CHAPTER IV

DISCUSSION

The isothiazolopyrimidine derivatives were synthesized here via 2 steps :

1. The synthesis of isothiazolo [3,4-d] pyrimidine ring, and

2. Derivatization of isothiazolopyrimidine derivatives

Synthesis of Isothiazolo [3,4-d] pyrimidine Ring

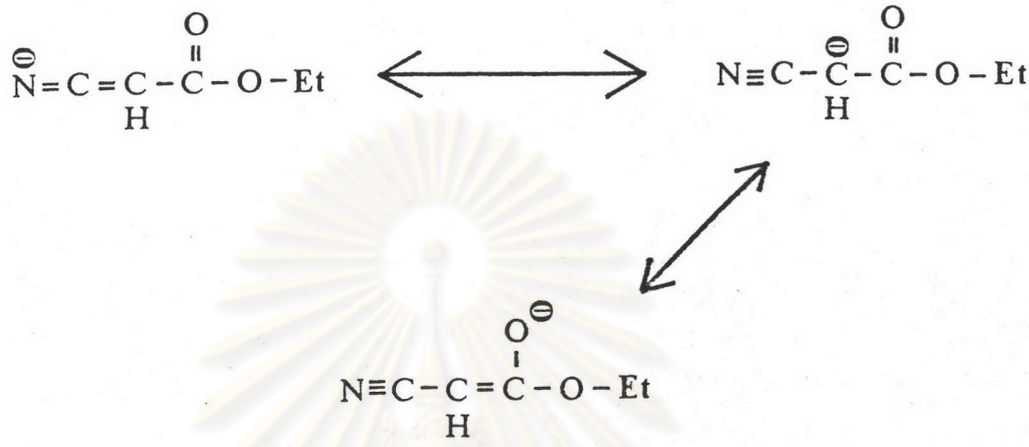
1. Synthesis of 3,5-Diamino-4-carbethoxyisothiazoles

The synthesis of 3,5-diamino-4-carbethoxy isothiazoles started with the formation of carbanion of ethyl cyanoacetate in the presence of sodium ethoxide.

$$NC-CH_2-COOEt \underset{C_2H_5OH}{\overset{NaOEt/}{\rightleftharpoons}} NC-\overset{\ominus}{C}H-COOEt$$

In general, the C-H bond is quite difficult to break, but in the presence of the electron withdrawing group attached at the α-position, the carbanion can be formed. The electron withdrawing group affects the acidity of α-hydrogen by helping to accomodate the

negative charge of the anion. In the case of ethyl cyanoacetate, carbanion is stabilized by the resonance hybrid :

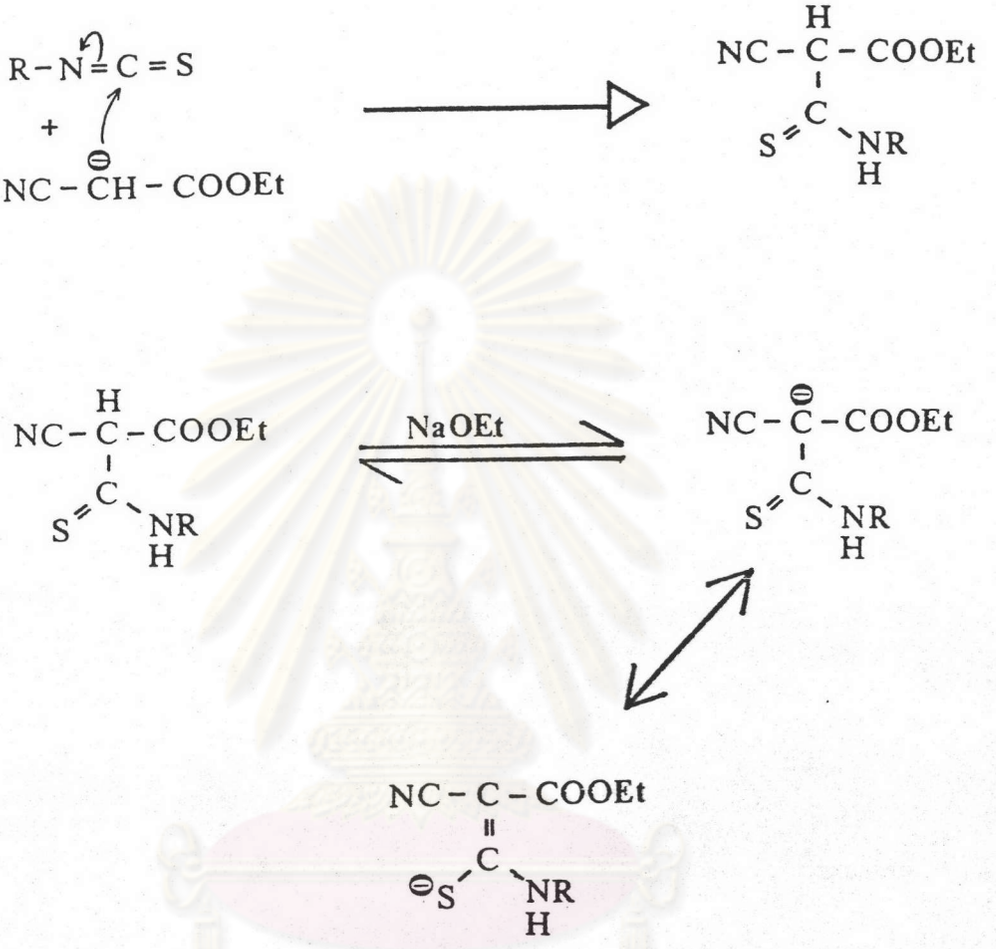


Though, the significant concentration of the carbanion can be generated from ethyl cyanoacetate which is only a weakly acidic compound. However, strong base is needed to promote a large amount of the carbanion formation.

