the greater is the energy absorbed . Consequently , drug degradation is more like to occur when radiation is absorbed in the ultraviolet and lower visible regions of the spectrum. The chemical reactions occurring are complex but include oxidation-reduction , ring rearrangement and modification , and polymerisation.

In photosensitiser or secondary photochemical reactions , the energy is absorbed by non-drug molecules (B) which impart their increased energy to the drug molecules (A) with subsequent degradation (Eqs. 7 , 8). The molecules absorbed radiant energy are called photosensitisers and act as catalyst for drug decomposition.

$$B \xrightarrow{\;hv\;} B^* \qquad\qquad (\text{Eq.7})$$

$$B^* + A \longrightarrow A^* + B \qquad (\text{Eq.8})$$
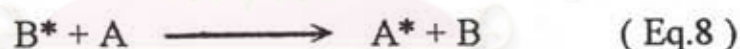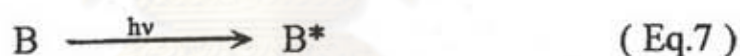
The kinetics of photochemical reactions is more complicated than kinetics of thermal reactions . (Lachman et al., 1986) This is due to (1) the complex nature of most photochemical reactions (Lin and Lachman ,1969) ; (2) various factors which include formulation factors (nature of solute , solvent , pH , buffer type , concentration and excipients), and storage factors (radiation sources , time and intensity of irradiation , temperature and packaging) (Stewart and Tucker,1985) and(3) the photochemical reaction may be enhanced by , inhibited by ,

or independent of simultaneous thermal reactions.(Lin and Lachman , 1969)

As a result of the complexity of photolytic reactions , zero-order , first-order and second-order are possible in photodegradative reactions. Photolysis reactions are usually associated with oxidation because the latter class of reaction is often initiated by light (Mollica , Ahuja and Cohen , 1978 ; Banker and Rhodes , 1990).

Prevention of photolytic reactions

To prevent degradation of drugs by photolysis , the use of appropriate light resistant containers offers the best form of protection against decomposition. Generally amber bottles will restrict the incident energy below 470 nm. (Lachman , Swartz ,and Cooper , 1960) In addition , depending on the type of chemical reaction caused by light absorption , the formulation can be manipulated to stabilize the drug (Stewart and Tucker,1985).
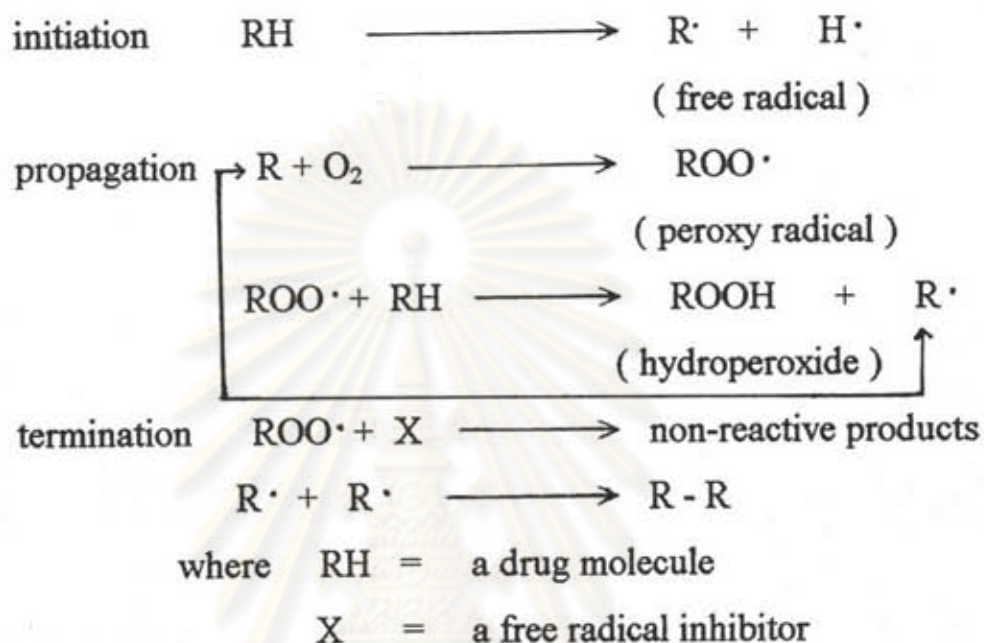
Oxidation

Oxidation is one of the major causes of drug instability. Two basic mechanisms of oxidation exist :

(a) autoxidation which involves reaction with molecular oxygen, chain reactions and free radical formation ; and

(b) the reversible loss of electrons without the addition of oxygen.

