CHAPTER IV

RESULTS AND DISCUSSIONS

In this research, 6-chlorochromone-3-carboxylic acid and 7-chloro-6-fluorochromone-3-carboxylic acid were synthesized. The cyclization to chromone-3-carboxaldehyde intermediate was prepared using Vielmeier-Hacck Reaction and converted to chromone-3-carboaldoxime by adding hydroxylamine hydrochloride. Chromone-3-carbonitrile was obtained from dehydration of chromone-3-carboaldoxime by acetic anhydride . The hydrolysis of nitrile produced chromone-3-carboxylic acid with good yield.

6-CHLOROCHROMONE-3-CARBOXYLIC ACID (7A)

The 7A was selected as a model for studying the preparation of chromone-3-carboxylic acid. Various individual steps in the overall synthesis of 6-cholro-chromone-3-carboxylic acid was discussed as followed :

1) 4-CHLOROPHENOL (1A)

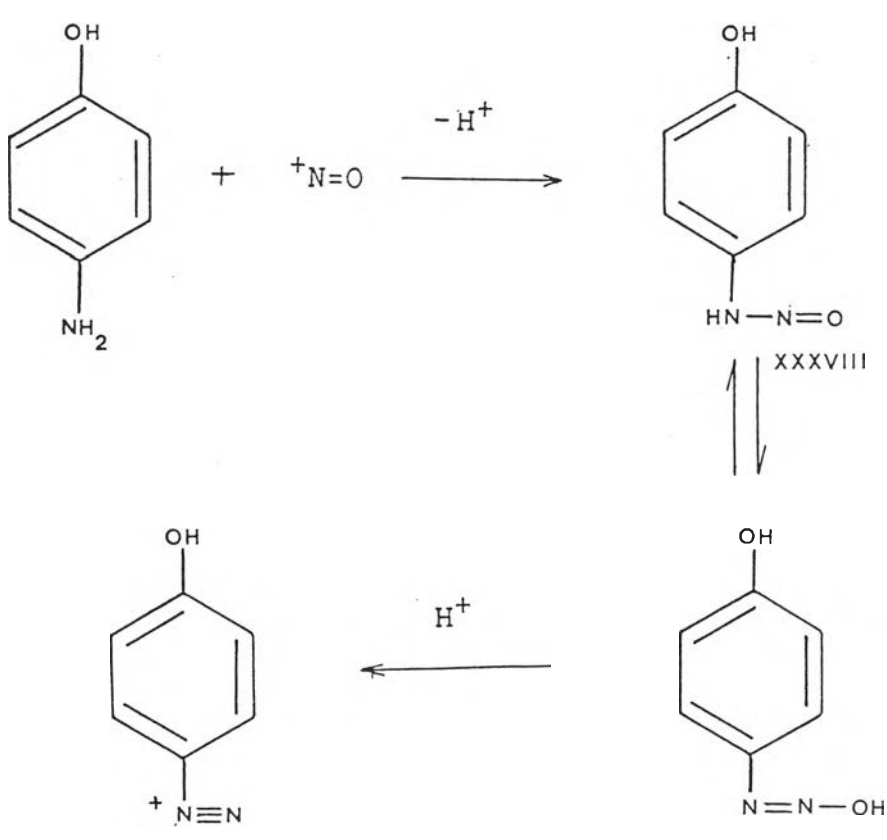
1A was synthesized following the Sandmeyer method which contained two steps. The first step involved a diazotization reaction between 4-aminophenol,

concentrated hydrochloric acid and sodium nitrite at $0^{o}c$ to yield 4-chlorophenodiazonium chloride. Mechanistically, this process resulted from the acidified nitrite solution provided a source of nitrosonium ion(XXXVII)which electrophilically replaced the hydrogen in the primary amino group to form the N-nitroso derivative (XXXVIII). This had a tautomeric structure, the hydroxydiazo form (XXXIX) yielded the diazonium ion (XXXX) under acidic condition.

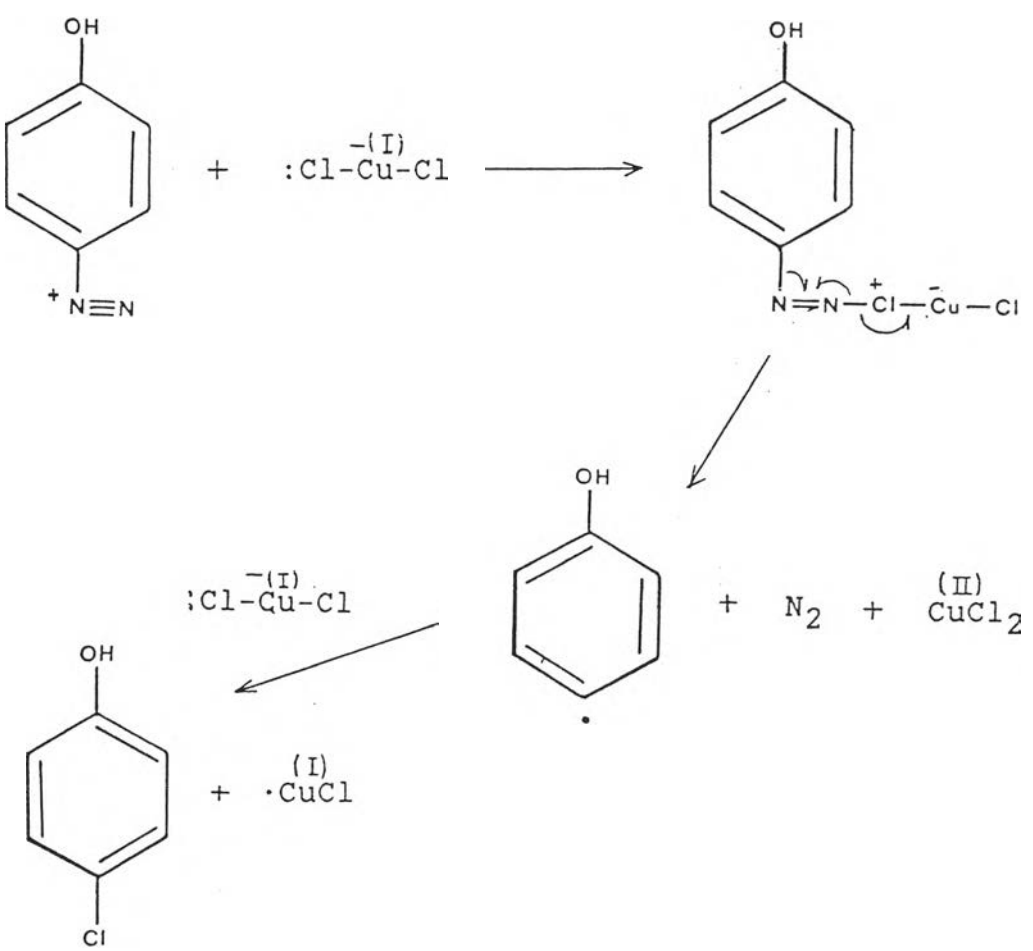
$$2\ NO_2^- + 2\ H^+ \longrightarrow 2\ HNO_2 \rightleftharpoons O{=}N{-}O{-}N{=}O + H_2O$$

$$O{=}N{-}O{-}N{=}O \rightleftharpoons O{=}N^+ + {}^-O{-}N{=}O$$

XXXVII



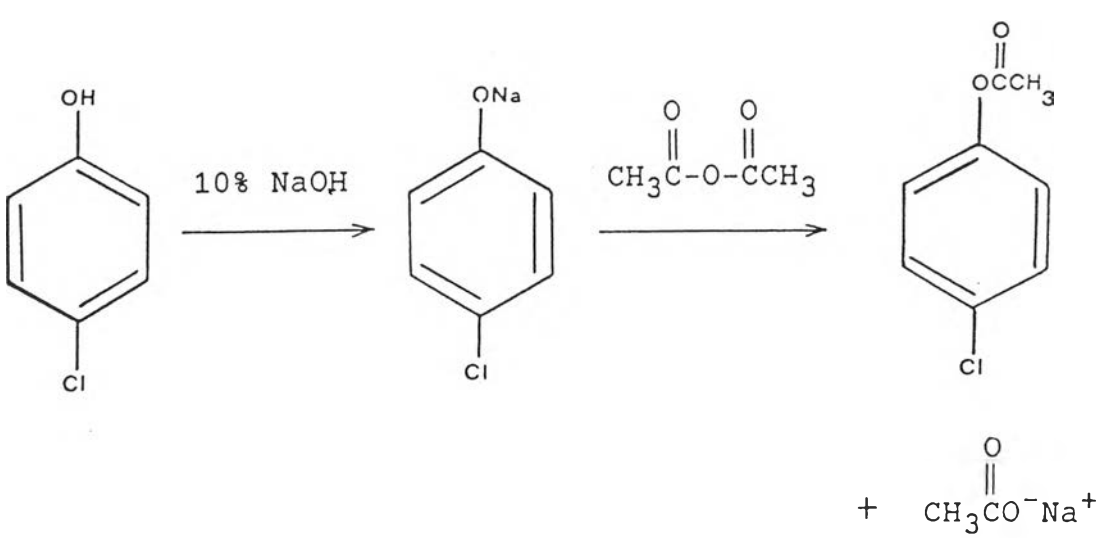
The second step of reaction was replacement of the diazonium group by chloride. Phenodiazonium salt had been reacted with copper(I) chloride to yield phenodiazonium-copper(I) chloride complex and when the temperature was raised, decomposition ensues accompanied by the evolution of nitrogen, the disappearance of the solid complex and separation of the oily layer of 4-chlorophenol. 4-Chlorophenol was formed when diazonium-copper (I) chloride complex decomposed by a radical mechanism summarized below . Copper catalysed this decomposition because it could under-go interconversion between the +1 and +2 oxidation states as a result of eletron-tranfer.



The IR spectrum of 4-chlorophenol (Figure 9) showed a broad peak at 3360 cm^{-1} for O-H stretching, peak at 1240 cm^{-1} for C-OH stretching and a strong peak at 830 cm^{-1} was represented for para substitution on aromatic ring. The ^{1}H-NMR of 4-chlorophenol (Figure 10) showed the peak at chemical shift 4.67 ppm. (singlet,1H) for a hydroxy proton. The peak at chemical shift 6.71 ppm. (doublet of doublet, 2H, J=2.3,6.6 Hz) represented for aromatic proton ortho with a hydroxy group. The peak at chemical shift 7.10 ppm.(doublet of doublet, 2H, J=2.3,6.6 Hz) for aromatic protons ortho with chloro group.

2) 4-CHLOROPHENYL ACETATE (2A)

This step was the acetylation of 4-chlorophenol with acetic anhydride in 10 % sodium hydroxide solution. The mechanism for this reaction involved nucleophilic substitution of phenolate ion which generated by dissolved 4-chlorophenol in 10% sodium hydroxide solution, and the acetic anhydride.