The carbanion formed will act as the nucleophile which can undergo nucleophilic substitution or addition. With methyl or phenyl isothiocyanate, which is the electrophile, anion of ethyl cyanoacetate attacked at the electrophilic carbon under reflux condition to give 2-carbethoxy-3-amino-3-thioacrylonitrile.

The 2-carbethoxy-3-amino-3-thioacrylonitrile still contained one ionizable hydrogen, and on the presence of sodium ethoxide it, too, could be converted into the anion. This anion could tautomerize to the

thiolate anion form.

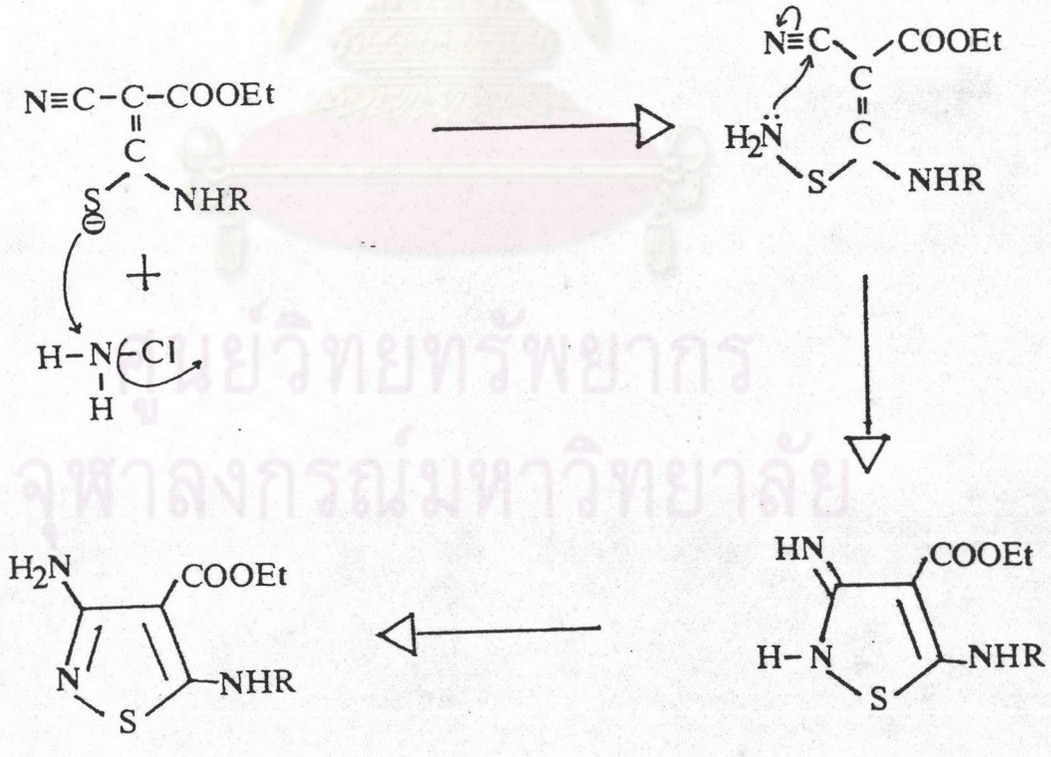


R = methyl, phenyl

Chloramine, which was prepared by dropwise addition of sodium hypochlorite solution at low temperature to the dilute aqueous ammonia, was then used for aminative cyclization of the thiolate anion. The reaction involved a sequence of steps initiated by a nucleophilic displacement at the chloramine nitrogen.

Then, the nucleophile nitrogen attacked at the electrophile cyano carbon. Electron rearrangement occured to give 3,5-diamino-4-carbethoxyisothiazoles as aromatic compound.

From the experiments, when R= methyl and phenyl, the yield of the former product was less than the latter (see table 1). This may be due to the bulky effect of the phenyl group which forced the intermediate to form intramolecular H-bonding between NH and the oxygen atom of the carbethoxy. Hence, more concentration of such intermediate formed and caused the oxidative cyclisation.



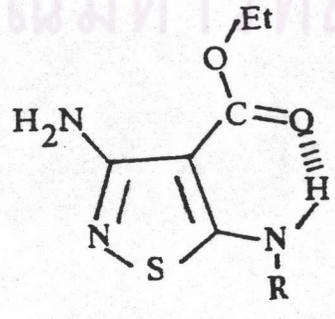
R = methyl, phenyl

2. Synthesis of 5-Substituted-3-(substituted) amino isothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione

Reaction of 3-amino-4-carbethoxy-5-(substituted) aminoisothiazole with methyl or phenylisothiocyanate under reflux condition was undertaken to give the target product. The reaction concerned with the electrophilic substitution to form N-isothiazolylthiourea and followed by cyclization.

3-Amino-4-carbethoxy-5-(substituted) aminoiso thiazole possess two nucleophilic sites on the side chain that are able to react with electrophilic carbon in methyl or phenyl isothiocyanate ie: the 3-amino nitrogen and the 5-(substituted) amino nitrogen. However, 3-amino nitrogen is likely to undergo the reaction because of :

1) The formation of intramolecular hydrogen bonding between the carbonyl oxygen of the carbethoxy group and the hydrogen of amine group in the 5 position to form six-membered ring conformation. So, steric hindrance occured and reduced the reactivity of the 5-amino nitrogen.