(a) Autoxidation occurs in three phases : initiation , propagation and termination , as in scheme 3

initiation $\quad$ RH $\quad\longrightarrow\quad$ R· + H·

(free radical)

propagation $\quad$ R + O₂ $\quad\longrightarrow\quad$ ROO·

(peroxy radical)

ROO· + RH $\quad\longrightarrow\quad$ ROOH + R·

(hydroperoxide)

termination $\quad$ ROO· + X $\quad\longrightarrow\quad$ non-reactive products

R· + R· $\quad\longrightarrow\quad$ R - R

where $\quad$ RH $\quad=\quad$ a drug molecule

X $\quad=\quad$ a free radical inhibitor

Scheme 3

Initiation process is catalyzed by heat and light (Mollica , Ahuja and Cohen ,1978). In addition , autoxidation is catalyzed by hydrogen ion concentration , trace metals or peroxides and presence of oxygen (Aker ,1982 ;Stewart and Tucker ,1985).

(b) Oxidation occurs by the reversible loss of electrons without the addition of oxygen. This process of oxidation involves the transfer of electrons and protons. A chemical oxidation-reduction half-reaction can be expressed by :

$$\text{reduced form} \rightleftharpoons \text{oxidized form} + \text{n electrons} \quad (\text{Eq.9})$$
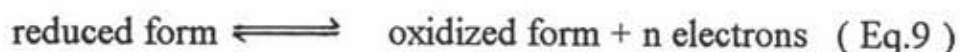
The Nernst equation is used to compute standard oxidation potential (E°). The greater the standard oxidation potential of the cell, e.g. , the greater the difference between the oxidation and reduction half- cell potentials , the more readily will oxidation occur.(Stewart and Tucker , 1985)

Photolysis of Imidazole

Because imidazole is the important part in the structure of ketoconazole , so photochemical reaction of imidazole must be considerable . When imidazole or imidazole derivative was exposed to light , it could be changed as following :