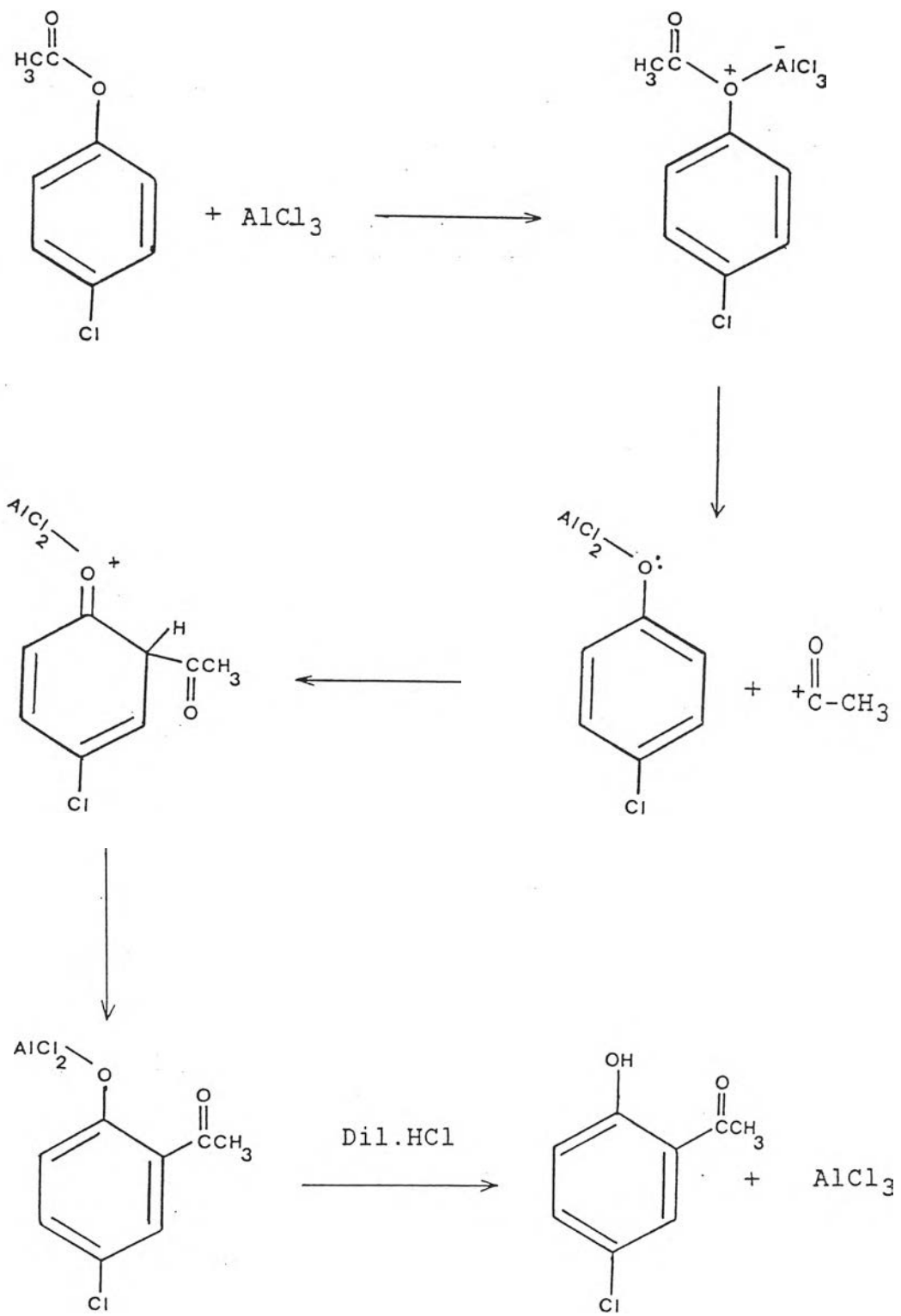
The IR spectrum of 4-chlorophenyl acetate (Figure 11) showed a strong peak at 1770 cm^{-1} for C=O stretching of ester group, broad peak at 1200 cm^{-1} for Ar-O-C=O group and peak at 850 cm^{-1} for para substitution on aromatic ring. The ^{1}H-NMR of 4-chlorophenyl acetate (Figure 12) showed the peak at 2.23 ppm (singlet, 3H) for proton of acetyl group. The peak at chemical shift 6.94 ppm.(doublet of doublet, 2H, J=2.2,9.0 Hz) for aromatic protons ortho to acetyl group and the peak at chemical shift 7.25 ppm. (doublet of doublet, 2H, J=2.2, 9.0 Hz) aromatic protons ortho to chloro group.

3) 5-CHLORO-2-HYDROXYACETOPHENONE (3A)

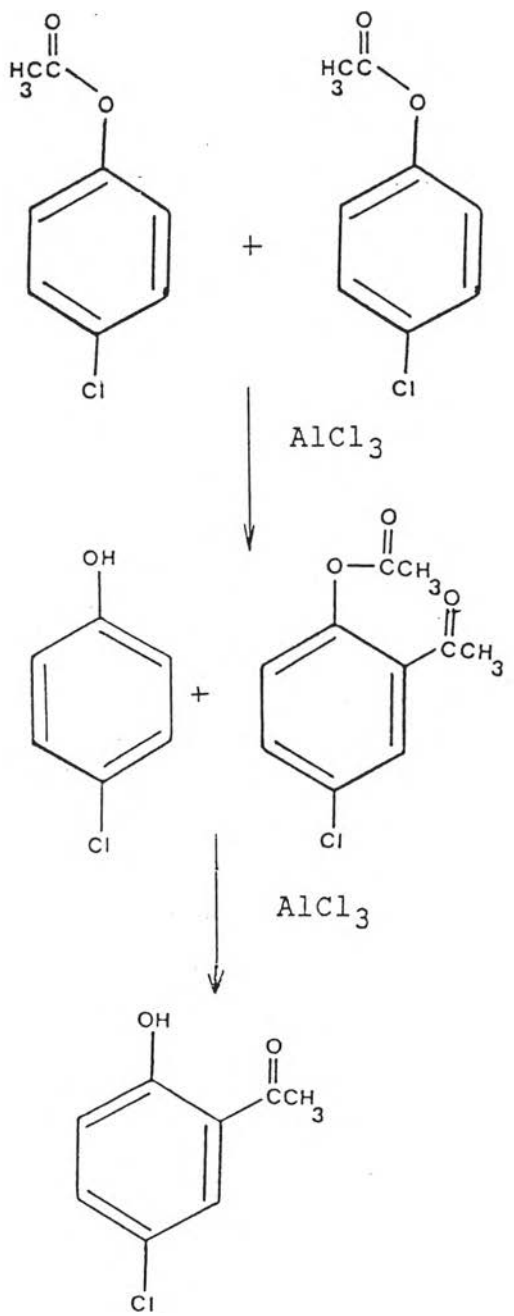
The synthesis of 3A was readily accomplished by the Fried's rearrangement when heating a mixture of 4-chlorophenyl acetate with aluminium chloride at 140^{o}c for 60 minutes. The precipitation of 5-chloro-2-hydroxyacetophenone was obtained by adding diluted hydrochloric acid (1:1) and ice-water to decompose the excess aluminium chloride and aluminium chloride complex.

Three difference mechanisms for the Fried's rearrangement have been described (Blatt, 1942). In one of them, the ester was assumed to react with aluminium chloride to give acid chloride and a phenoxyaluminium

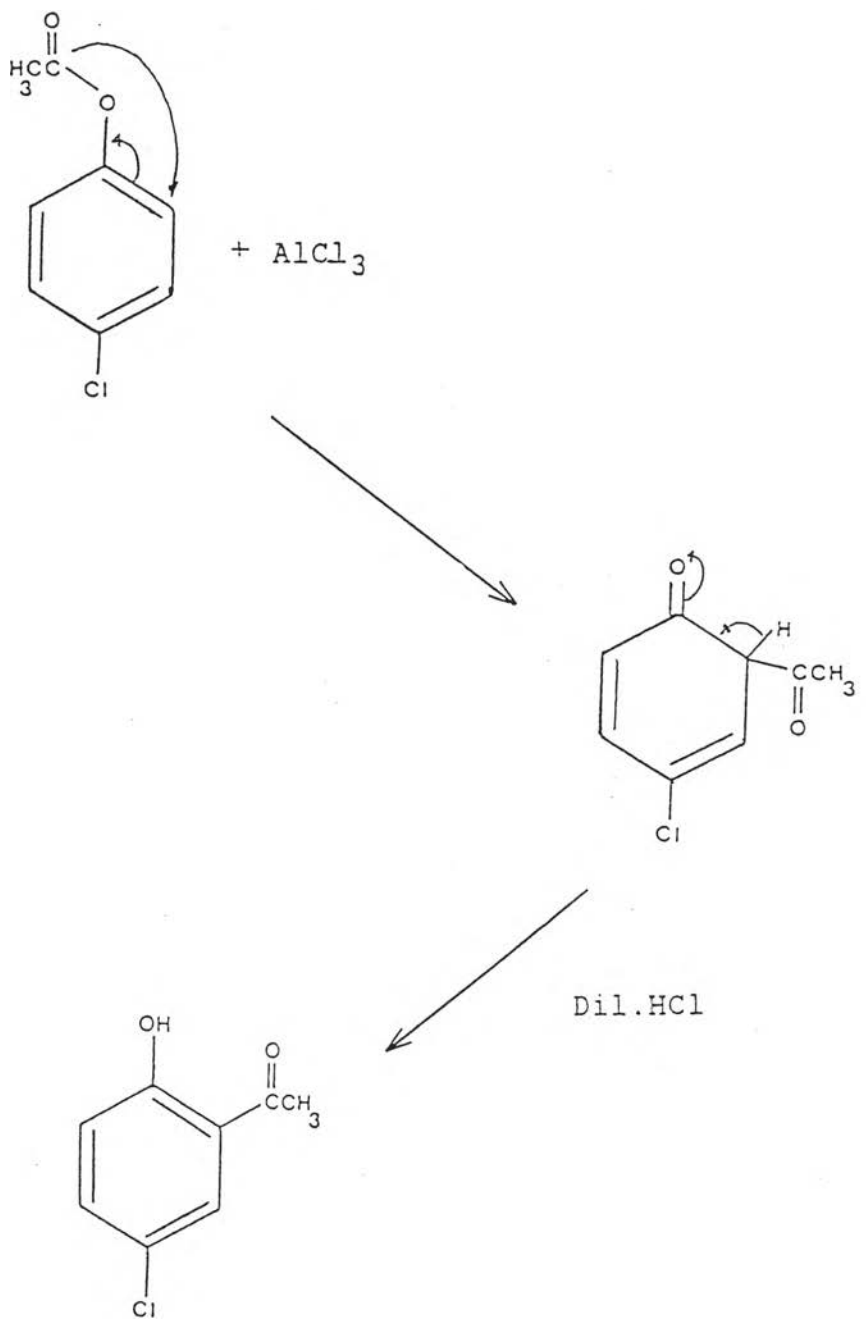
chloride which combined to form a derivative of hydroxy-ketone.