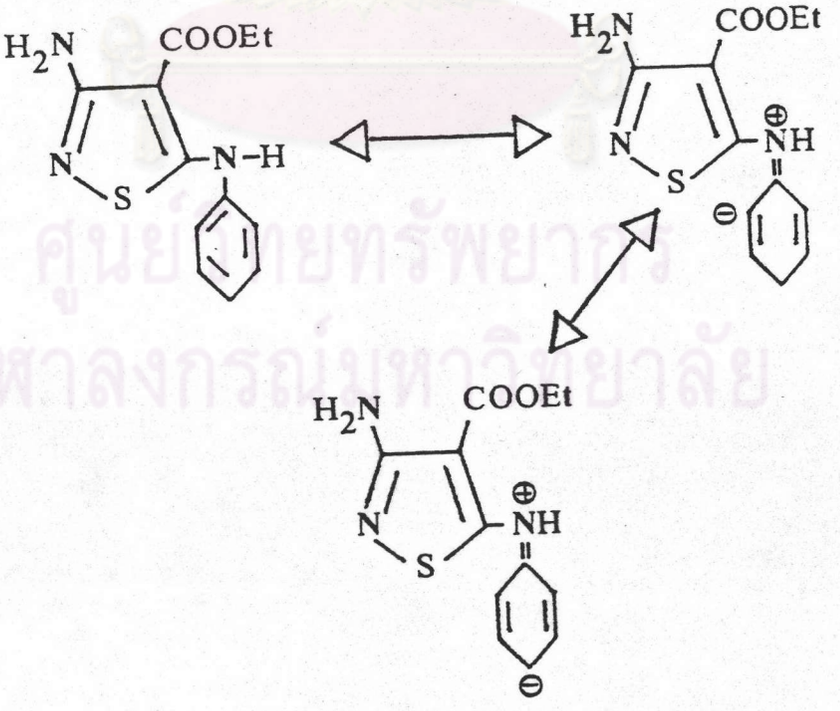


R = methyl, phenyl

2) Methyl group and phenyl group attached at the 5 amino nitrogen is bulky and cause the steric effect for the electrophilic substitution reaction

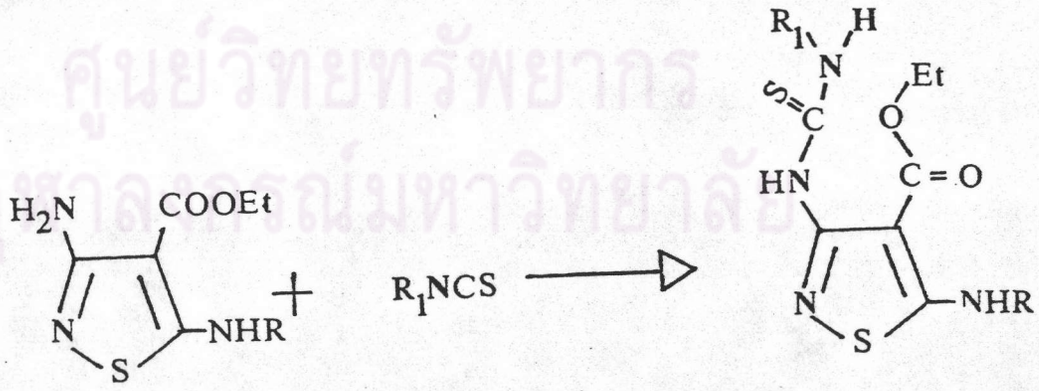
3) In the case of 3-amino-4-carbethoxy-5-phenylaminoisothiazole, phenyl group of the 5 amino function possess the electron withdrawing property. So, the electron density at nitrogen is reduced. The basicity of the 5 amino nitrogen is, therefore, lessen.

Furthermore, electrons at nitrogen can delocalize into the phenyl ring by the conjugation effect. This cause the reduction of the basicity of the 5 amino nitrogen.



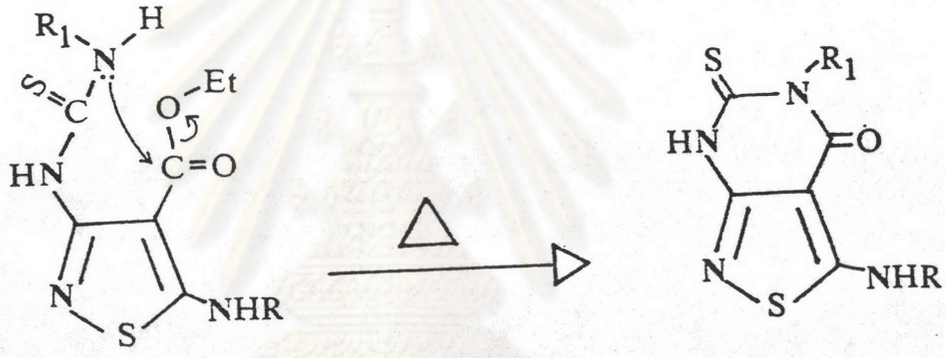
However, in the case of 3-amino-4-carbethoxy-5-methylaminoisothiazole, the inductive effect and conjugation effect were not likely to occur because the methyl group has positive inductive and positive conjugation effect. The steric effect and intramolecular hydrogen bonding may be the predominate reasons for the electrophilic substitution to occur at the 3-amino nitrogen.