A. <u>Fragmentation</u>

Many studies about the photochemical fragmentation product of imidazole derivatives have been reported (Ogata et al . ,1970 , Haddadin , Hawi , and Nazer , 1978 ; Gainsford and Woolhouse , 1978) . For example , in 1970 , Ogata and coworkers reported the formation of N - Benzylidene - N' -propionyl - O - phenylenediamine (18.14 %)   as the major product from the irradiation of 1 - benzyl - 2 - ethylbenzimidazole - 3 - oxide (Scheme 4) .
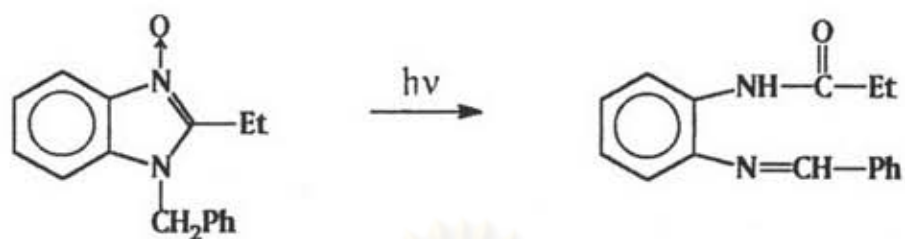
Scheme 4 : The photodegraded product of 1-benzyl - 2 - ethybenzimidazole - 3 - oxide
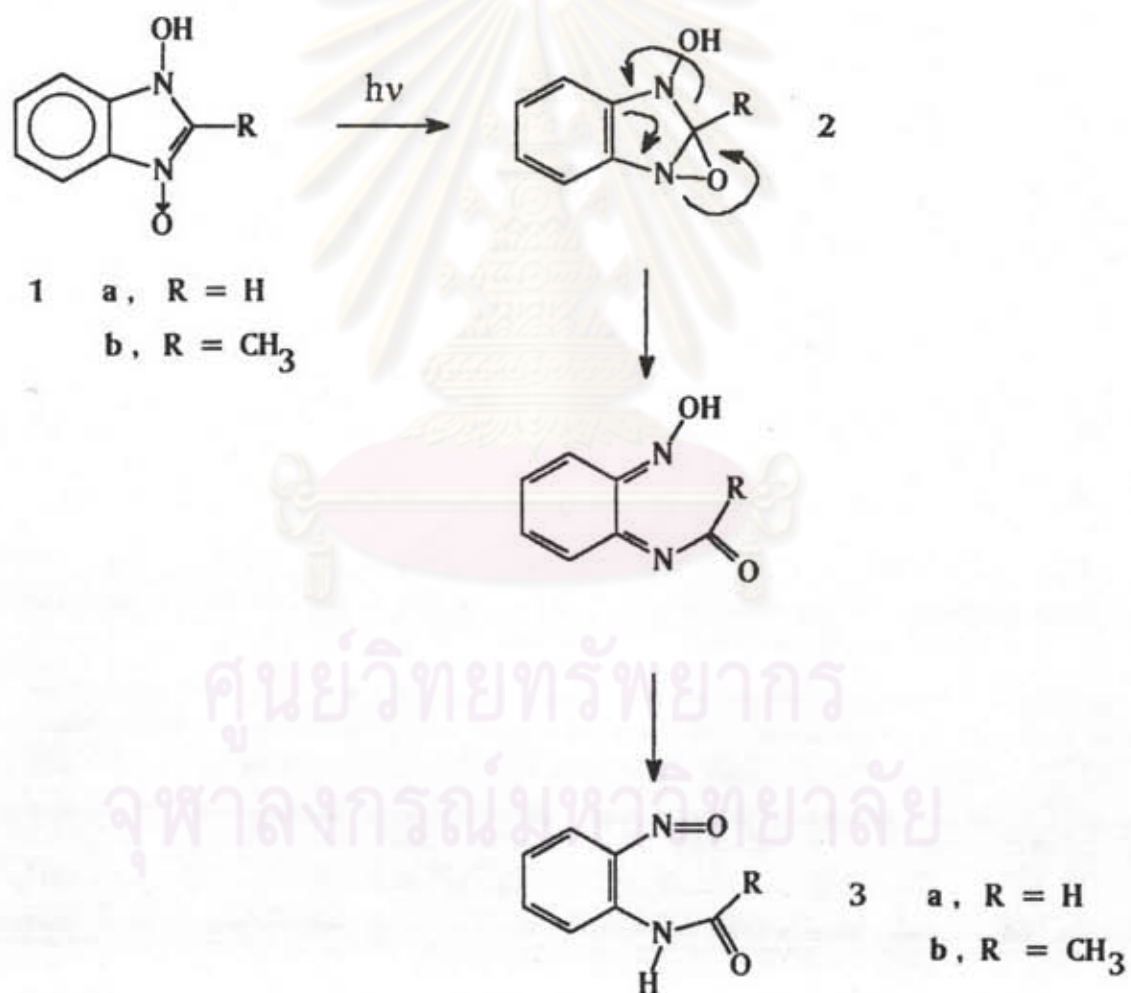
Scheme : 5 The photodegraded product of benzimidazole 3-oxide

Similarly , Haddadin and his colleaques found that , when 1 - hydroxy - 2 -methylbenzimidazole 3-oxide (1b) was photolyzed , the product was o-nitrosoacetanilide (3b) and whereas there is no substituent at C-2 (1a) , the product isolated was o - nitrosoformanilide (3a). This reaction was believed to proceed via a fused oxaziridine intermediate (2) (Scheme 5).
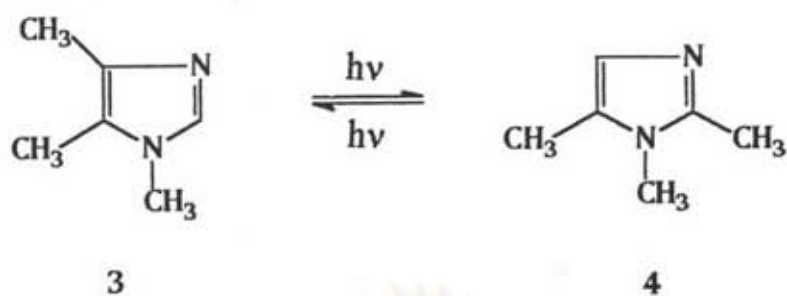
B. Rearrangememt

