

## CHAPTER III

### RESULTS AND DISCUSSION

#### 1. Optimization of the Thermal Reaction

The thermal reaction of N,S-diacyl-2-mercaptoethylguanidine hydrochlorides were studied under different conditions (see Table II). While chemical transformation did not occur at room temperature, or under 160°C when heated either with or without a solvent. On the other hand, heating above 160° resulted in the formation of three major products (A-, B- and C-series compounds). Longer heating times and higher temperatures resulted in the production of by-products and decomposition, particularly above 190°C. It was found that the best conditions were 180°C under N<sub>2</sub> atmosphere for 2-7 hours. As expected, reduced pressure without total exclusion of oxygen was worse than completely anaerobic conditions.

#### 2. Separation and Purification of Major Products

According to the TLC upon optimization of the thermal reaction of N,S-diacyl-2-mercaptoethylguanidine hydrochlorides, this thermal reaction gave three major products namely A-, B- and C-series compounds in the order of their R<sub>f</sub> values from low to high using alumina (Art. 1092, Merck) as absorbent and a mixed solvent of 80% hexane and 20% acetone as eluent. This means that the A-series compound appeared to be the most polar compound. While the C-series compounds would be the least. It is interesting to note that the R<sub>f</sub> of the A-series compound was unchanged from 0.04 no matter which starting materials were used but the R<sub>f</sub> values of B- and C-series compounds were varied and quite close to each

other as their ranges appeared to be 0.46-0.64 and 0.61-0.74 respectively. The separation of these three major products was carried out by taking advantage of their different polarities. The A-series compound was precipitated out from the reaction mixture by the addition of acetone and recrystallized from a mixed solvent of methanol and acetone. However, it was unable to separate the other two major products in the same manner but they were successfully done by means of column chromatography using alumina (Art. 1076, Merck) as an absorbent and hexane-acetone mixture as an eluent. The details of this separation are shown in Table VII. Each of the B- and C-series compounds was purified by recrystallization from a mixed solvent of hexane and chloroform.

