CHAPTER IV

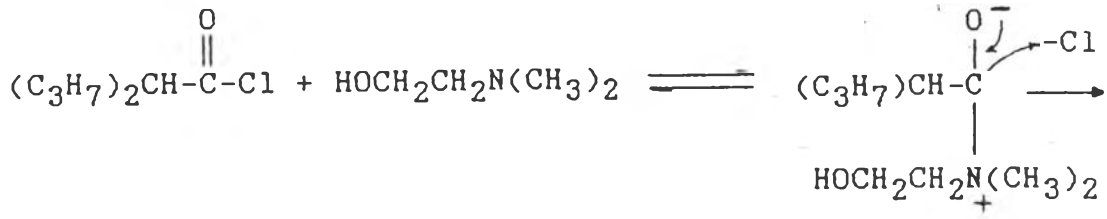
RESULTS AND DISCUSSIONS

In this research, potential prodrugs of valproic acid and monoureide analogues of valproic acid were synthesized. Valproic acid was synthesized according to the procedure of Porubek *et al*. (1989) Valproic acid was, then, converted to the acid chloride, which may be prepared by any conventional methods. Here, valproic acid was allowed to react with thionyl chloride at room temperature. This method was preferably attractive due to the smooth reaction and the products can be used in the next reaction without purification.

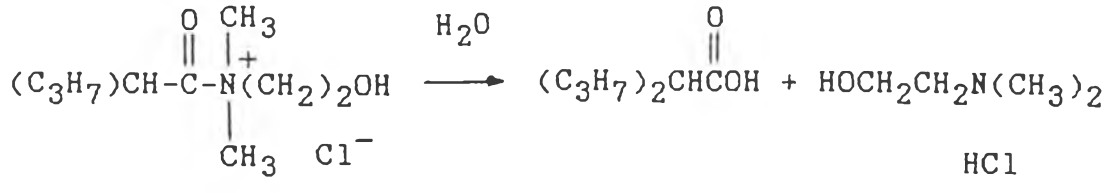
(N,N-dimethylaminoethyl)-2-propylpentanoate

This compound represents ester type, the acid chloride was allowed to react directly with N,N-dimethylaminoethanol in benzene. In fact, N,N-dimethylaminoethanol possesses two nucleophilic moieties in its molecule, (hydroxyl and amino groups). Dimethylamino moiety, in contrast to hydroxyl moiety, is better nucleophile, but it cannot be converted to an amide, for this requires loss of proton from the nitrogen atom and tertiary amine intermediate (XXXIX) has no proton to lose. As the amino moiety attacks to electrophilic

carbonyl of acid chloride, the intermediate formed may decompose into its parent compounds, or it may lose chloride ion to form an N-acylammonium ion (XL) which is unstable, sensitive to moisture, and readily hydrolysed as following.

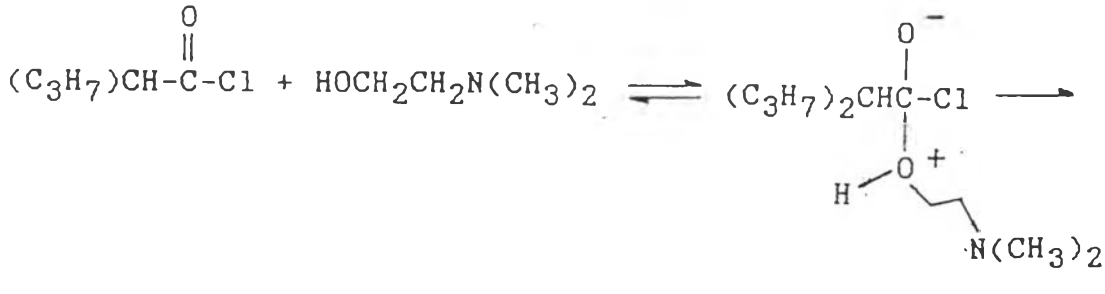


XXXIX

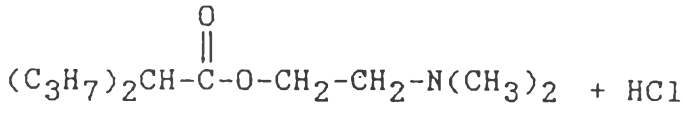


XL

On the other hand, when the hydroxyl moiety of N,N-dimethylaminoethanol attacks to carbonyl of acid chloride as following.