In another mechanism, it was proposed that one molecule of phenyl ester was acetylated by another molecule.



In the third mechanism, the Fried's Reaction was considered to be a true intramolecular rearrangement in which the acyl group shift directly from the oxygen atom to carbon atom of the ring.



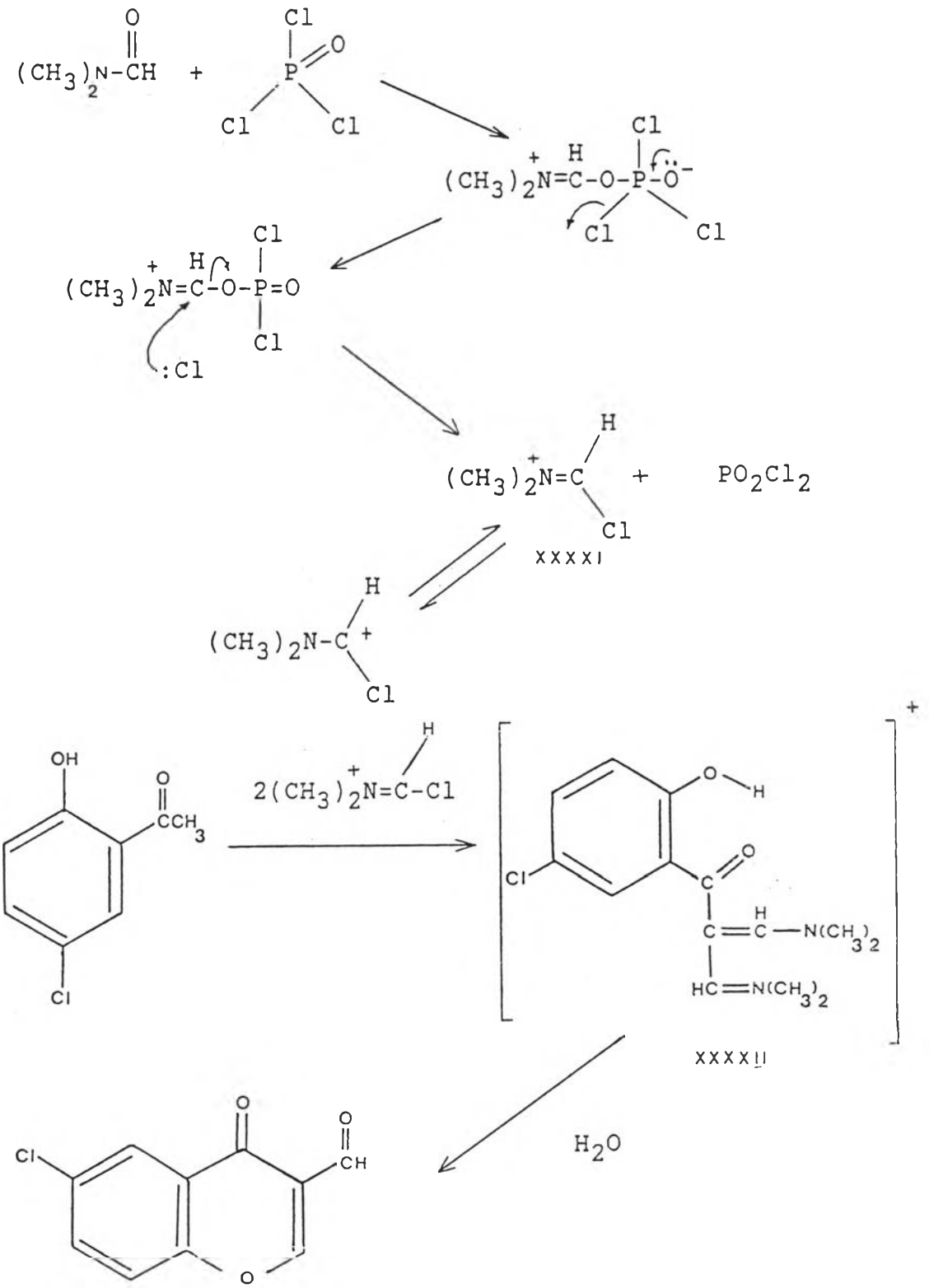
The IR spectrum of 5-chloro-2-hydroxyacetophenone (3A) (Figure 13) showed the peak at 3400 cm^{-1} for O-H stretching, the peak at 1660 was assigned for C=O stretching of keto group.

The ^{1}H-NMR spectrum of 3A (Figure 14) showed the peak at chemical shift 2.59 ppm.(3H, singlet) represents for acetyl proton. The peak at chemical shift 6.90 ppm.(doublet, 1H, J=9 Hz) for aromatic proton position 3, the peak at chemical shift 7.35 ppm. (doublet of doublet, 1H, J=2.6, 9.0 Hz) for aromatic proton position 4 and the peak at chemical shift 7.66 ppm. (doublet, 1H, J= 2.6 Hz) for aromatic proton position 3. The hydroxy proton was showed the peak at 12.11 ppm.

4) 6-CHLOROCHROMONE-3-CARBOXALDEHYDE (4A)

The synthesis of 4A was synthesized followed the method of Vielsmeier-Hacck reaction. The synthesis of 4A started by reaction of 5-chloro-2-hydroxyacetophenone, N,N-dimethylformamide and phosphorous oxychloride. The proposed mechanism of this reaction involved the chlorination of N,N-dimethyl formamide by phosphorous oxychloride to yield the intermediate (XXXXI) which had two mesomeric forms . The 5-chloro-2-hydroxyacetophenone was formylated with two moles of this intermediate to form the intermediate

(XXXXII). The cyclization was followed by adding ice-water to obtain 6-chlorochromone-3-carboxaldehyde.

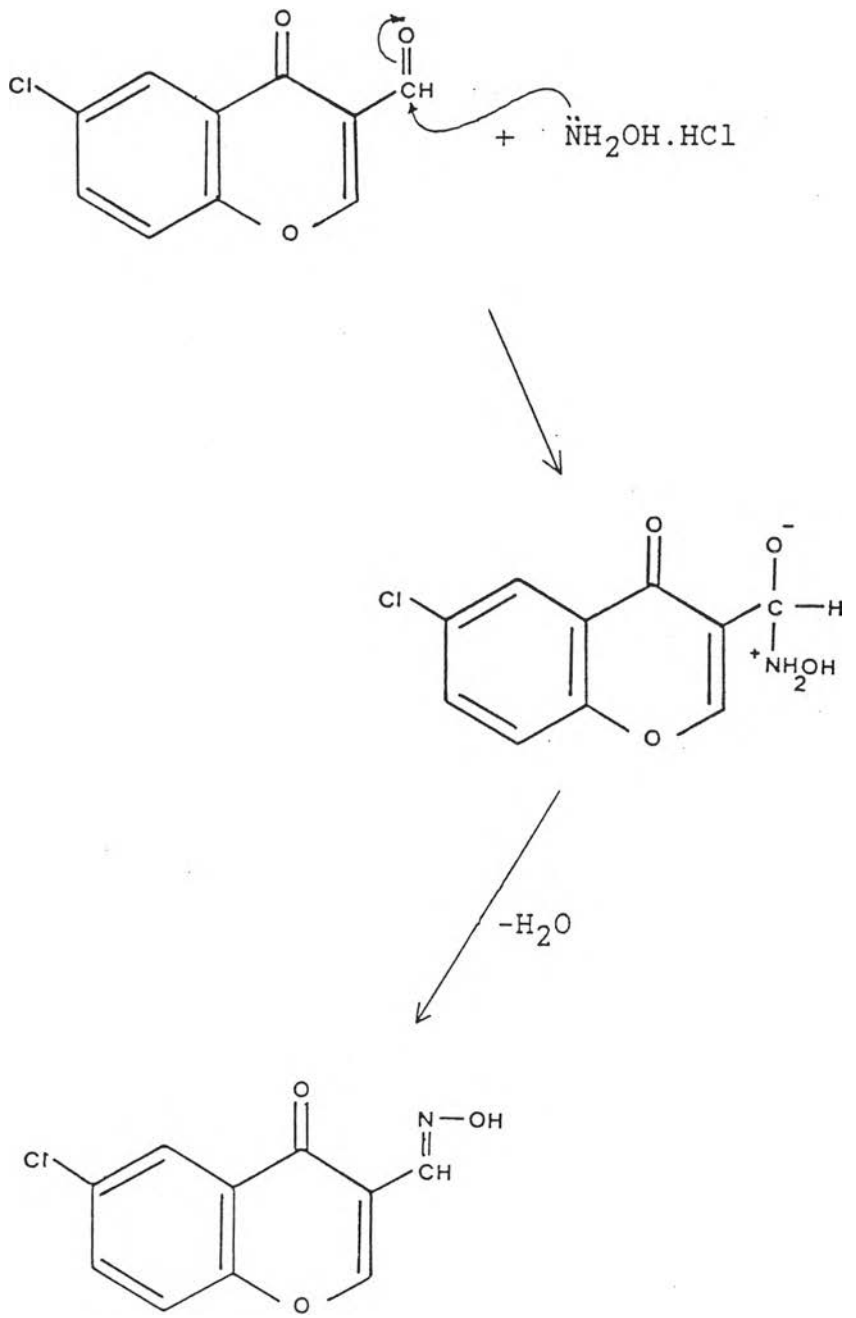


The IR spectrum of 6-chlorochromone-carboxaldehyde (4A) (Figure 15) showed the aldehyde proton at 2860 cm^{-1}. Strong peak at 1700 cm^{-1} and 1660 cm^{-1} represents for C=O stretching of aldehyde and pyrone, respectively.

The ^{1}H-NMR spectrum of 6-chlorochromone-3-carboxaldehyde (Figure 16) showed the characteristic proton at chemical shift 7.43 ppm.(doublet, 1H, J=9 Hz) was identified for proton position 8. Proton position 7 was found the peak at chemical shift 7.60 ppm.(doublet of doublet, 1H, J =2,9 Hz). The peak at 8.23 ppm. (doublet, 1H, J =2 Hz) was assigned for proton position 5. The peak at chemical shift 8.52 ppm. (singlet, 1H) was identified for proton position 2 and peak at 10.35 ppm.(singlet, 1H) for aldehyde proton.

5) 6-CHLOROCHROMONE-3-CARBOALDOXIME (5A)

The synthesis of 5A was accomplished by reaction of 6-chlorochromone-3-carboxaldehyde and hydroxylamine hydrochloride in absolute ethanol. The reaction mechanism involved nucleophilic attack of a nitrogen atom of hydroxylamine hydrochloride on a partial positive-charge carbon of aldehyde group and subsequently lost one molecule of water to form aldoxime derivative.