### 3. Structural Elucidation of 2-Amino-2-thiazoline hydrochloride (A-series compound)

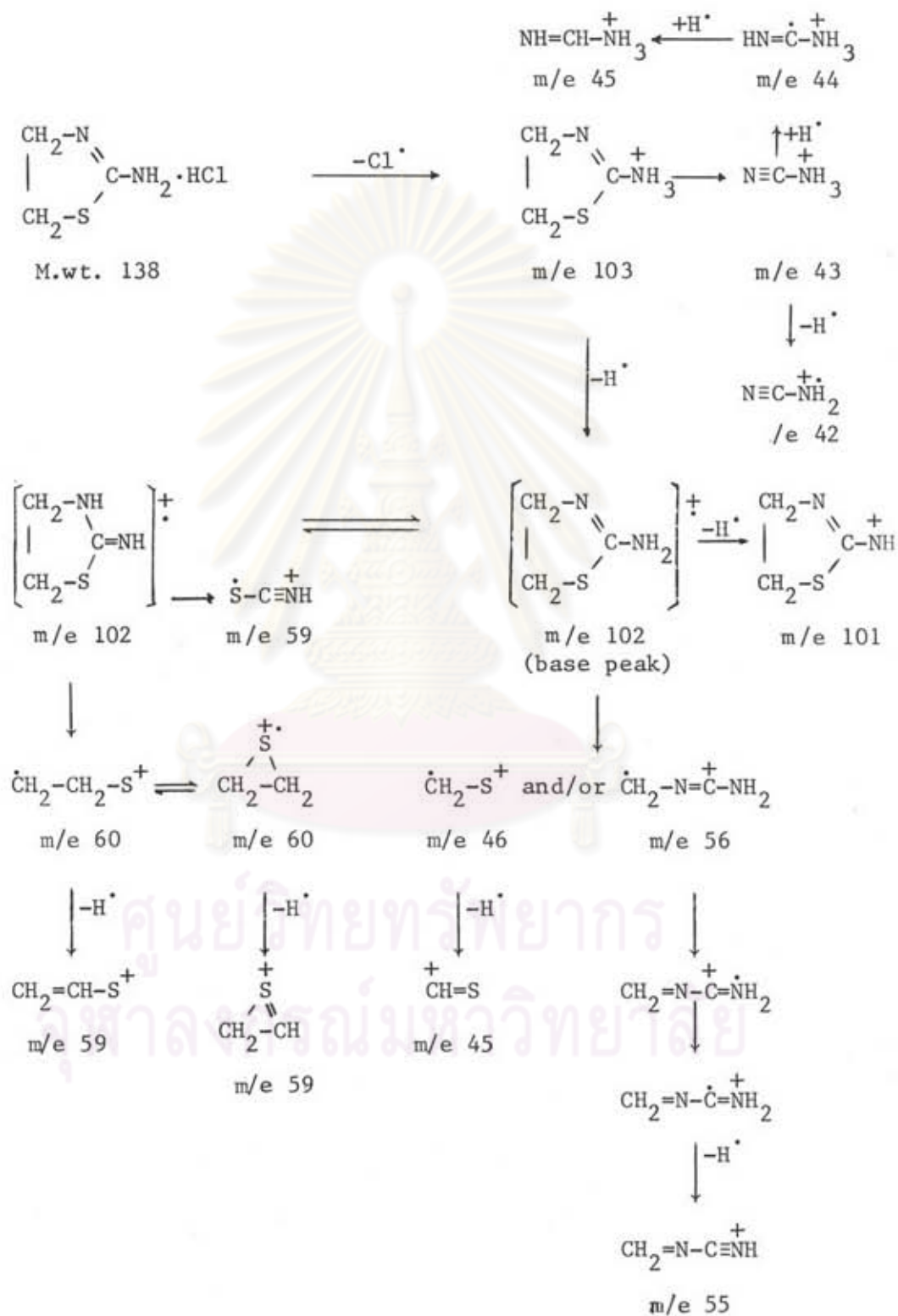
As mentioned above the thermal reaction of N,S-diacyl-2-mercaptoethylguanidine hydrochlorides produced the same  $R_f$  for all the products in this series. Although their melting points from each experiment were varied from 194-203°C, both NMR and IR spectra showed no differences from each other. There was no depression of an admixture with other A-compound in this series. It was confirmed by the elemental analysis and mass spectra that the products of A-series compound from all the thermal reactions were in fact the same. They were all very hydrophilic and hygroscopic and proved to be a hydrochloride salt by the fact that it liberated an acidic gas upon heating and chlorine was found by both elemental analysis and by the Beilstein Test. Elemental analysis revealed the molecular formula to be  $C_3H_7N_2SCl$ . The  $^1H$  NMR spectrum showed an example of an  $A_2B_2$  coupling system for the  $\underline{CH}_2$ -S- and  $\underline{CH}_2$ -N-. There was also a broad peak at  $\delta$  9.76 with an integration of two protons indicating

the presence of an  $\text{NH}_2$  group. The number of carbon atoms in the molecule was confirmed by the appearance of 3 peaks in the  $^{13}\text{C}$  NMR at 172.73, 48.38, 30.83 ppm. Two of the three carbon atoms were clearly methylene carbons next to a heteroatom. The peak at 172.73 ppm could be due to  $\text{C}=\text{O}$  or  $\text{C}=\text{N}$ . The presence of a double band was confirmed by UV absorption at 240.5 nm. Since there was no oxygen present (elemental analysis), this low field carbon atom must be  $\text{C}=\text{N}$ . The assignments for all the spectroscopic data is shown in Table IV. Mass-spectral fragmentation is proposed as shown in Scheme II which corresponds to the previous report<sup>(160)</sup>.

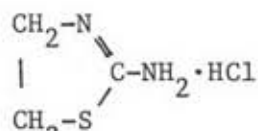


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Scheme II Proposed mechanism for mass spectral fragmentation of A-series compound