From these reasons, methyl or phenyl isothiocyanate, when refluxed with 3-amino-4-carbethoxy-5-substitutedaminoisothiazole, substituted to the 3-amino nitrogen to from N-Methyl-N^{I}-(4-carbethoxy)-5-methylamino-3-isothiazolyl)-thiourea; N-phenyl-N^{I}-(4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea; N-Methyl-N^{I}-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea and N-phenyl-N^{I}-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea.



R , R_1 = methyl, phenyl

These N-isothiazolyl thioureas undergo further heat catalytic cyclization to obtain 5-methyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione; 5-phenyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine -4-one-6(7H)-thione; 5-methyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione and 5-phenyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione.



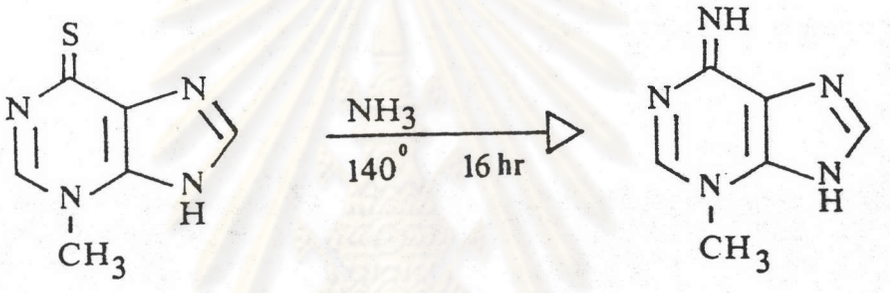
R , R_1 = methyl , phenyl

Derivatization of Isothiazolopyrimidine Derivatives

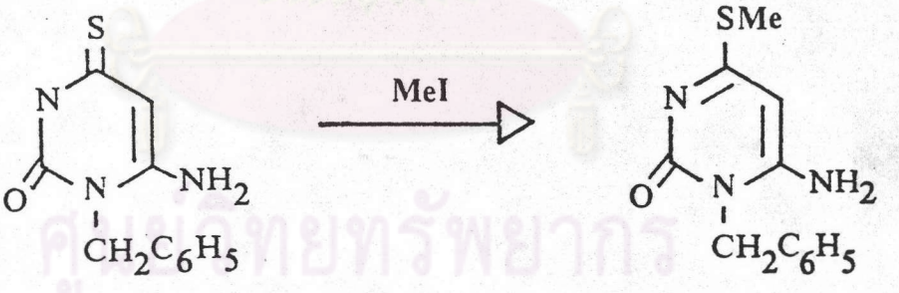
1. Synthesis of Methylsulphide of Isothiazolo pyrimidine Derivatives.

The methylsulphide of isothiazolopyrimidine derivatives have been synthesized here as an intermediate in the synthesis of the 6-substituted isothiazolopyrimidine derivatives. Although some thio derivatives have been shown to undergo substitution reaction (26), some of them

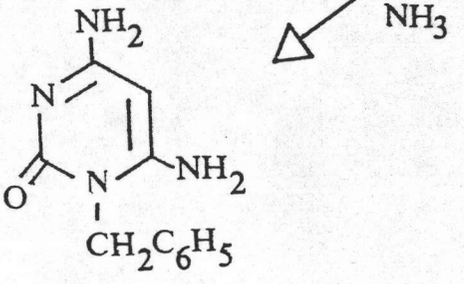
are inactive. However, after the transformation to the methylsulphide derivatives, their reactivity increase and the reaction is going on. For example, Furukawa *et al* (21) directly substituted the ammonia to the (XXXVIII) to obtain the corresponding amine (XXXIX). Whereas (XL) and (XLIII) have to change to the methylsulphide (XLI) and (XLIV) then substituted by the amines to the corresponding products (XLII, XLV) (22,24).



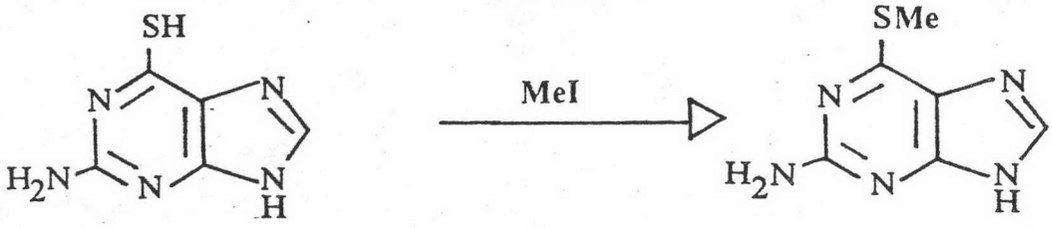
(XXXVIII) (XXXIX)



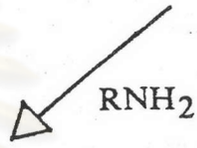
(XL) (XLI)

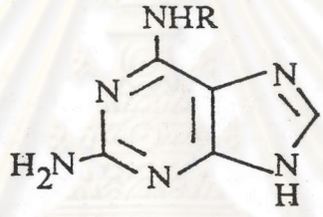


(XLII)



(XLIII) (XLIV)