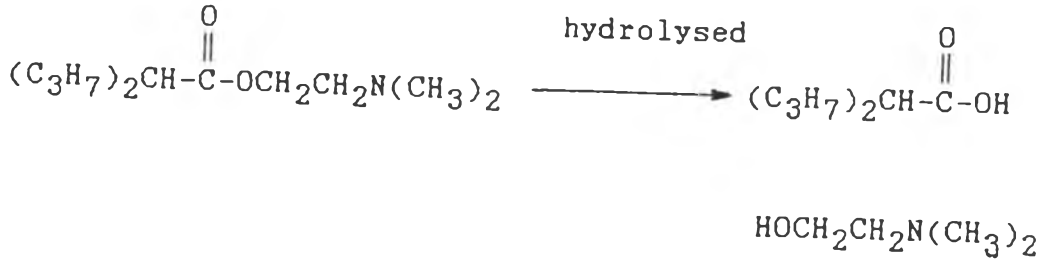
XLI



The intermediate (XLI) loses the oxygenated proton while the chlorine is expelled to form hydrogen chloride and the corresponding amino ester. The hydrogen chloride formed is neutralized with potassium carbonate.

The IR spectrum of (N,N-dimethylaminoethyl)-2-propylpentanoate (Figure 3) showed a strong C=O stretching absorption peak at 1735 cm^{-1}, a peak at 1163 cm^{-1} for the C-O stretching vibration, the peak at 1458 cm^{-1} for $(CH_3)_2$-N scissoring vibration and the C-H stretching vibrations showed in the region 2780-2980 cm^{-1}. The ^{1}H-NMR spectrum (Figure 4) showed the peak at chemical shift 4.19 ppm (triplet, 2H) for methylene protons adjacent to oxygen ester, the peak at 2.57 ppm (triplet, 2H) for methylene protons adjacent to nitrogen atom, the peaks at 0.92 ppm (multiplet, 6H) for two methyl protons and the peaks at 1.38 - 1.61 ppm (multiplet, 8H) for two ethylene protons of the alkyl side chain. The peak at 2.29 ppm (singlet, 7H) represented 6 methyl protons adjacent to nitrogen atom and 1 methine proton which appeared at about the same chemical shift. By comparison with ^{1}H-NMR spectrum of 2-propylpentanoic acid and its derivatives, the methine proton of each compound showed the chemical shift at about 2.1 - 2.4 ppm. This observations confirmed the above assignment.

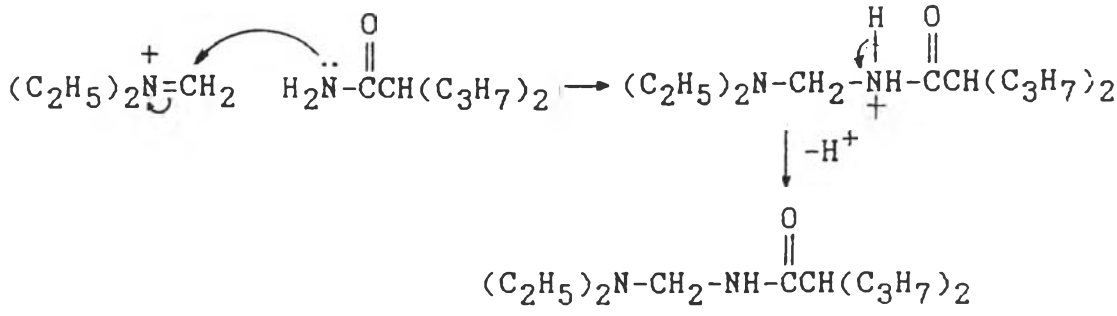
The compound obtained was a liquid state, unstable and easily vaporize at room temperature. Attempt to convert this compound into solid state by formation into inorganic salts such as HCl, H_2SO_4 or organic salts, succinamate, cinnamate was unsuccessful. This compound was expected to be hydrolysed to valproic acid as following.