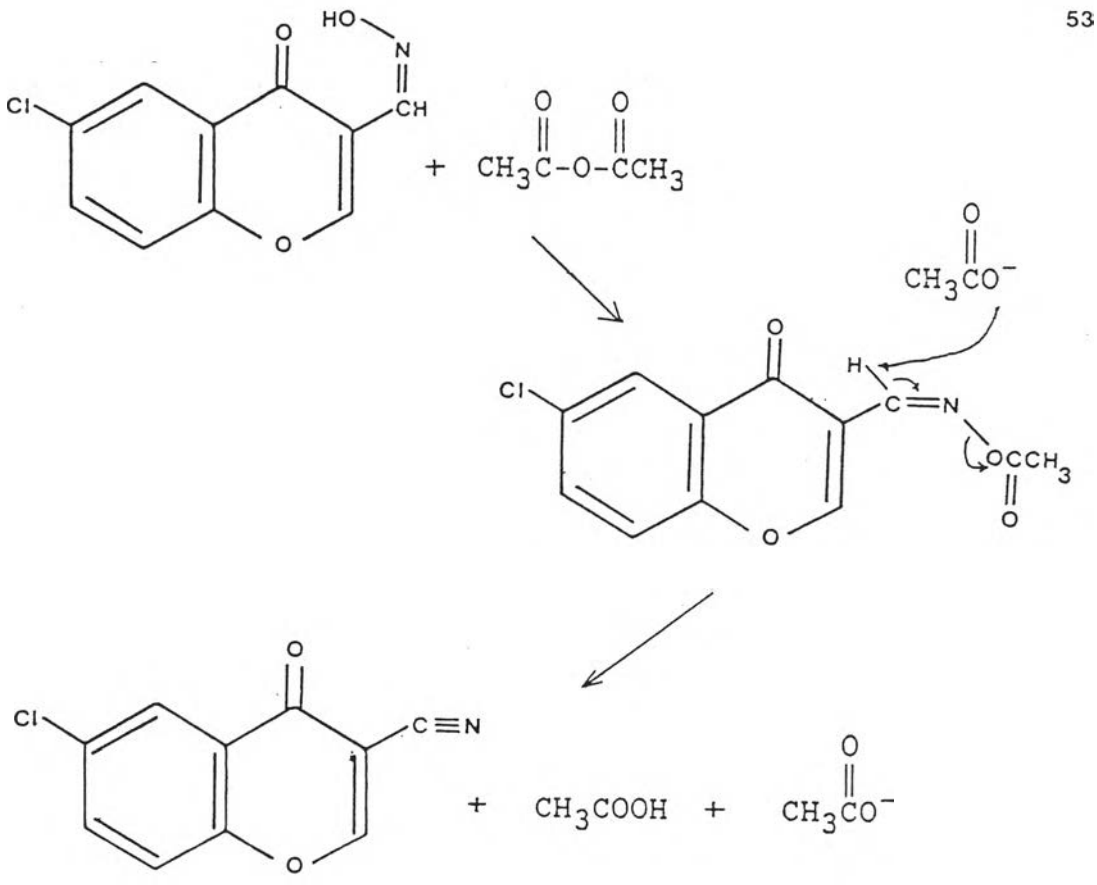


The IR spectrum of 6-chlorochromone-3-carboaldoxime (5A) (Figure 17) showed the broad peak of O-H stretching at 3200 cm^{-1}, the strong peak at 1650 cm^{-1} for C=O stretching of pyrone and C=N stretching.

The ^{1}H-NMR spectrum of 5A (Figure 18) showed the peak at chemical shift 7.64 ppm. (doublet, 1H, J= 9 Hz) for proton position 8, the peak at chemical shift 7.77 ppm. (doublet of doublet, 1H, J= 2.4,9 Hz) for proton position 7, the peak at chemical shift 8.07 (doublet, 1H, J =2.4 Hz) for proton position 5. The singlet peak at chemical shift 8.53 ppm. (1H) was assigned as proton position 2. The peak at chemical shift 10.53 ppm.(singlet, 1H) for hydroxy proton of aldoxime group and the peak at chemical shift 8.17 ppm. (singlet, 1H) for proton adjacent to C=N group.

6) 6-CHLOROCHROMONE-3-CARBONITRILE(6A)

The synthesis of 6A was accomplished by dehydration of 6-chlorochromone-3-carboaldoxime with acetic anhydride. The mechanism for this reaction involved acetic anhydride as dehydrating agent which probably affected on initial acetylation of oximino group followed by elimination of acetic acid. Moreover, the reaction was most successful when the H and O-H atom of oximino group were in opposite direction.

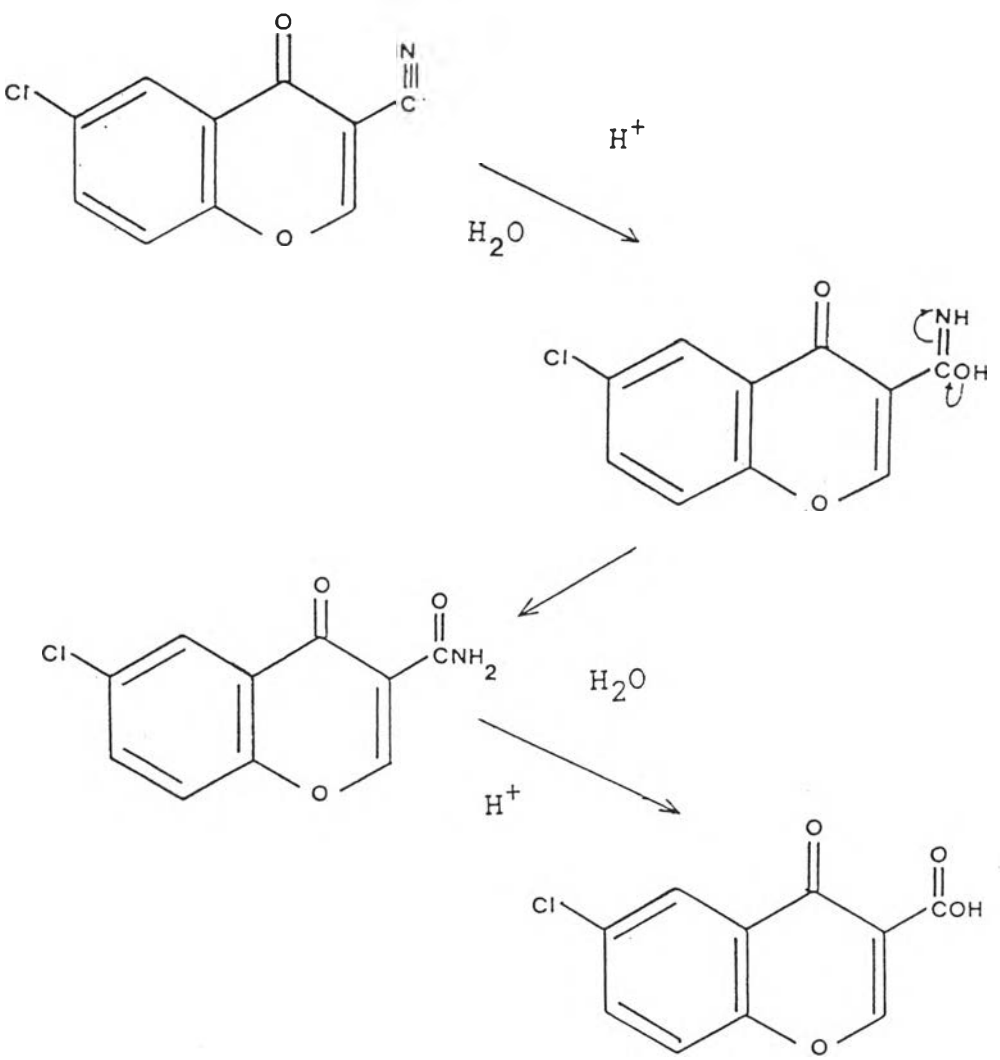


The IR spectrum of 6-chlorochromone-3-carbonitrile (6A) (Figure 19) showed the peak of C-H stretching vibration between 3000-3100 cm^{-1}, the strong peak at 2240 cm^{-1} for C≡N stretching and strong peak at 1660 cm^{-1} for C=O stretching of pyrone.

The ^{1}H-NMR spectrum of 6A (Figure 20) showed the peak at chemical shift 7.73 ppm. (doublet, 1H, J= 9 Hz) for proton position 8, peak at chemical shift 7.85 ppm. (doublet of doublet, 1H, J= 2.4,9 Hz) for proton position 7, the peak at chemical shift 7.92 ppm. (doublet, 1H, J=2.4 Hz) for proton position 5. The singlet peak at chemical shift 9.17 ppm. (1H) was identified for proton position 2.

7) 6-CHLOROCHROMONE-3-CARBOXYLIC ACID (7A)