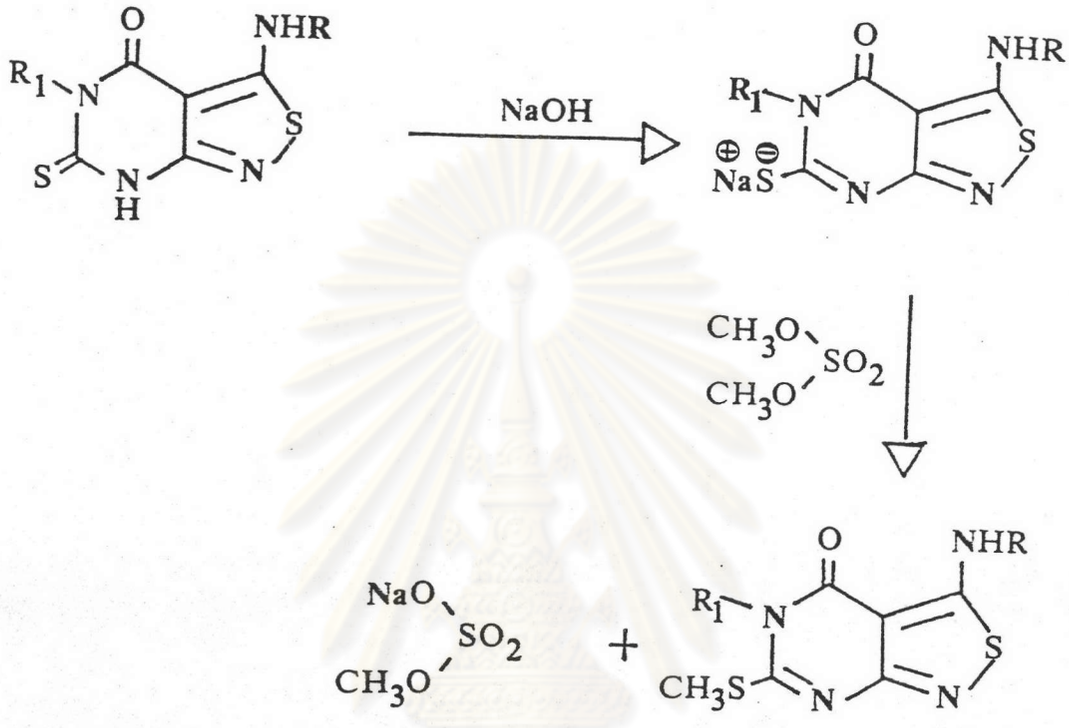




(XLV)

R = methyl, phenyl

Methylation at the thio functional group of isothiazolopyrimidine derivatives were performed by the reaction of dimethyl sulphate under alkali condition. At first, the thio forms were tautomerized under alkali condition to form the sodium salt of thiolate anions. Then, the electrophilic replacement of methyl group from dimethyl sulphate occured.



R , R_1= methyl, phenyl

To this method, four methylthio derivatives, 5-methyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one; 5-phenyl-6-methylthio-3-phenylamino isothiazolo [3,4-d] pyrimidine-4-one; 5-methyl-6-methyl thio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one and 5-phenyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one, were obtained.

The IR spectrum of 5-methyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one (figure 23) showed the NH stretching of secondary amine at 3250 cm^{-1}, stretching of carbonyl group at 1650 cm^{-1}, stretching and bending of the carbon-carbon in the aromatic benzene at 1600, 1580, 1500 and 1450 cm^{-1}, asymetric bending of the methyl in the methylthio functional group at 1460 cm^{-1}, and C-H out of plane bending of the aromatic benzene at 750 and 690 cm^{-1} which represented the monosubstituted benzene. The ^{1}H-NMR spectrum (Figure 5) showed the peak of amino hydrogen at 9.84 ppm (broad, 1H), the peak for phenyl hydrogen at the 3-phenylamino at 7.40 ppm (multiplet, 5H), the peak for methyl hydrogen at position 5 at 3.52 ppm (singlet, 3H) and the peak for methyl hydrogen of the 6-methylthio at 2.66 ppm (singlet, 3H).

The IR spectrum of 5-phenyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one (figure 24) showed the NH streching of secondary amine at 3250 cm^{-1}, streching of carbonyl group at 1680 cm^{-1}, asymmetric bending of methyl in the methylthio functional group at 1460 cm^{-1}, symmetric bending of methyl in the methylthio functional group at 1340 cm^{-1} and C-H out of plane bending of the aromatic benzene at 750 cm^{-1} (doublet) and 690 cm^{-1} (doublet) which represented the two monosubstituted benzene. The ^{1}H-NMR spectrum (Figure 6) showed the peak of amino hydrogen at 9.81 ppm