(N,N-diethylaminomethyl)-2-propylpentamide

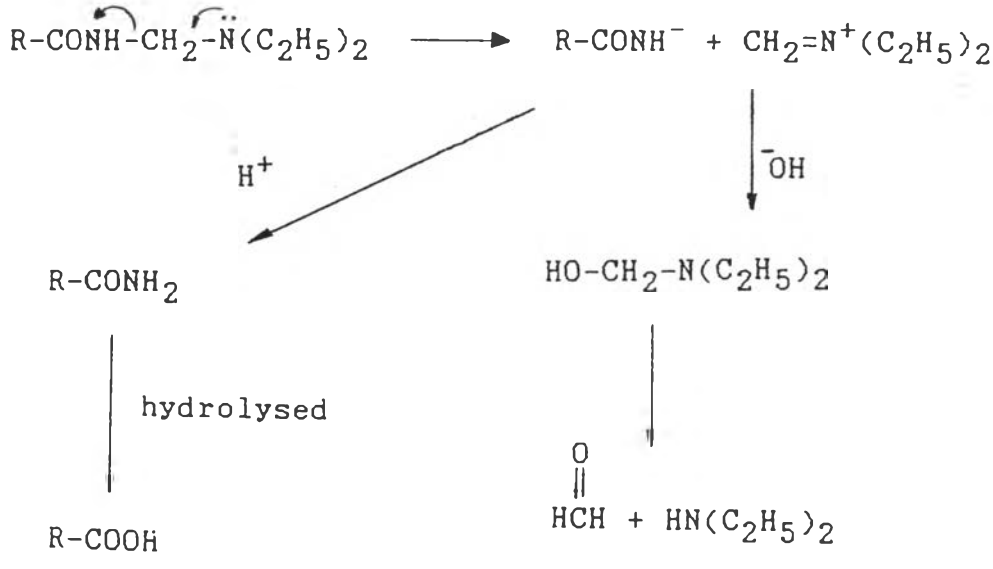
This compound represents N-Mannich base derivative. It was known that Mannich reaction concerns the reaction of amine (1^{o} or 2^{o}), formaldehyde and a compound containing a highly active hydrogen atom, such as various amides, imides, carbamates or hydantoins. In order to synthesize this type of prodrug, valproic acid was converted to its primary amide derivative, valpramide, by treating valproyl chloride with concentrated ammonia solution. Then, the N-Mannich base derivative obtained was accomplished by the reaction of valpramide, formaldehyde and diethylamine in ethanol. The addition of the amine to formaldehyde under the usual slightly acidic reaction condition has been considered as a possible primary step (House, 1972).

The reaction involved nucleophilic attack of nitrogen lone pair electrons to carbonyl carbon of formaldehyde to give diethylamino methanol, which later underwent dehydration to give the corresponding iminium ion. Then, the methylene carbon of iminium ion was attacked by the lone pair electron of amide nitrogen to give (N,N-diethylaminomethyl)-2-propylpentamide