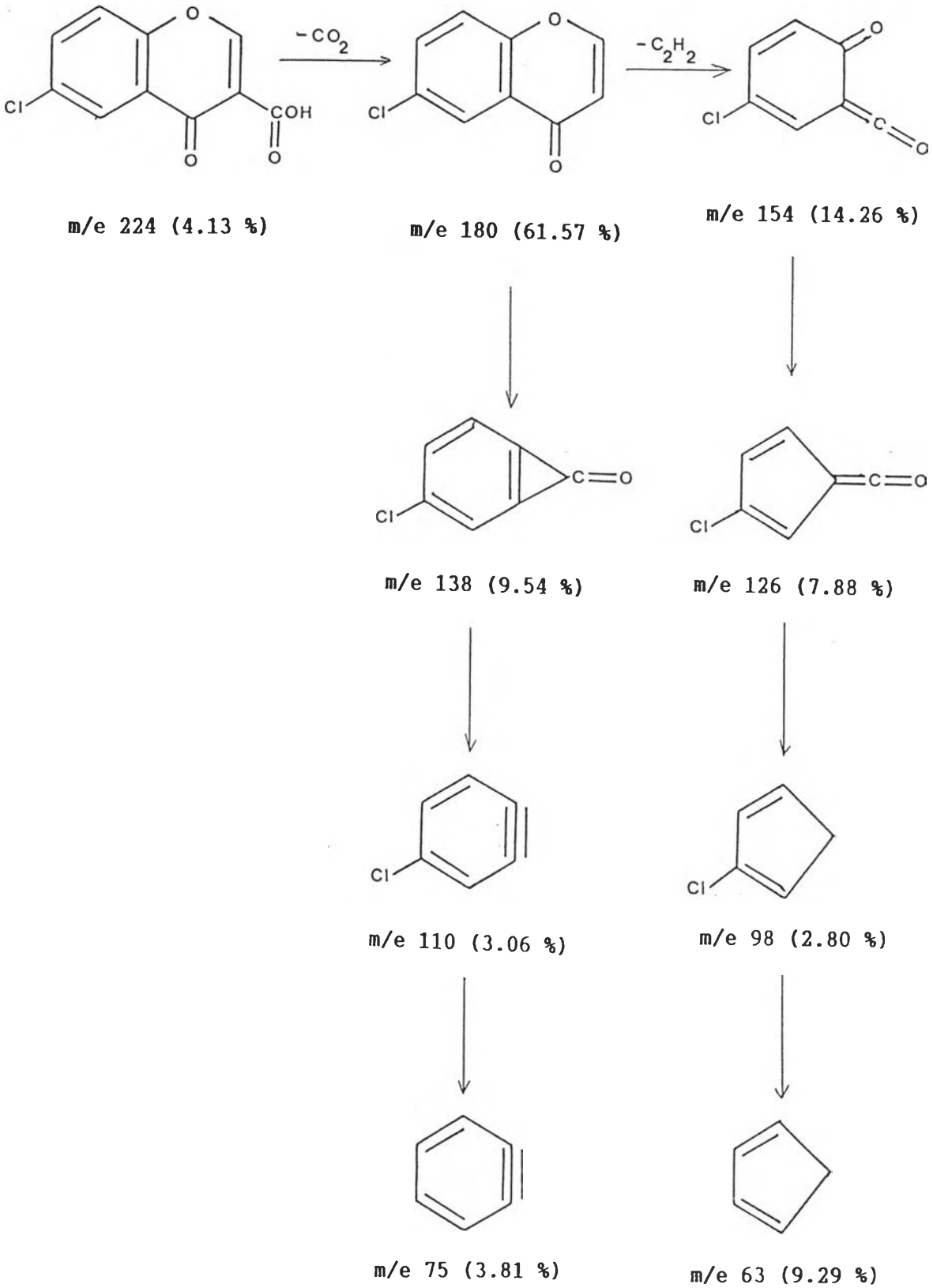
The final product of this derivative 7A was synthesized by hydrolysis 6-chlorochromone-3-carbonitrile with 55% sulfuric acid. The mechanism of this hydrolysis involved initial attack of water on nitrile group which was catalysed by acid. The reaction proceeded via the formation of an amide intermediate , which was finally hydrolyzed to obtained 6-chloro-chromone-3-carboxylic acid.



The IR spectrum of 6-chlorochromone-3-carboxylic acid (Figure 21) showed C-H stretching vibration at 3080 cm^{-1}, the broad peak in the region 2600 - 3150 cm^{-1} was assigned for O-H of carboxylic acid with intramolecular hydrogen bonding. The peak at 1770 $cm^{-1}$and 1650 cm^{-1} was identified for C=O stretching of carboxylic acid and carbonyl group of pyrone ring, respectively. The peak at 1140 cm^{-1} was assigned for ARC(=O)-OH group.

The ^{1}H-NMR spectrum of 6-chlorochromone-3-carboxylic acid (Figure 22) showed the peak at chemical shift 7.50 ppm. (doublet, 1H, J= 9 Hz) for proton position 8, the peak at chemical shift 7.75 ppm. (doublet of doublet, 1H, J=2.4,9.0 Hz) for proton position 7, the peak at chemical shift 8.30 ppm. (doublet, 1H, 2.4 Hz) for proton position 5. The singlet peak at chemical shift 9.00 ppm. (1H) was assigned for proton position 2.

The mass spectrum of 7A (Figure 23) showed peak at m/e 224 for molecular ion peak. The fragment ion peak at m/e 180 exhibited the loss of CO_2 . The characteristic peak at m/e 63, 75, 98, 110, 126, 138, 154 were proposed as follows :



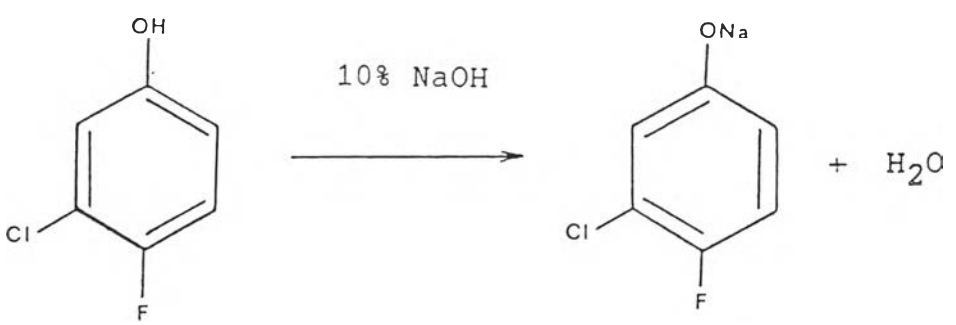
Scheme II Fragmentation pattern in ei mass spectrum of 6-Chlorochromone-3-carboxylic acid

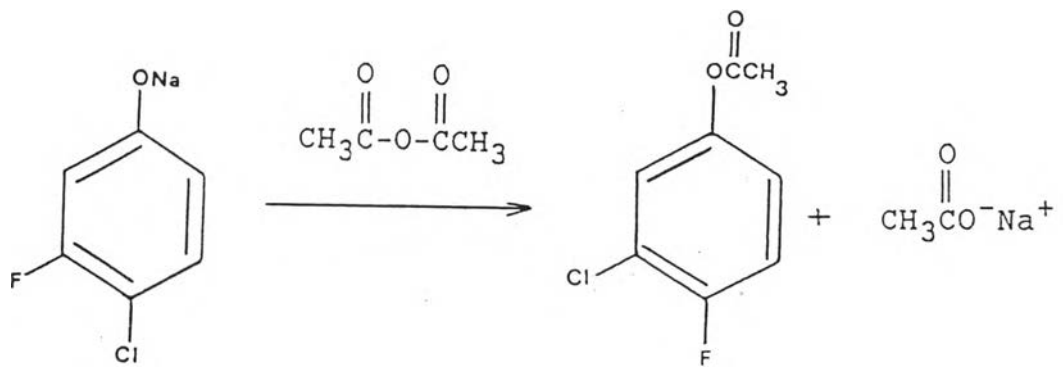
7-CHLORO-6-FLUOROCHROMONE-3-CARBOXYLIC ACID (6B)

The 7-chloro-6-fluorochromone-3-carboxylic acid (6B) was synthesized by the pathway similar to that of 6-chlorochromone-3-carboxylic acid . This derivative was selected as a new compound for potential antimicrobial agent. The overall synthesis of 7-chloro-6-fluorochromone-3- carboxylic acid was discussed as follows :

1) 3-CHLORO-4-FLUOROPHENYL ACETATE (1B)

This step was the acetylation of 3-chloro-4-fluorophenol with acetic anhydride in 10 % sodium hydroxide solution. The mechanism for this reaction involved nucleophilic substitution of phenolate ion which generated by dissolving 3-chloro-4-fluorophenol in 10 %sodium hydroxide solution, and the acetic anhydride.



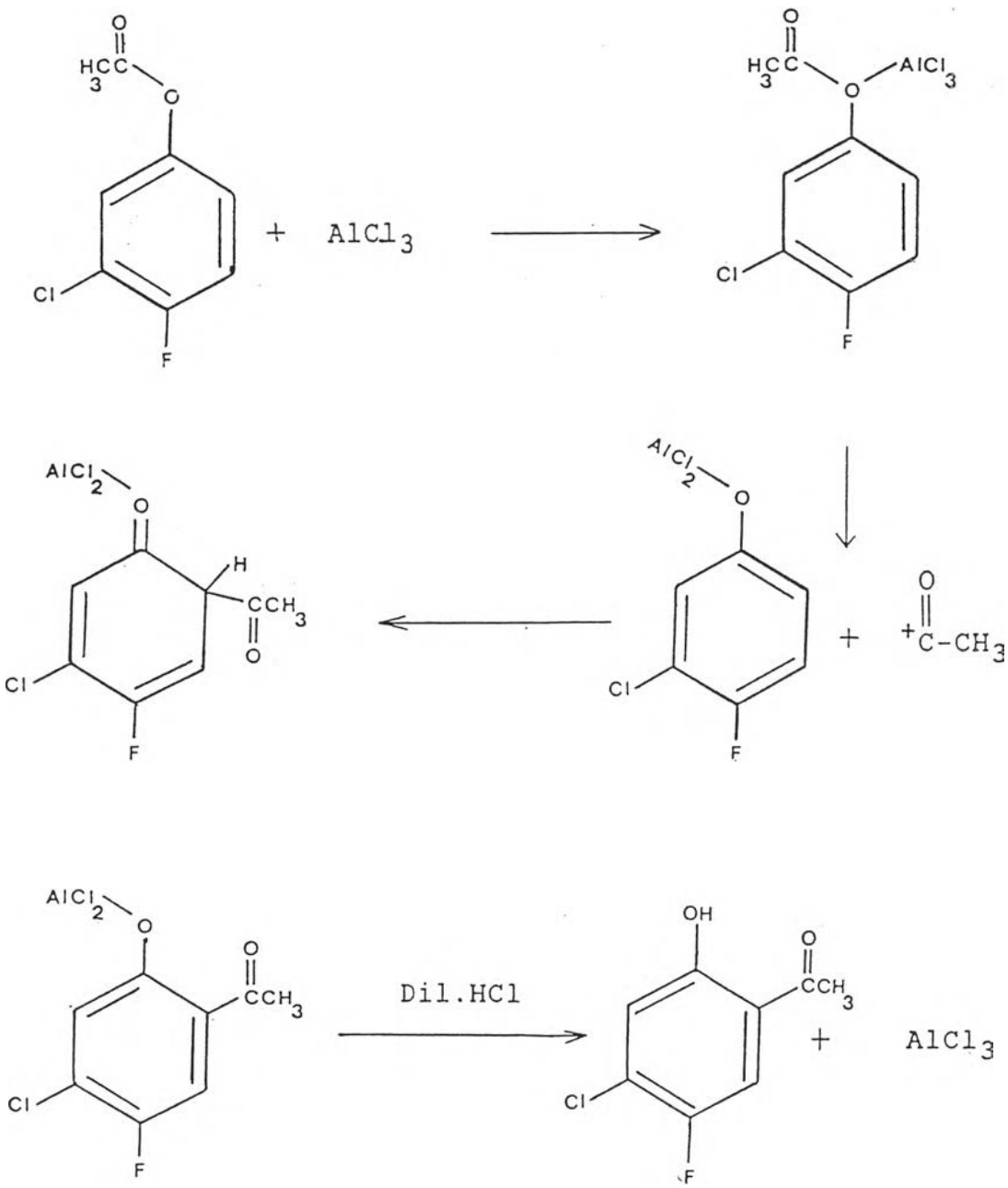


The IR spectrum of 3-chloro-4-fluorophenyl acetate (1B) (Figure 24) showed a strong peak at 1775 cm^{-1} for C=O stretching of ester group, peak at 1190 cm^{-1} for Ar-O-$\overset{O}{\overset{\|}{C}}CH_3$ group . The ^{1}H-NMR of 1B (Figure 25) showed the peak at chemical shift 2.27 ppm.(singlet, 3H) for proton of acetyl group. The peak at chemical shift 6.94 - 7.36 ppm. (multiplet, 3H) was identified for protons on aromatic ring.