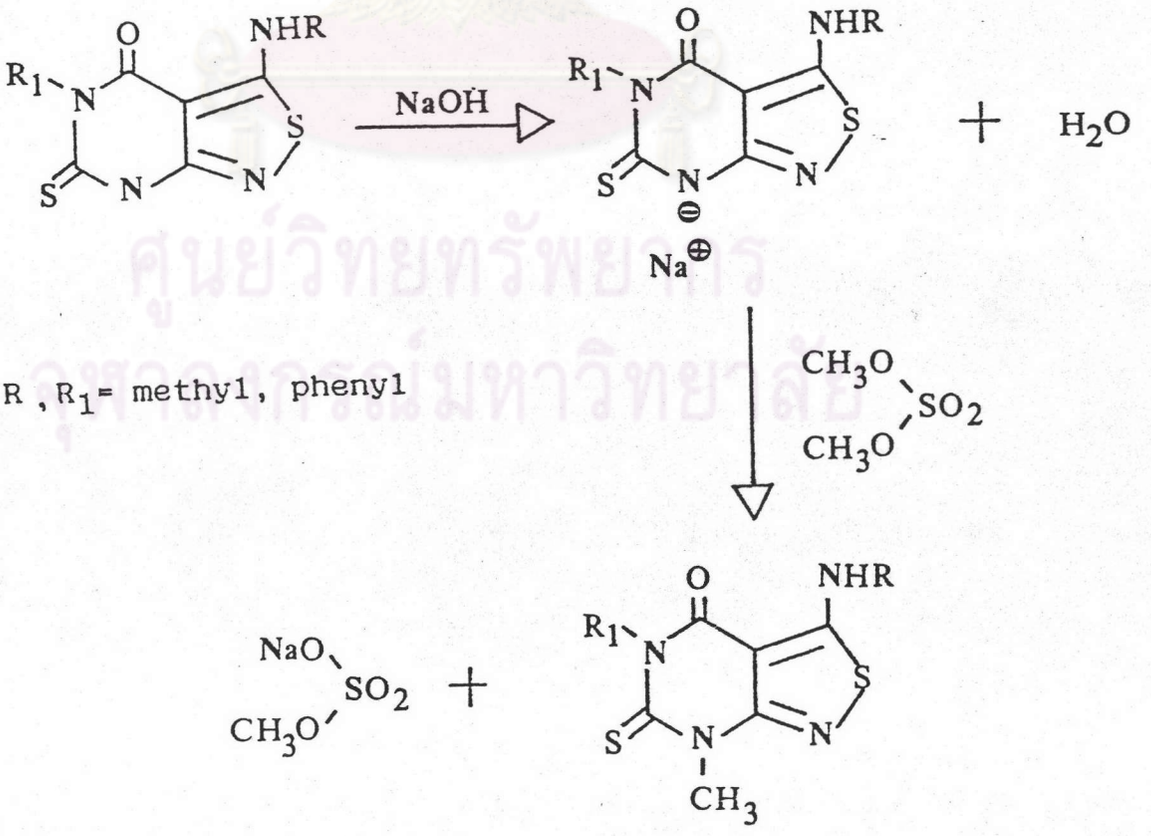
(broad,1H), the peak for phenyl hydrogen at the 3-phenylamino and 5-phenyl at 7.50 ppm (multiplet, 10H), the peak for methyl hydrogen of the 6-methylthio at 2.53 ppm (singlet, 3H).

The IR spectrum of 5-methyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one (figure 26) showed the NH streching of secondary amine at 3250 cm^{-1}, streching of carbonyl group at 1660 cm^{-1}, asymmetric bending of methyl in the methylthio functional group at 1415 cm^{-1} and symmetric bending of methyl in the methylthio functional group at 1330 cm^{-1}. The ^{1}H-NMR spectrum (Figure 8,9) showed the broad peak of amino hydrogen at 7.30 ppm, the peak for methyl hydrogen at position at 3.47 ppm (singlet, 3H), the peak for methyl hydrogen of 3-methylamino at 3.07 ppm (doublet, 3H, J= 5.3) and the peak for methyl hydrogen of the 6-methylthio at 2.63 ppm (singlet, 3H).

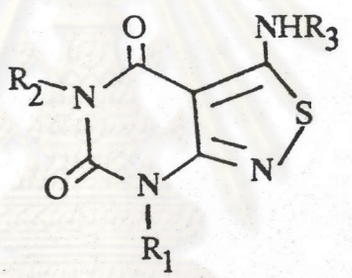
The IR spectrum of 5-phenyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one (figure 28) showed the NH streching of secondary amine at 3330 cm^{-1}, streching of carbonyl group at 1670 cm^{-1}, asymmetric bending of methyl in the methylthio functional group at 1415 cm^{-1}, symmetric bending of methyl in the methylthio functional group at 1340 cm^{-1} and C-H out of plane bending of the aromatic benzene at 750 cm^{-1} and 690 cm^{-1} which represented the monosubstituted bending.

The ^{1}H-NMR spectrum (Figure 11) showed the peak of the 5-phenyl hydrogen at 7.50 ppm (multiplet, 5H), the peak for methyl hydrogen at the 3-methylamino at 3.01 ppm (doublet, 3H, J = 5.4), the peak for methyl hydrogen of the 6-methylthio at 2.50 ppm. The peak of amino hydrogen at position 3 was not observed. It was expected to show the peak at about 7.30 ppm which interfere with the phenyl hydrogen position.

Owing to the non-selective methylating agent property of dimethyl sulphate, it may undergo both O-methylation and N-methylation. From the starting compound used, the hydrogen at the 7-nitrogen may be substituted by methyl group of dimethyl sulphate according to this mechanism :



Although the hydrogen of the NH at the position 3 is likely to be substituted by methyl group of dimethyl sulfate, such compound were not obtained. This can be described by the formation of hydrogen bond of amino hydrogen and the oxygen of the carbonyl at the position 4. This event has also been found by Furukawa Y. and Shima S.(14), when they synthesized 3-(monosubstituted) aminoisothiazole [3,4-d] pyrimidine-4,6 (5H,7H)-diones (XLVI).



(XLVI)

R_1 = Bz, p-ClPh, Ph

R_2 = H

R_3 = Et, Me

When R_2 was hydrogen, it was alkylated within shorter time whereas alkylation of the amino group of the 3-position required longer reaction time, so that the selective alkylation of N-5 position was possible.

5,7-Dimethyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6-thione showed the IR spectrum (Figure 22) of NH streching of the secondary amine at 3250 cm^{-1}, streching of carbonyl group at 1650 cm^{-1}, thione group at 1350 cm^{-1}, and C-H out of plane bending of the aromatic benzene at 750 cm^{-1} and 680 cm^{-1} represented the monosubstituted benzene. The ^{1}H-NMR spectrum (Figure 4) showed the broad peak for amino hydrogen at 9.95 ppm, the peak for phenyl hydrogen of 3-phenylamino at 7.40 ppm (multiplet, 5H) , the peak for methyl hydrogen at position 7 at 3.97 ppm (singlet, 3H) and the peak for methyl hydrogen at position 5 at 3.78 ppm (singlet, 3H).