Although the reverse reaction was well known , the photochemical conversion of imidazoles into pyrazoles and benzimidazole into indazoles does not take place . However , 1,4 - and 1,2 - disubstituted imidazoles were interconverted in *t* - butanol , while 1,4,5- trimethyl imidazole gives the 1,2,5 - isomer in ethanol , *t* - butanol or cyclohexane .In 1969 , Beak and Messer reported that , irradiation of 1,4 -dimethylimidazole (1) in *t* - butanol for 41 hours gives 1,2 - dimethylimidazole (2) in 40 % conversion which under the same condition 2 is converted to 1 with 50 % conversion after 30.5 hours (Scheme 6) and photolysis 1,4,5 - trimethylimidazole (3) in ethanol or cyclohexane gives 1,2,5 - trimethylimidazole (4) (Scheme 7) . The photorearrangements of 1 , 2 and 3 to isomeric imidazole may involves an initial disrotatory formation of bicyclic isomer (6) followed by a 1,3 - sigmatropic shift to a second bicyclic isomer (7) which undergoes a disrotatory ring opening to the product (Scheme 8) . In 1976 ,Copper and Ervin reported that under irradiation at 300 nm , *trans* -1-

styrylimidazoles (1) were transformed into the cis - isomers (2) which subsequently undergo photocyclization to 3 (Scheme 9). This was probably a radical cyclization on to the imidazole ring gains credence both from the position of attack at C-2 .

The photo - Fries rearrangement of N - substituted imidazoles, where the substituent on nitrogen was an acyl group , to give 2- and 4-substituted isomers could involve either a dissociative path (A) or an intramolecular process (B) . The excited species could be a radical or a radical cation . More complex acyl groups , e.g. stearoyl, tend to undergo cleavage in the side chain , but the 1- acylimidazole (1), derived from dehydroabietic acid appears to be subject only to migration , perhaps via a cyclobutanol intermediate , to give 2 - and 4 - acyl derivatives (2) (Scheme 10).
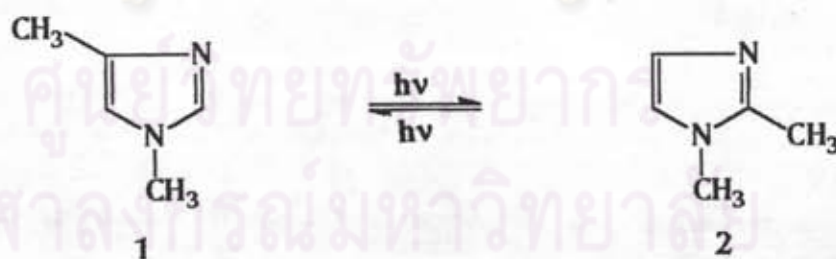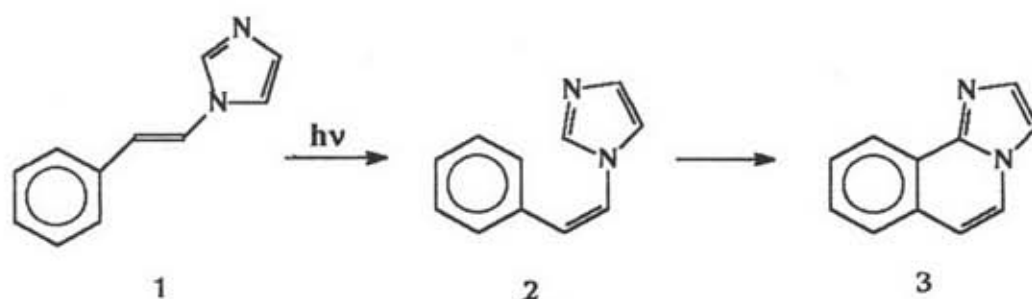