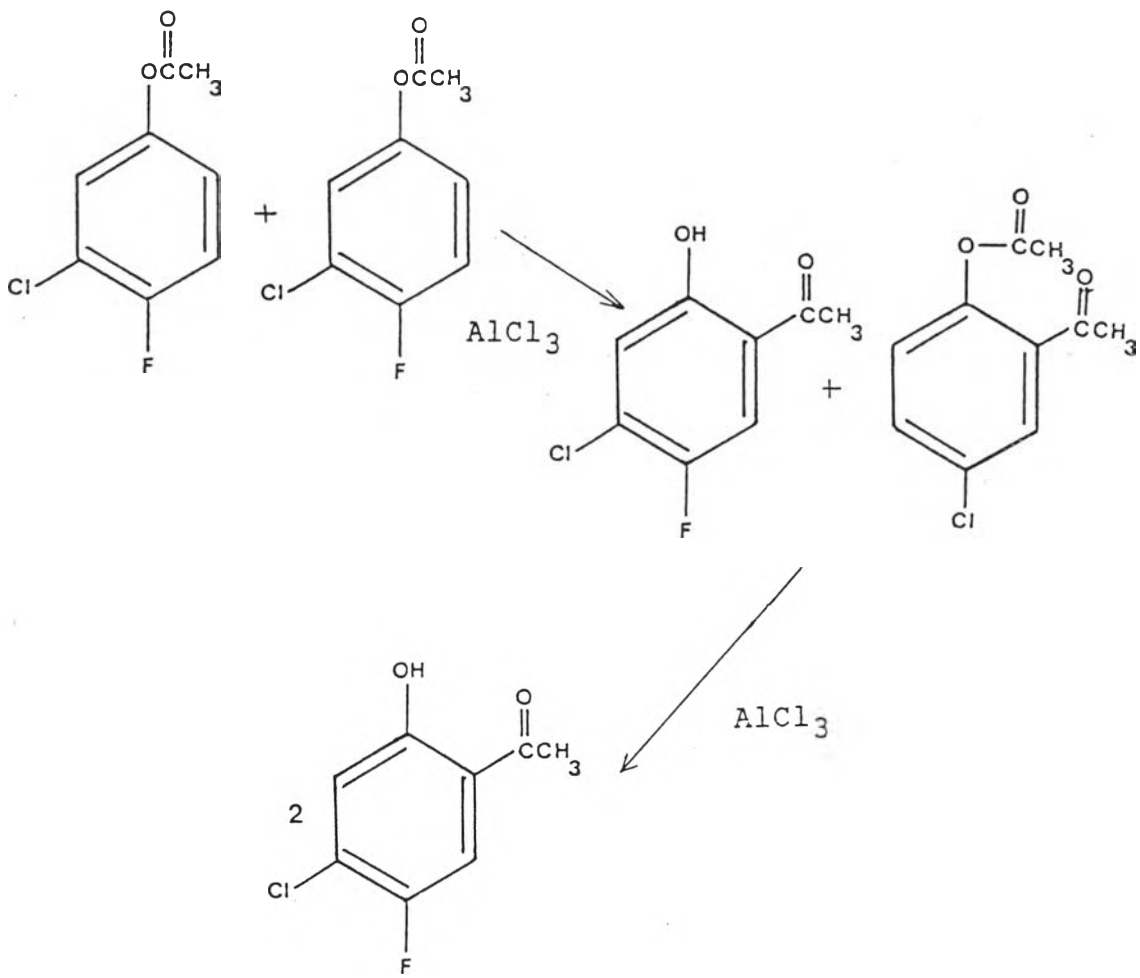
2) <u>4-CHLORO-5-FLUORO-2-HYDROXYACETOPHENONE</u> (2B)

The synthesis of 2B was readily accomplished by Fried's rearrangement by heating a mixture of 3-chloro-4-fluorophenyl acetate with anhydrous aluminium chloride at 120 ^{o}c for 15 minutes. The precipitation of 4-chloro-5-fluoro-2-hydroxyacetophenone was obtained by adding diluted hydrochloric acid (1:1) and ice-water to decompose the excess aluminium chloride and 3-chloro-4-fluorophenolate-aluminium chloride complex.

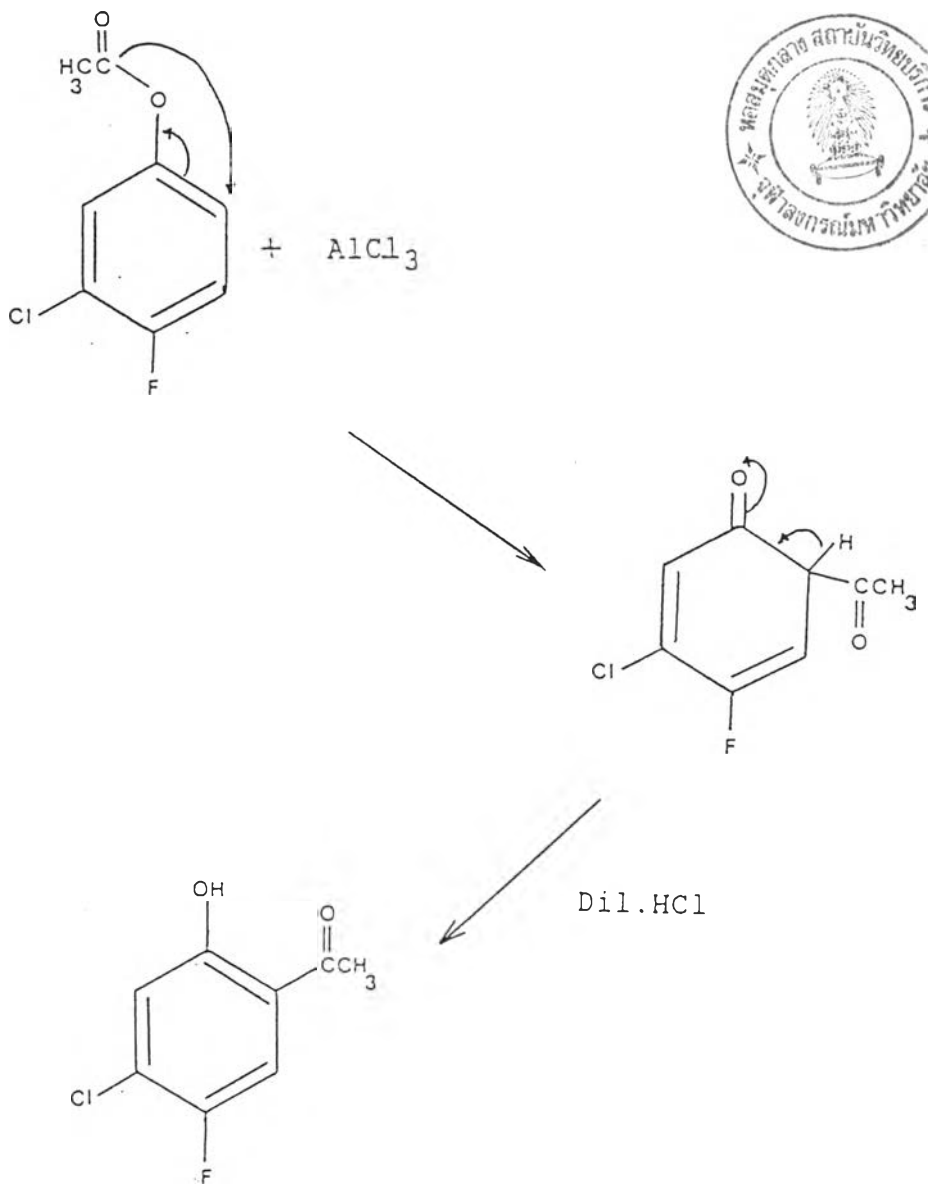
Three different mechanisms for the Fried rearrangement had been reported. In one of them, the ester was assumed to react with aluminium chloride to give acid chloride and phenoxy aluminium chloride which combine to form a derivative of hydroxyketone.



In another mechanism, it was proposed that one molecule of phenyl ester is acetylated by another molecule.



In the third mechanism, the Fried reaction was considered to be a true intramolecular rearrangement in which acetyl group shift directly from the oxygen atom to carbon atom of the ring.



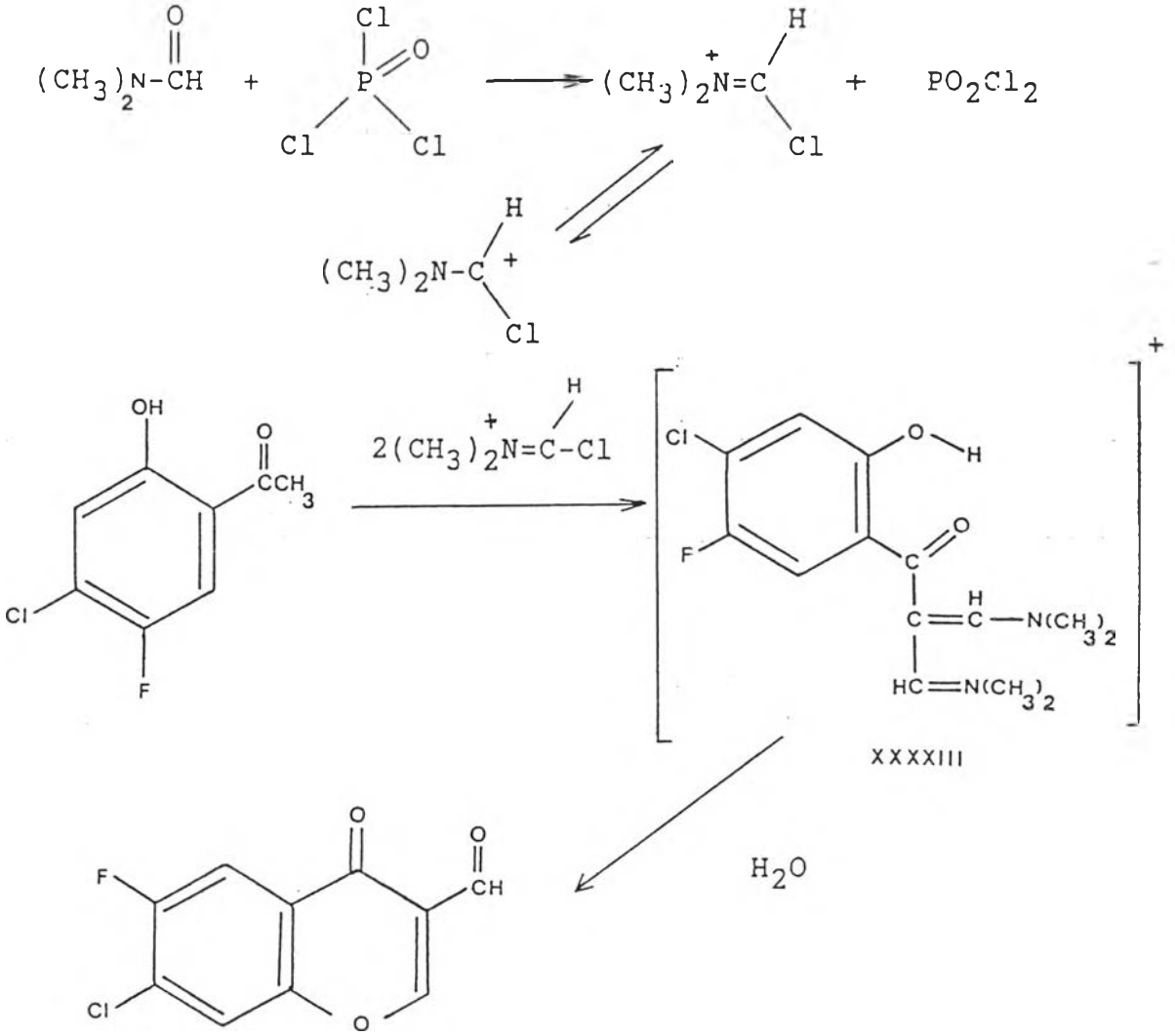
The IR spectrum of 4-chloro-5-fluoro-2-hydroxyacetophenone (2B) (Figure 26) showed the peak at 3000 cm^{-1} for O-H stretching with intramolecular hydrogen