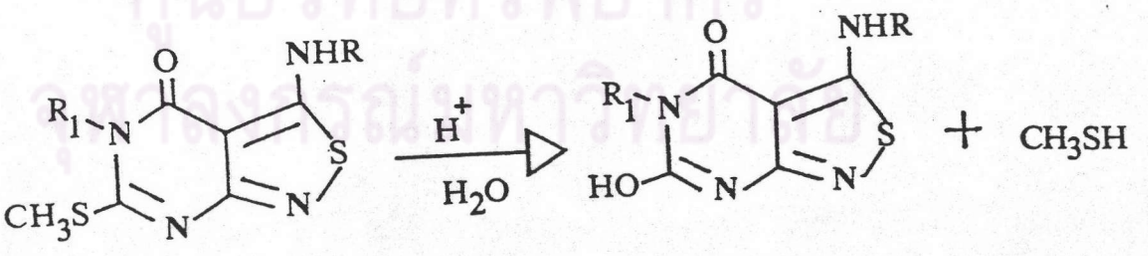
5,7-Dimethyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6-thione showed the IR spectrum (figure 25) of NH sreching of the secondary amine at 3320 cm^{-1}, streching of carbonyl group at 1670 cm^{-1} and thione group at 1360 cm^{-1}. The ^{1}H-NMR spectrum (Figure 7) showed the broad peak of amino hydrogen at 7.52 ppm, the peak for methyl hydrogen at position 7 at 3.94 ppm (singlet, 3H), the peak for methyl hydrogen at position 5 at 3.74 ppm (singlet, 3H) and the peak for methyl hydrogen of 3-methyl amino at 3.08 ppm (doublet, 3H, J = 5.4).

7-Methyl-5-phenylaminoisothiazolo [3,4-d] pyrimidine -4-one-6-thione showed the IR spectrum (Figure 27) of NH stretching at 3350 cm^{-1}, the stretching of carbonyl group at 1680 cm^{-1}, thione group at 1350 cm^{-1} and C-H out of

plane bending of the aromatic benzene at 730 cm^{-1} and 690 cm^{-1} represented the monosubstituted benzene. The ^{1}H-NMR spectrum (Figure 10) showed the peak for phenyl hydrogen at position 5 at 7.40 ppm (multiplet, 5H), the peak for methyl hydrogen at 7 position at 3.97 ppm (singlet, 3H), the peak of methyl hydrogen at 3-methylamino at 3.04 ppm (doublet, 3H, J = 5.2). The peak of aminohydrogen at position 3 was not observed. It was expected to show the peak at about 7.5 ppm which interfere with the phenyl hydrogen position.

2. Replacement of Methylthio Substituents by Hydroxyl Group

According to the method for the replacement, acid hydrolysis was chosen. Methylsulphide of isothiazolopyrimidine derivatives were hydrolyzed using acid as catalyst to form the corresponding products.



R = methyl, phenyl

Therefore, the obtained products were 5-methyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one, 5-phenyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one, 5-methyl-3-methylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one and 5-phenyl-3-methylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one.

Due to the poor solubility in almost all the organic solvent of the obtained products, the poor ^{1}H-NMR spectra were obtained. However, the IR spectra and elemental analyses verified the structure of these products. The IR spectrum of 5-methyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one (figure 29) showed the peak of OH stretching vibration at 3200 cm^{-1}, the stretching of carbonyl group at 1720 cm^{-1}, CO stretching at 1190 cm^{-1} and C-H out of plane bending of the aromatic benzene at 750 and 690 cm^{-1} which represented the monosubstituted benzene. The ^{1}H-NMR spectrum (Figure 12) showed the peak of phenyl hydrogen of 3-phenylamino group at 7.40 ppm (multiplet), the peak for methyl hydrogen at position 5 at 3.40 ppm (singlet), the peaks of amino hydrogen and hydroxy hydrogen were not observed.

The IR spectrum of 5-phenyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one (figure 30) showed the peak of OH stretching vibration at 3400 cm^{-1}, stretching of carbonyl group at 1710 cm^{-1}, CO stretching

at 1190 cm^{-1} and CH out of plane bending of the aromatic benzene at 725 and 695 cm^{-1} which represented the monosubstituted benzene. The ^{1}H-NMR spectrum (Figure 13) showed the peak of hydrogen of the two phenyl groups at 7.40 ppm (multiplet). The peaks of amino hydrogen and hydroxyl hydrogen were not observed.

The IR spectrum of 5-methyl-3-methylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one (figure 31) showed the peak of NH stretching at 3250 cm^{-1}, OH stretching vibration at 3200 cm^{-1}, the stretching of carbonyl group at 1705 cm^{-1} and CO stretching at 1200 cm^{-1} The ^{1}H-NMR spectrum (Figure 14, 15) showed the peak of methyl hydrogen at position 5 at 3.30 ppm and the peak of methyl hydrogen of the 3-methylamino group at 2.93 ppm (doublet). The peaks of amino hydrogen and hydroxyl hydrogen were not observed.