The IR spectrum of (N,N-diethylaminomethyl)-2-propylpentamide (Figure 6) showed the peak of NH stretching vibration of secondary amide at 3320 cm^{-1}, CH stretching vibration at 2890 - 2980 cm^{-1}, a strong absorption peak of C=O stretching vibration (amide I) of secondary amide at 1650 cm^{-1}, a strong NH bending vibration (amide II) at 1540 cm^{-1}, C-N stretching vibration of aliphatic amine at 1200 cm^{-1} and NH out of plane bending at 600 - 800 cm^{-1}. The ^{1}H-NMR spectrum (Figure 7) showed the peaks at chemical shift 0.92 ppm (multiplet, 6H) for two methyl proton and the peaks at 1.38 - 1.61 ppm (multiplet, 8H) for two ethylene protons of alkyl side chain, the broad peak of secondary amide proton at 5.89 ppm (broad, 1H), doublet of methylene protons at 4.2 ppm (doublet, 2H), two methylene protons of amino group at 2.50 ppm (quartet, $N(CH_2-)_2$, 4H) and methine proton at 2.10 ppm (multiplet, 1H). The mass spectrum (Figure 8) showed peak at m/e 228 for molecular ion peak.

(N,N-diethylaminomethyl)-2-propylpentamide was expected to act possibly as a prodrug which converted spontaneously to 2-propylpentamide as the following scheme III.



$R = (C_3H_7)_2CH-$

Scheme III Proposed breakdown pathway of (N,N-diethylaminomethyl)-2-propylpentamide

2-propylpentamide, expected to obtain from the spontaneous breakdown of (N,N-diethylaminomethyl)-2-propylpentamide, is a potent anticonvulsant agent, the further hydrolysis of this compound results in the parent anticonvulsant agent, valproic acid.

N(2′-Propylpentanoyl)-2-pyrrolidinone

This compound represents amide type, though its chemical structure is imide. The synthesis of this compound was accomplished by the reaction of 2-propylpentanoyl chloride and 2-pyrrolidinone sodium in benzene. 2-pyrrolidinone, a liquid state at above 25^{o}C, was first converted to its sodium salt by treating with sodium hydride. Then, N(2′-propylpentanoyl)-2-pyrrolidinone was obtained upon one-step reaction of the above reactants. With this synthetic procedure the overall yield was about 90 percents.

N(2′-propylpentanoyl)-2-pyrrolidinone obtained was an oily liquid and was purified by column chromatography. The chemical structure of the compound was determined by using ^{1}H-NMR, ^{13}C-NMR, IR and mass spectrometric technique.

The IR spectrum of N(2′-propylpentanoyl)-2-pyrrolidinone (Figure 9) showed two strong C=O stretching vibrations at 1680 cm^{-1} and 1740 cm^{-1}. The peak at 1680

cm^{-1} represented amide carbonyl and the peak at 1740 cm^{-1} represented lactam carbonyl which absorbed at higher frequency than amide carbonyl due to the decrement of resonance effect in rigid molecule. The peaks at 2890 - 2980 cm^{-1} represented CH stretching vibration of normal alkane. The peak at 1350 cm^{-1} represented C-N stretching of lactam ring.

The ^{13}C-NMR spectrum of N(2′-propylpentanoyl)-2-pyrrolidinone (Figure 10) indicated 9 kinds of chemical shifts, which were assigned as following, the peak at 13.83 ppm represented two carbons at position 5′and 3", the peak at 16.70 ppm for carbon at position 4 and the peak at 20.17 ppm for two carbons at position 4′and 2". The chemical shifts at 33.77, 34.04, 43.08 and 45.52 ppm represented carbons at position 3, 3′, 2′ and 5 respectively. The carbonyl carbons for amide and lactam showed at 174.67 and 179.65 ppm respectively. The ^{1}H-NMR spectrum of N(2′-propylpentanoyl)-2-pyrrolidinone (Figure 11) showed three characteristic peaks of methylene protons in 2-pyrrolidinone. The peak at about 2.01 ppm (quintet, 2H) represented methylene protons at position 4, the peak at 2.61 ppm (triplet, 2H) for methylene protons at posiiton 3 and the peak at 3.81 ppm (triplet, 3H) for methylene protons at position 5. The fact that, intensity of protons ratio at 3.81 ppm was not corresponded for methylene protons (it should be only two protons) the exceeding proton may be the methine proton at position 2′

that showed broad peak (quintet, 1H) at about the same chemical shift. In order to prove this assumption, the spin-spin decoupling technique was used (Figure 12).