bonding, the peak at 1640 cm^{-1} for C=O stretching of ketone group.

The ^{1}H-NMR of 2A (Figure 27) was found the peak at chemical shift 2.30 ppm. (singlet, 3H) for proton of acetyl group. The peak at chemical shift 7.01 ppm. (doublet, 1H, J=6 Hz) was identified for proton position 3, the peak at chemical shift 7.42 ppm. (doublet, 1H, J= 9 Hz) for proton position 6 on aromatic ring. The hydroxy proton was found the peak at chemical shift 12.03 ppm.(singlet, 1H).

3) 7-CHLORO-6-FLUOROCHROMONE-3-CARBOXALDEHYDE (3B)

The synthesis of 3B was following the method of Vielmeier-Hacck Reaction . It started by the reaction of 4-chloro-5-fluoro-3-hydroxyacetophenone, N,N-dimethylformamide and phosphorous oxychloride. The mechanism of this reaction involved the chlorination of N,N-dimethylformamide by phosphorous oxychloride to yield the intermediate(XXXXI)which had two mesomerics. The 4-chloro-5-fluoro-2-hydroxyacetophenone was formylated with two moles of this intermediate to form the intermediate(XXXXIII). The cyclization was occurred by adding ice-water to obtain 7-chloro-6-fluoro-chromone-3-carboxaldehyde.



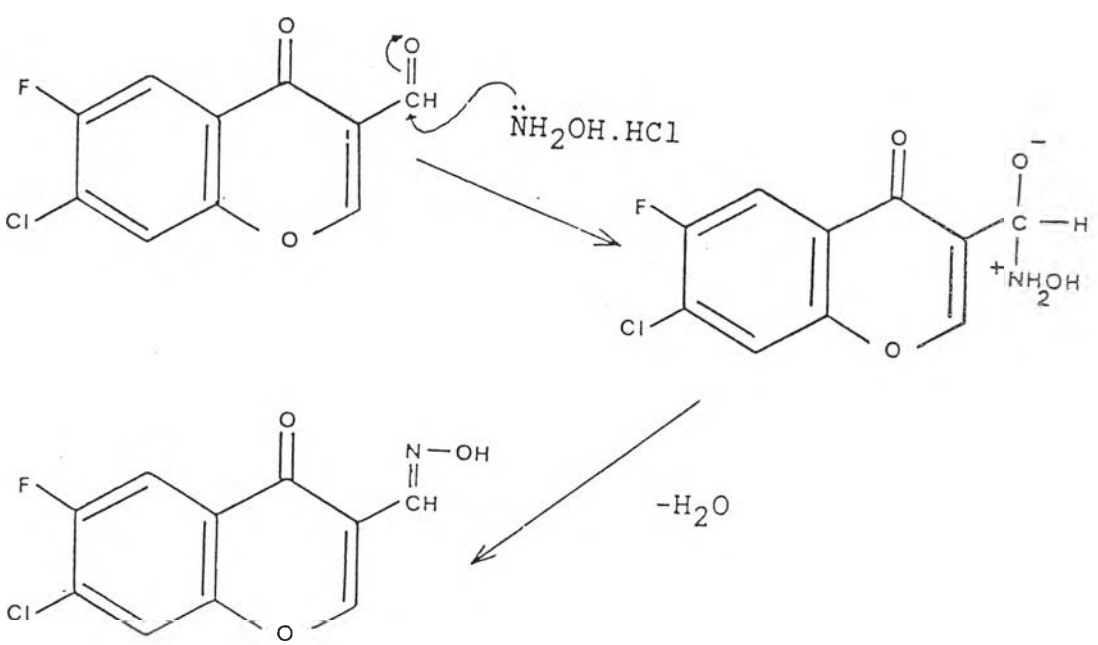
The IR spectrum of 7-chloro-6-fluorochromone-3-carboxaldehyde (Figure 28) showed the aldehyde proton at 2880 cm^{-1} . The strong peak at 1710 cm^{-1} and 1650 cm^{-1} were assigned for C=O stretching of aldehyde and ketone of pyrone ring, respectively.

The ^{1}H-NMR spectrum of 7-chloro-6-fluorochromone-3-carboxaldehyde (Figure 29) exhibited the characteristic proton at chemical shift 7.58 ppm.

(doublet, 1H, J=6 Hz) for the proton position 8, The peak at chemical shift 7.92 ppm. (doublet, 1H, J=9 Hz) for the proton position 5. The singlet peak at chemical shift 8.45 ppm. (1H) and 10.28 ppm. (1H) were identified for the proton position 2 and aldehyde proton, respectively.

4) 7-CHLORO-6-FLUOROCHROMONE-3-CARBOALDOXIME (4B)

The synthesis of 4B was accomplished by the reaction of 7-chloro-6-fluorochromone-3-carboxaldehyde and hydroxylamine hydrochloride in absolute ethanol. The reaction mechanism involved nucleophilic attack of nitrogen atom of hydroxylamine hydrochloride on partial positive charge carbon atom of aldehyde group and then it lost one molecule of water to form aldoxime derivative.

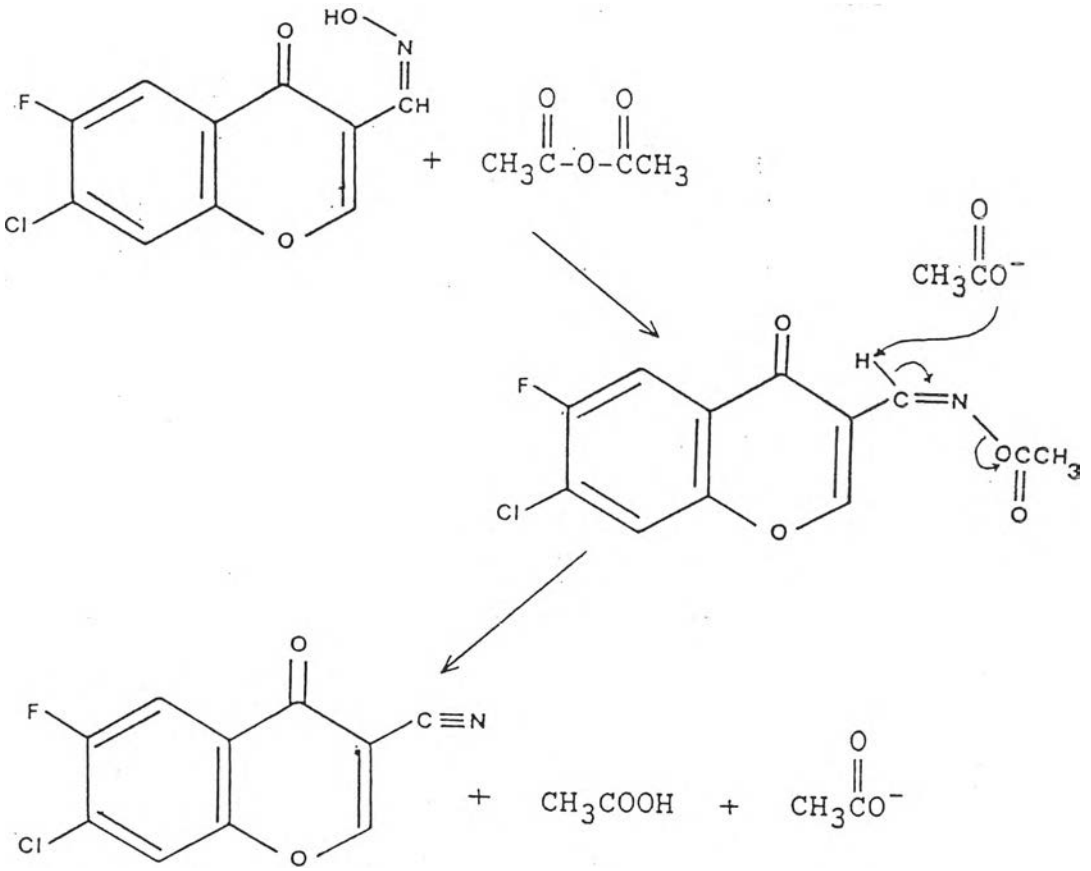


The IR spectrum of 7-chloro-6-fluorochromone-3-carboaldoxime (Figure 30) showed the broad peak at 3200 cm^{-1} for O-H stretching of aldoxime group, the strong peak at 1620 cm^{-1} and 1650 cm^{-1} for C=O stretching of carbonyl of pyrone ring and C=N stretching of aldoxime group, respectively.

The ^{1}H-NMR spectrum of 7-chloro-6-fluorochromone-3-carboaldoxime (Figure 31) showed the peak at chemical shift 7.93 ppm. (doublet, 1H, J=9.0 Hz) for the proton position 5, the peak at chemical shift 8.18 ppm. (doublet, 1H, J=6.0 Hz)for the proton position 8. The singlet peak at chemical shift 11.49 ppm. (1H), 8.70 ppm. (1H) and 8.03 ppm. (1H) were assigned for the hydroxyproton, proton position 2 and proton adjacent to oximino group, respectively.