It is clear that the structure of the compound is



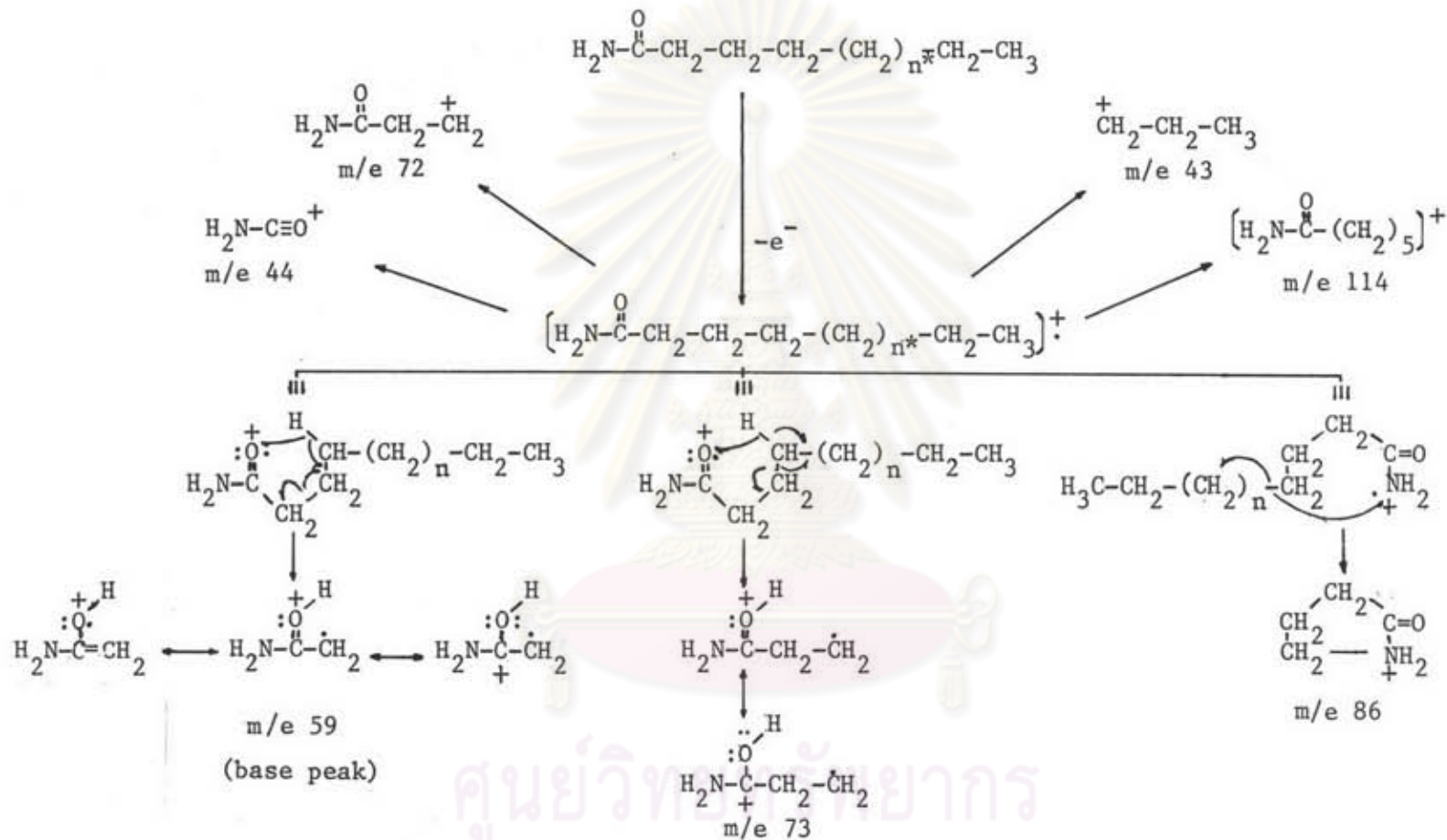
4. Structural Elucidation of the Aliphatic primary amides (B-series compounds)

Elemental analysis<sup>(67,72)</sup> clearly showed the existence of an oxygen atom in the molecular formula. The products, made from the saturated acid chlorides in the initial starting materials, agreed that the formula in this series is  $C_n H_{2n+1} NO$ . The presence of a carbonyl group was clearly indicated by both IR spectra at around  $1660\text{ cm}^{-1}$  and  $^{13}\text{C}$  NMR spectra at  $175.92\text{--}175.70\text{ ppm}$ . The  $^{13}\text{C}$  NMR revealed the number of methylene groups in the molecule. All the spectroscopic data of these products are shown in Table IX to Table XIII. According to the mass spectra the fragmentation of the molecules could be proposed as follows:



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Scheme III Proposed mechanism for mass spectral fragmentation of B-series compounds