Decoupling the two protons triplet at 2.02 ppm caused the triplet at 2.61 ppm to collapse to a sharp singlet and the triplet at 3.81 ppm to collapse to about five peaks. Decoupling the triplet at 2.61 ppm retained the triplet at 2.02 ppm and 3.81 ppm and decoupling the triplet at 3.81 ppm also retained the triplets at 2.02 ppm and 2.61 ppm. This investigation indicated that the two protons at 2.02 ppm, represented the proton at position 4, coupled with the protons at 2.61 ppm and 3.81 ppm. The two protons at chemical shift 2.61 ppm coupled with the protons at 2.02 ppm and the peak at chemical shift 3.81 ppm coupled with the protons at 2.61 ppm represented protons adjacent to nitrogen atom. Therefore, it was obvious that the methine proton at position 2′ also appeared at about 3.8 ppm. The chemical shifts assignments of ^{13}C-NMR and ^{1}H-NMR were summerized in Table II.

The mass spectrum of this compound (Figure 13) showed peak at m/e 211 for molecular ion peak. The fragment ion peak at m/e 126 was characterized by loss of C_4H_7NO.

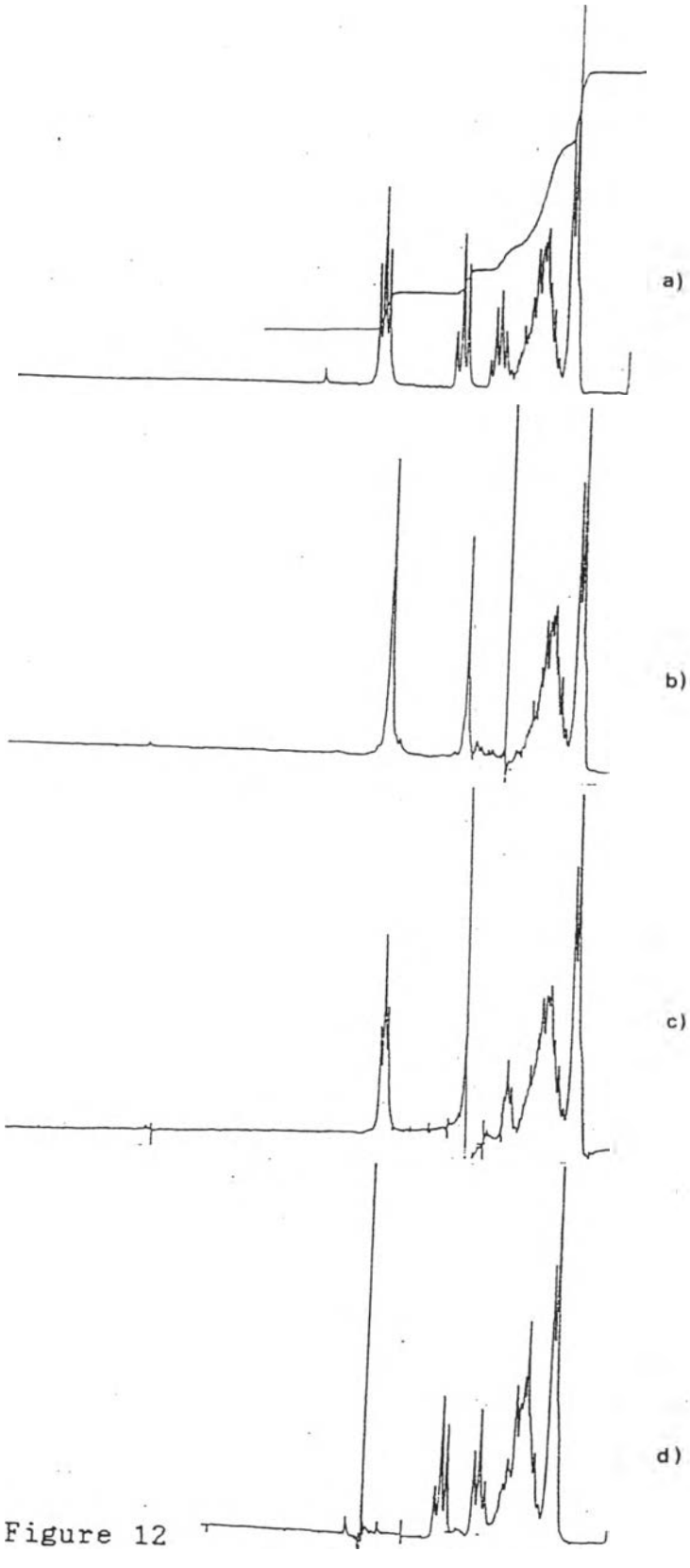