5) 7-CHLORO-6-FLUOROCHROMONE-3-CARBONITRILE (5B)

The synthesis of 5B was prepared by dehydration of 7-chloro-6-fluorochromone-3-carboxaldoxime. The dehydrating agent was acetic anhydride which probably initiated the acetylation of oximino group followed by elimination of acetic acid. Moreover, the reaction was most sucessful when the H and O-H of oximino group were on the opposite side.

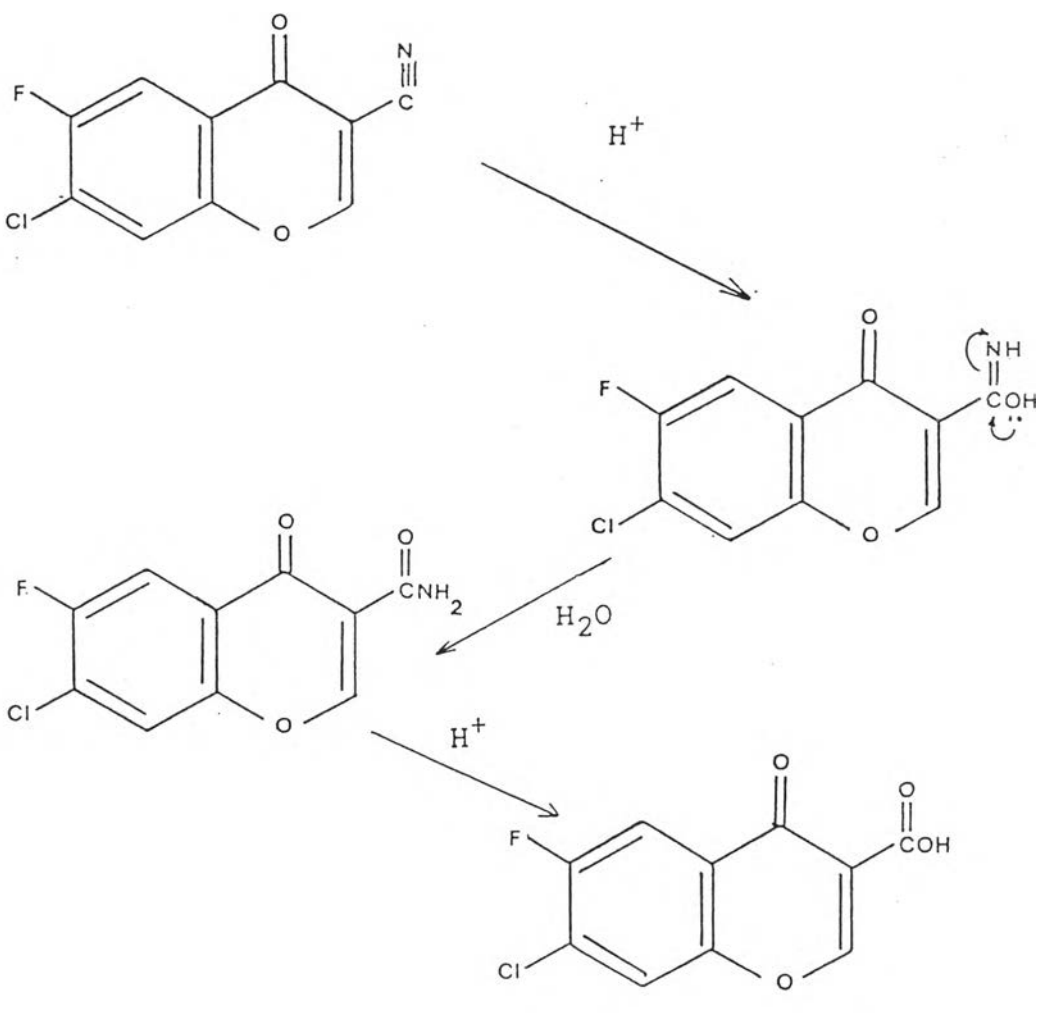


The IR spectrum of 7-chloro-6-fluorochromone-3-carbonitrile (5B) (Figure 32) showed the peak of C-H stretching vibration between 3060-3090 cm^{-1}, the strong peak at 2240 cm^{-1} for C≡N stretching. The C=O stretching of carbonyl of pyrone ring was found the peak at 1660 cm^{-1}.

The ^{1}H-NMR of 5B (Figure 33) showed the peak at chemical shift 7.91 ppm. (doublet, 1H, J=9.0 Hz) for the proton position 5, the peak at chemical shift 8.23 ppm. (doublet, 1H, J= 6 Hz) for the proton position 8. The singlet peak was found at chemical shift 9.19 ppm. for the proton position 2.

6) 7-CHLORO-6-FLUOROCHROMONE-3-CARBOXYLIC ACID (6B)

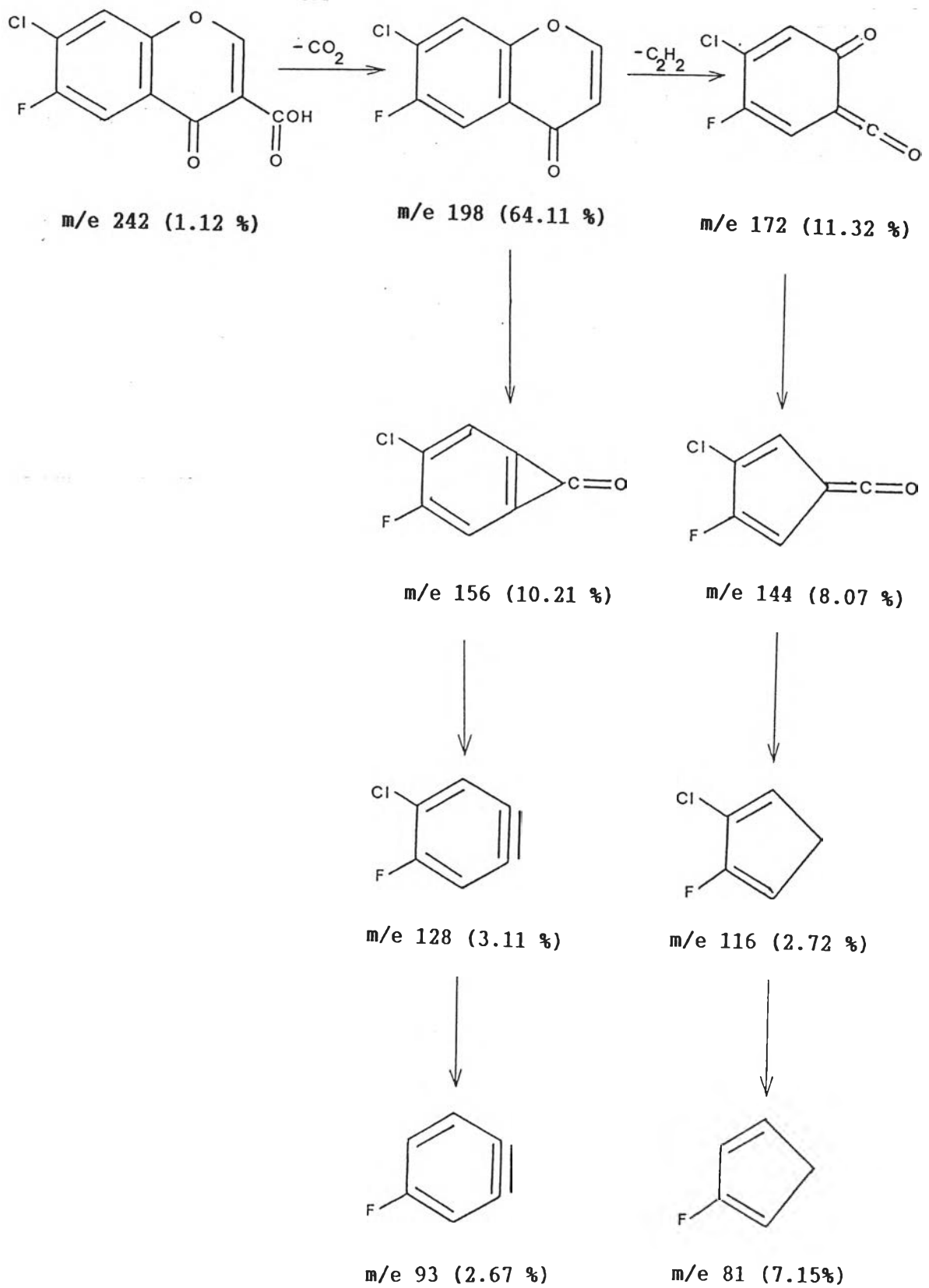
This product was synthesized by hydrolysis 7-chloro-6-fluorochromone-3-carbonitrile with 55 % sulfuric acid. The mechanism of this hydrolysis involved initial attack of water on the nitrile group. The reaction proceeded via the formation of an amide intermediate. Then the amide intermediate was finally hydrolyzed by acid catalyse to obtained 7-chloro-6-fluorochromone-3-carboxylic acid.



The IR spectrum of 7-chloro-6-fluorochromone-3-carboxylic acid (6B) (Figure 34) showed the broad peak in the region 2500 - 2700 cm^{-1} for O-H stretching of carboxylic acid which had intramolecular hydrogen bonding. The peak at 1760 cm^{-1} and 1610 cm^{-1} were identified for C=O stretching of carboxylic acid and ketone of pyrone ring, respectively.

The ^{1}H-NMR spectrum of 6B (Figure 35) was found the peak at chemical shift 7.74 ppm. (doublet, 1H, J=5.7 Hz) for the proton position 8, the peak at chemical shift 8.00 ppm. (doublet, 1H, J=8.0 Hz) for the proton position 5. The singlet peak at chemical shift 8.96 ppm. (1H) and 12.96 ppm. (1H) were identified for the proton position 2 and hydroxy proton of carboxylic acid group.

The mass spectrum of 6B (Figure 36) showed peak at m/e 242 for molecular ion peak. The fragment ion peak at m/e 198 exhibited the loss of CO_2 group. The characteristic peak at m/e 62, 74, 81, 93, 116, 128, 144, 156, 172 were proposed as follows :



Scheme III Fragmentation pattern in ei mass spectrum of 7-Chloro-6-fluorochromone-3-carboxylic acid

Table 1 Results of the antibacterial activity of the two compounds.

Conc. of Cpd. A (μg/cup)	Types of Microorganism		
	S. aureus	E. coli	Ps. aeruginosa
50	+	-	-
100	+	-	-
200	+	-	-

Conc. of Cpd. B (μg/cup)	Types of Microorganism		
	S. aureus	E. coli	Ps. aeruginosa
50	-	-	-
100	-	+	-
200	-	+	-

Cpd. A = 6-Chlorochromone-3-carboxylic Acid

Cpd. B = 7-Chloro-6-fluorochromone-3-carboxylic Acid

\+ = the presence of the inhibition zone

\- = the absence of the inhibition zone