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n	m/e ( $M^+$ )	Designated compounds
1	129	B-7
2	143	B-8
3	157	B-9
4	171	B-10
5	185	B-12



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## 5. Structural Elucidation of N,N-Diacyl-cystamines (C-series compounds)

One of the most difficult tasks to identify the reaction products of N,S-diacyl-2-mercaptoethylguanidine hydrochlorides was the structural elucidation of the compounds in this C-series compounds. However, it was successfully done by the assistance of modern instruments such as UV, IR, NMR (both  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) and also mass spectrometer. IR spectra clearly exhibited the characteristic of an amide by showing all the peaks for amide I-VI bands. Other assignments for the IR absorption peaks were described in Table XVI. The presence of a double bond was also indicated by the UV absorption at the range of 241.0-248.5 nm (see Table XV). The most significant indication for the presence of a carbonyl group was shown by the  $^{13}\text{C}$  NMR. All the products in this series showed the absorption peak of a carbonyl carbon atom at the range of 173.65-173.48 ppm. In addition there was always a pair of absorption peaks at the ranges of 38.58-38.25 ppm in the  $^{13}\text{C}$  NMR spectra. They were assigned for C-N at the lower field and a C-S at the higher one in respect to their electronegativity. Other assignments for the  $^{13}\text{C}$  NMR as well as for the  $^1\text{H}$  NMR were set in Table XVII and Table XVIII respectively.

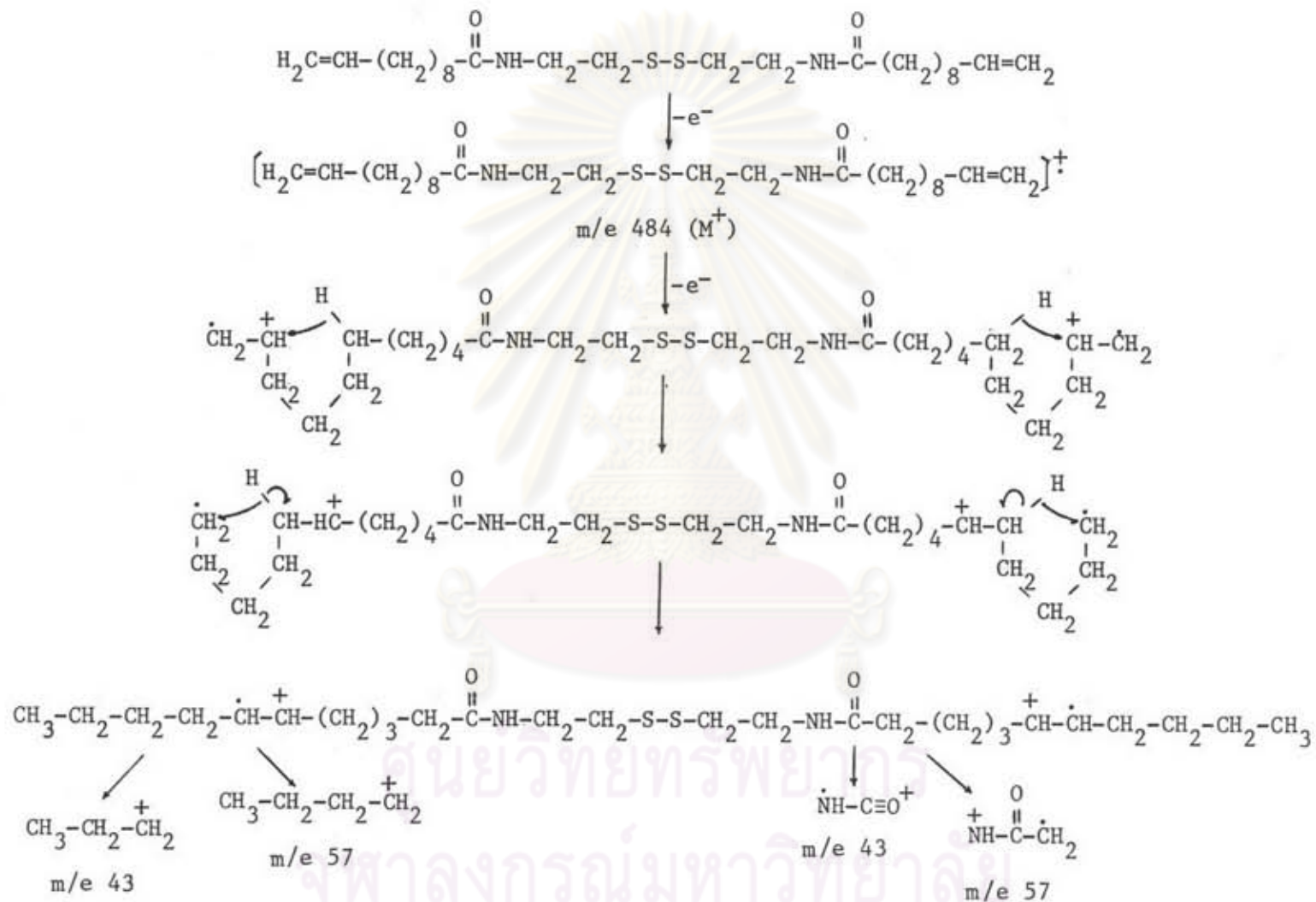
Nevertheless all the spectroscopic datum as mentioned above can not be used to identify specifically whether the products are thiol compounds or their respective disulfids. As a matter of fact a disulfide possesses a symmetrical molecular structure it differs from its thiol compound only by one hydrogen atom. We cannot rely solely on either the NMR, both  $^1\text{H}$  and  $^{13}\text{C}$ , or the IR spectra to distinguish clearly the differences between the thiol and its disulfide. In fact some of the spectroscopic data created confusion so did elemental analysis. Fortunately we have the mass spectrometer to prove that the structure of the product is indeed a disulfide as indicated by its molecular ion peak and other fragmentations as shown in the proposed mechanism as follows:



n	m/e				Designated compounds
	$M^+$	$\frac{1}{2}M^+ + H$	$\frac{1}{2}M^+$	$\frac{1}{2}M^+ - S$ (base peaks)	
1	376	189	138	156	C-7
2	404	203	202	170	C-8
3	432	217	216	184	C-9
4	460	231	230	198	C-10
5	488	245	244	212	C-12

Similar to B-11 there is a rearrangement in C-11, due to the unsaturation, in order to get the following M/e peaks 43, 57 as shown below:

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In addition to the spectroscopic techniques being used to elucidate the structure of the products in this series, some chemical reactions were also employed to prove the absence of an -SH group. The following negative tests were obtained from all the specific reactions for the -SH group as follows:

5.1 Iodine test gave no change in the  $I_2$  colour.

5.2 Mercuric acetate test produced no complex formation.

5.3 Sbf-C1 (4-chloro-7-sulphobenzofurazan) which has been proved to be a thiol specific reagent<sup>(161)</sup> showed no fluorescence after the addition of any compound in this series.

It is interesting to note that Foys and his co-workers<sup>(142)</sup> reported the synthesis of N-octanoyl-2-mercaptoethylamine with its m.p. much lower than that of its corresponding disulfide found in this series. It is convincing that the compounds obtained from this series are obviously N,N'-diacyl-cystamines with the variation of the acyl group.

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The overall reaction was two moles of the starting materials after the thermal reaction they became one mole of 2-amino-2-thiazoline hydrochloride (A-series compound) two moles and half a mole of their corresponding aliphatic primary amides (B-series compounds) and N,N'-diacylcystamines (C-series compounds) respectively. It is interesting to note that under the normal atmosphere without nitrogen flow it took shorter time to produce relatively higher yield of the N,N'-diacylcystamines than it did under the nitrogen flow. Exception was made only once when the reaction went on with an extremely long period of time up to seven hours (see Table VI). This might be due to the fact that the precursor anion compounds were easily oxidized to their respective disulfides in air than they were under nitrogen flow. As mentioned in the optimization of the thermal reaction it was also noticeable that the longer times it spent for the reactions at the same temperature the higher yields of the three major products were obtained. However, if the temperatures exceeded 190°C it produced more unidentified products. Nevertheless, under the nitrogen flow it produced higher yields for both 2-amino-2-thiazoline hydrochloride (A-series compound) and the aliphatic primary amides (B-series compounds). Apparently the nitrogen flow prevented further oxidation and consequently produced less decomposition of the products.

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