Figure 12

The ^{1}H-NMR spectrum of N(2´-propylpentanoyl)-2-pyrrolidinone (a) showing proton irradiation at 2.02 PPM (b); proton irradiation at 2.61 PPM (c); and proton irradiation at 3.81 PPM (d)

The ^{1}H-NMR chemical shift of methine proton in the molecule of this compound appeared at about 3.8 ppm, it was supposed that the inductive effect caused by the electron withdrawing pyrrolidinone group and the possible hydrogen bonding (Figure 14) deshielded the methine proton, which caused its peak much more down field.

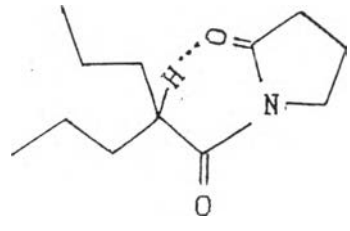
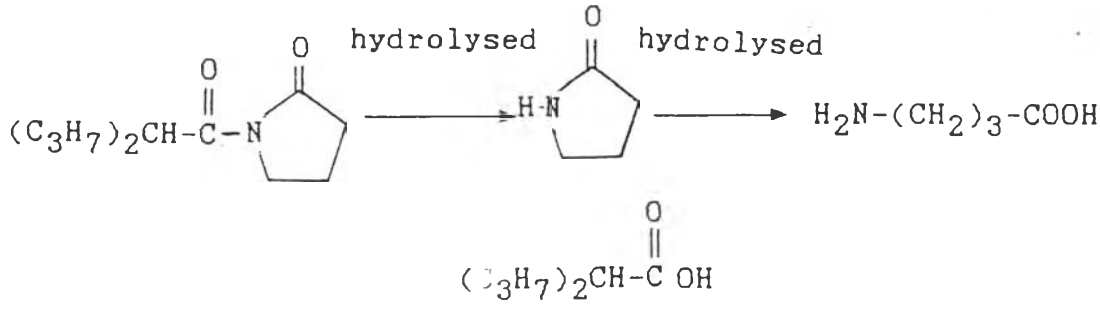


Figure 14 Proposed structure of N(2′-propylpentanoyl)-2-pyrrolidinone showing hydrogen bonding

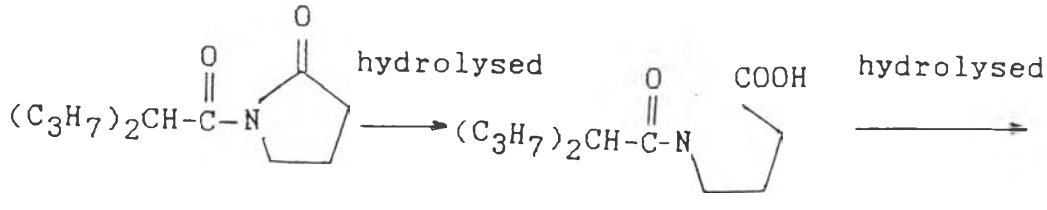
Recently, Sasaki et al. synthesized a series of 1-acyl-2-pyrrolidinone derivatives and evaluated the anticonvulsant activity of these compounds by intraperitoneally administration into the mice. The data indicated that some derivatives possessing anticonvulsant action were due to the release of GABA by hydrolysis.