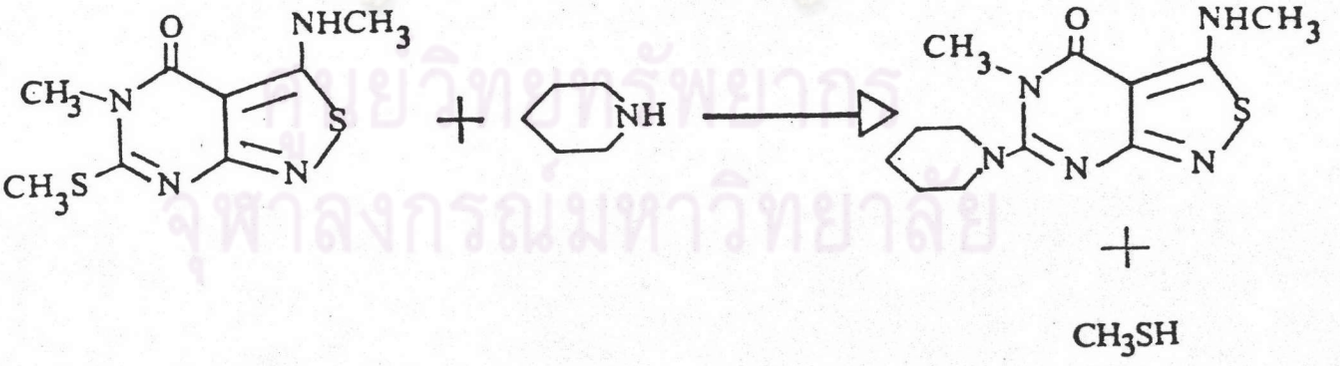
The IR spectrum of 5-phenyl-3-methylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one (figure 32) showed the peak of OH stretching vibration at 3450 cm^{-1}, NH stretching at 3380 cm^{-1}, the stretching of carbonyl group at 1710 cm^{-1}, CO stretching at 1200 cm^{-1} and CH out of plane bending of the aromatic benzene at 770 and 690 cm^{-1} which represented the monosubstituted benzne. The ^{1}H-NMR spectrum (Figure 16,17) showed the peak of phenyl hydrogen at position 5 at 7.50 ppm and the peak of methyl hydrogen at the 3-methylamino at 2.90 ppm (doublet).

The peaks of amino hydrogen and hydroxyl hydrogen were not observed.

3. Replacement of Methylthio Substituents by Amines

Several attempts have been performed in the reaction of 5-methyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one with many amines under various conditions such as toluidine, aniline, alcoholic ammonia, but no replacement reaction were observed. However,on the treatment with piperidine at 140°C for 24 hours an expected product of 5-methyl-3-methylamino-6-(1′-piperidino)isothiazolo [3,4-d] pyrimidine-4-one was obtained.

The reaction depended on the nucleophilic substitution of piperidine at position 6.



The ^{13}C NMR spectrum (figure 20,21) showed the peak at 190.37 ppm (singlet) which was assigned to the carbonyl carbon at position 4. The peak at 165.39,

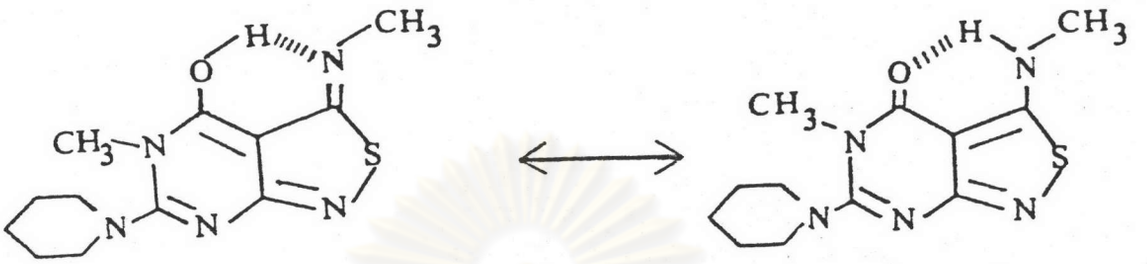
163.23, 157.19 and 92.75 ppm which were singlet, were assigned to the carbon at position 6, 7a, 3 and 3a respectively. The peak at 49.90 ppm (triplet) was assigned to the 2′ and 6′ carbon. The peak at 33.86 and 31.75 ppm which were quadret were assigned to the carbon at 5-methyl and 3-methyl. The peak at 25.41 and 24.16 ppm were assigned to the 3′ and 5′ carbon, respectively.

The ^{1}H-NMR spectra (Figure 18,19) showed the peak of methyl hydrogen at position 5 at 3.35 ppm (singlet, 3H). The peak of methylamino hydrogen at 3.23 ppm (singlet, 3H). The peak of 2′ and 6′ hydrogen at 3.18 ppm (singlet, 4H) and the peak of 3′ ,4′ ,5′ hydrogen at 1.66 ppm (broad, 6H). The peak of methylthio functional group (2.60 ppm) disappeared. The integration of the spectrum revealed that piperidine substituted to the 5-methyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one at methylthio position in the ratio of 1:1.

The IR spectrum (Figure 33) showed the peak at 3400 cm^{-1}. The peak of methylthio functional group at 1415 and 1330 cm^{-1} were disappeared.

From the spectra data obtained, it was expected that on the solid state the 5-methyl-3-methylamino-6-(1′-piperidino)isothiazolo [3,4-d] pyrimidine -4-one was in the form of (XLVI) of which the carbonyl peak in the IR spectrum cannot be observed. Whearas in

the solution state the form of (XLVII) was predominate.



(XLVI) (XLVII)

However, more information is needed for furthur confirmation of the structure.