Scheme 6 : The interconversion of 1,4 and 1,2 dimethyl imidazole on photolysis

Scheme : 7 The interconversion of 1,4,5 and 1,2,5 trimethyl imidazole on photolysis
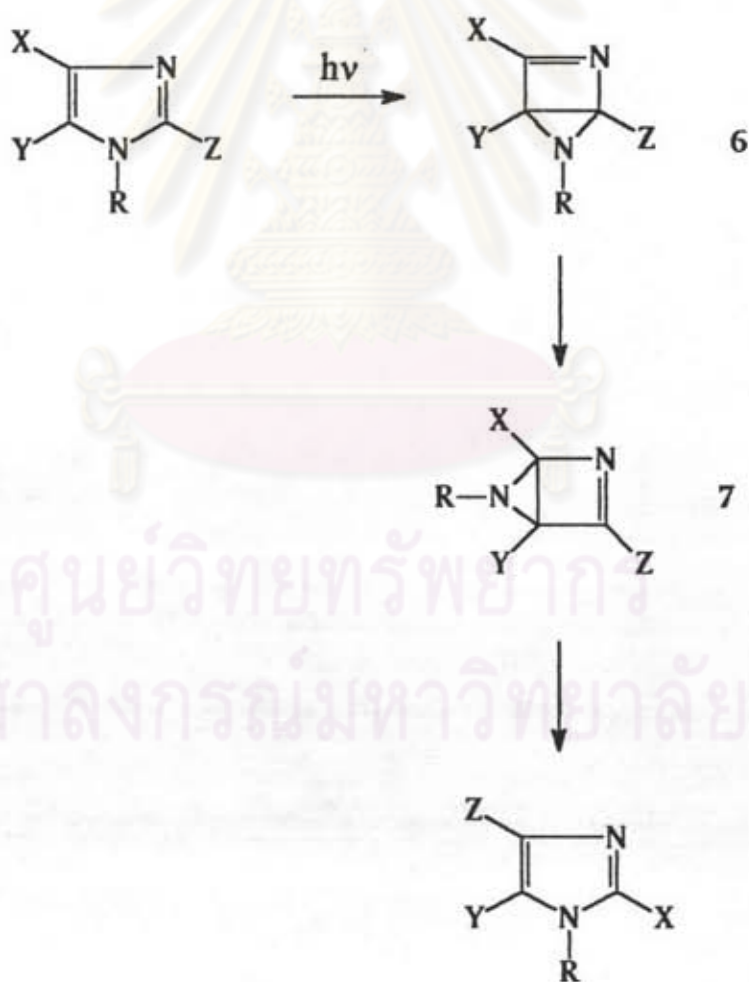