N(2′-propylpentanoyl)-2-pyrrolidinone was supposed to act possibly as a prodrug with dual anticonvulsant action. It was expected to be converted enzymatically to GABA and the corresponding anticonvulsant valproic acid as shown in Scheme IV.

PATHWAY A



PATHWAY B



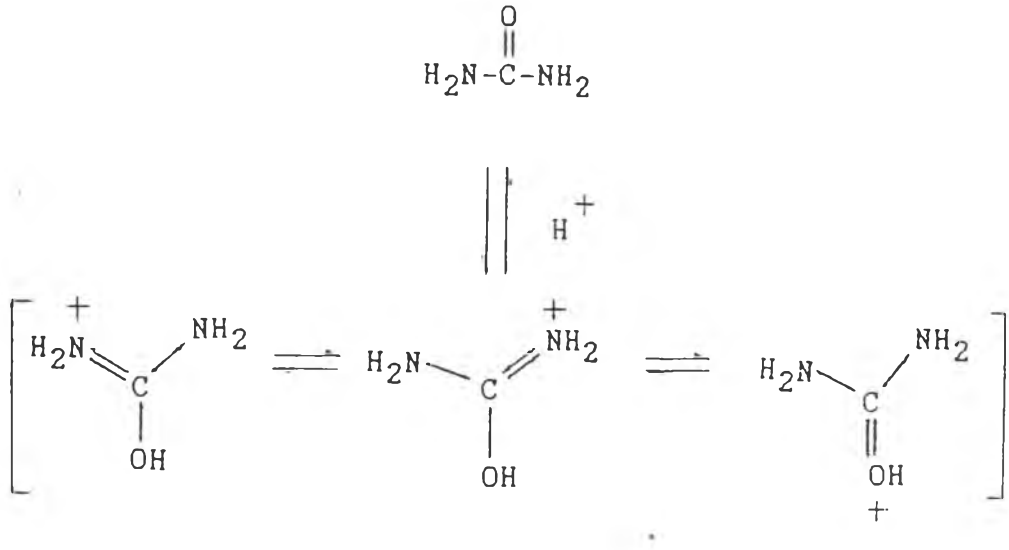
$(C_3H_7)_2CH-C-OH$ + $H_2N-(CH_2)_3-COOH$

Scheme IV Proposed hydrolytic pathways of N(2′-propylpentanoyl)-2-pyrrolidinone.

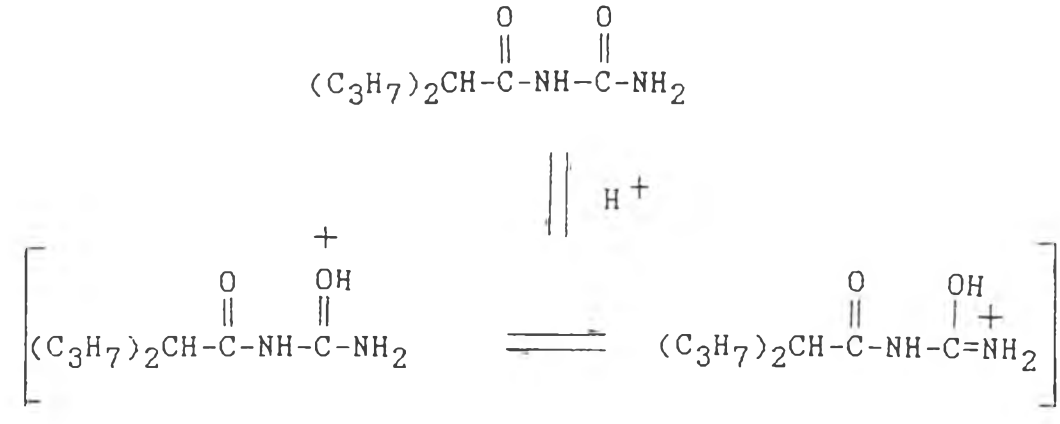
However, the mechanism of hydrolysis predominantly proceeds by which pathways must be elicited. In addition, pharmacological activity, kinetic study of the hydrolysis must be further investigated.