Scheme : 8 The photorearrangement mechanism of imidazole

Scheme : 9 The photocyclization of *trans* - 1 -styrylimidazole

C. Polymerization

When imidazoles and benzimidazoles have free NH groups, intermolecular hydrogen bonding gives rise to linear associates of molecules in the crystals and in non-protic solvents. Early determinations of molar masses and dipole moments gave anomalous results because of this phenomenon , particularly when concentrated solutions of the azoles were used. In fact , linear associates of as many as 20 molecules of imidazole were possible at high concentrations in solvents such as benzene. This intermolecular hydrogen bonding gave rise to broad NH signals in NMR spectra , and in solvents capable of exchange , e.g. D_2O , no signal at all for NH . A number of photochemical processes gave rise to dimeric products in which hydrogen is lost , sometimes by concurrent oxidation. In 1978 , Cole and workers reported that , irradiation benzimidazole in various solvents with free access to air gave the unsymmetrical dehydrodimer , 2,4' - and 2,5' - bibenzimidazole . this reaction probably proceeds through the intermediacy of a 2 - benzimidazolyl radical which substituted the benzene moiety unchanged (Scheme 11).
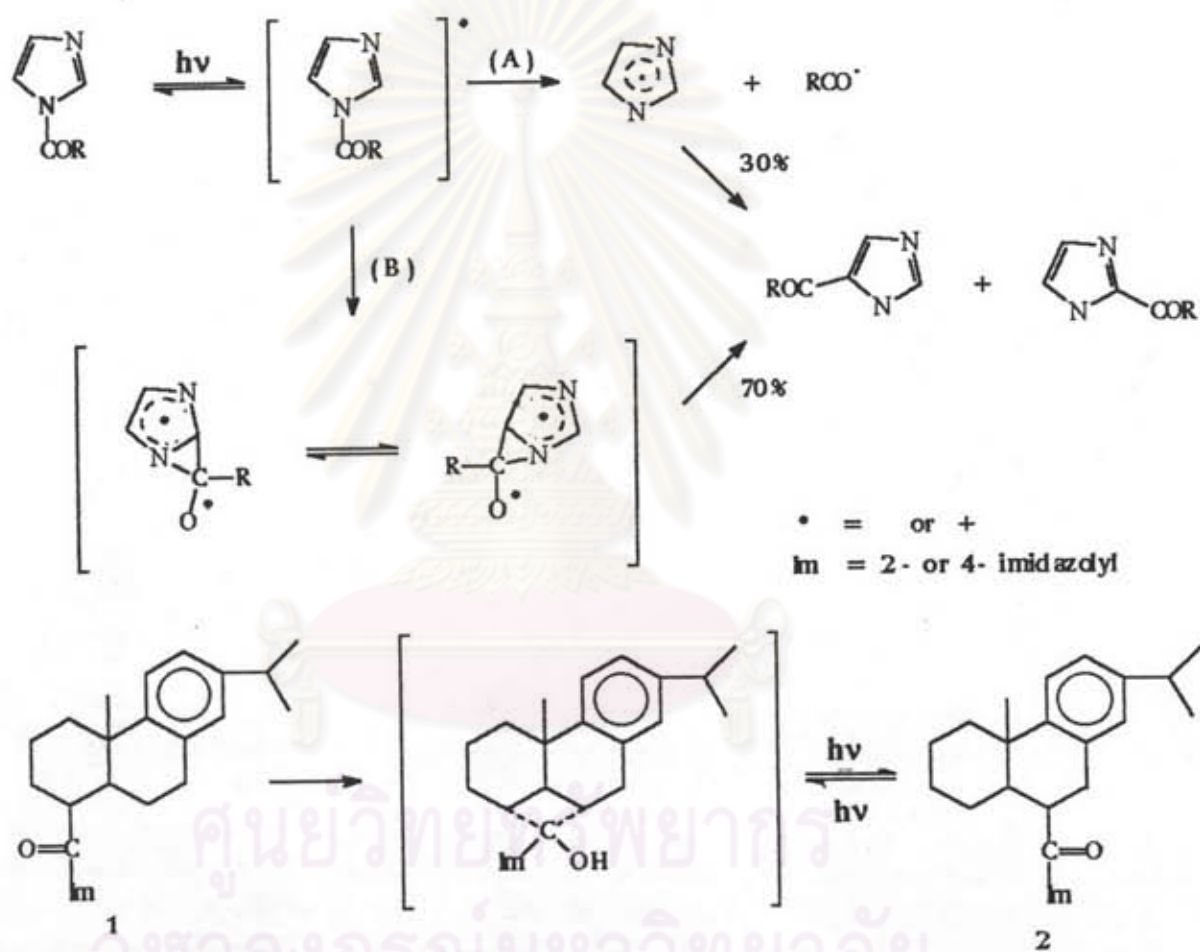
Scheme : 10 The photo - Fries rearrangement of N - substituted imidazole
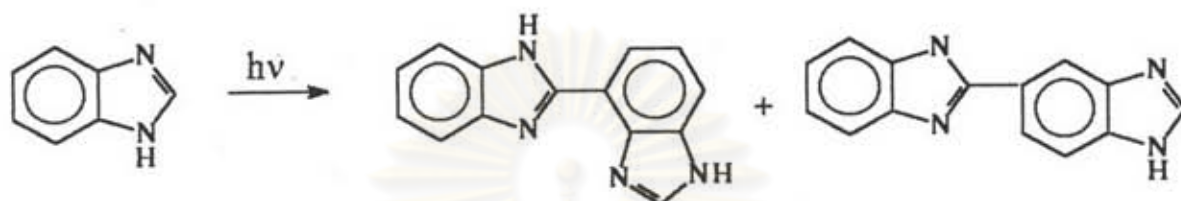
Scheme 11 : The degradation products of benzimidazole on photolysis