N(2-propylpentanoyl) Urea

This compound was a novel acylurea derivative. The synthesis of this compound was easily accomplished by the reaction of 2-propylpentanoyl chloride and urea in dry benzene with the presence of potassium carbonate granules. The reaction proceeds simple nucleophilic substitution. It is known that ordinary amides are neutral or weakly basic. On the other hand, they are poor nucleophiles. Urea is such a compound, the chemical structure of urea is a symmetric primary diamide. However, urea is stronger base than ordinary amide which is attributed to resonance stabilization of the cation.



Therefore, neucleophilic attack by urea nitrogen can occur, however, in slow rate. Heat accelerates the reaction rate. From the chemical structure of urea, it was noted that urea posseses two nucleophilic moieties in molecule, however, no diacylated compound was detected from this reaction. Since when one mole of acyl compound reacted with urea nitrogen, the monoacylated urea obtained was lower nucleophilicity due to the decrement of basicity in contrast to free urea molecule, thus the second acylation was difficul



The IR spectrum of N(2-propylpentanoyl) urea (Figure 15) showed the strong sharp peak at 3400 cm^{-1} for NH stretching vibration of imide, the peak at 3340 cm^{-1} and 3240 cm^{-1} for asymmetric and symmetric NH stretching vibration of primary amide, the strong peak at 1700 cm^{-1} represented C=O stretching vibration (amide I) of imide and the strong peak at 1680 cm^{-1} represented C=O stretching vibration (amide I) of primary amide, the peak at 1590 cm^{-1} represented NH bending vibration (amide II) and the peak at 1355 cm^{-1} for C-N stretching vibration of amide.

The ^{1}H-NMR ($CDCl_3$) of N(2-propylpentanoyl) urea (Figure 16) showed the peak at 0.90 - 0.97 ppm (multiplet, 6H) for two methyl protons of alkyl side chain, the peak at 1.20 - 1.66 ppm (complex, 8H) for two ethylene protons of alkyl side chain. The broad peak at 2.24 ppm (multiplet, 1H) represented methine proton. The three broad peaks at chemical shift 5.48 ppm (broad, 1H), 8.39 (broad, 1H) and 9.20 ppm (broad, 1H) represented NH protons. The peak at 9.20 ppm located at the most downfield should be the NH proton of imide NH proton which was most deshielded by the two carbonyl groups. The NH protons of primary amides usually show chemical shifts in the region 5-7 ppm and the two NH protons should appear at the same chemical shift or may appear at a little different signals due to the rotation around the CO-N bond which is so slow that the two separated signals are observed for the two conformers. In this case, the two NH protons, which should be the NH protons of primary amide moiety, showed two separated signals at chemical shifts 5.48 ppm and 8.39 ppm. It was supposed that some effects influence one of the two NH protons which deshield its electron environment causing the two separated signals much more different.

Hydrogen bonding can explain this phenomena, hydrogen bonding decrease the electron density around the proton and thus moves the proton absorption to lower field. Both intramolecular and intermolecular hydrogen

bonding were suspected to be involved, especially for intramolecular hydrogen bonding. Figure 17 showed proposed intramolecular hydrogen bonded structure of this compound, the intramolecular hydrogen bond formed built a stabilized six membered ring structure of the compound. It was stated that the ^{1}H-NMR chemical shift at 9.20 represented NH proton of imide, the chemical shift at 8.39 for NH proton formed hydrogen bond and the chemical shift at 5.48 for NH proton of intramolecular hydrogen bond.

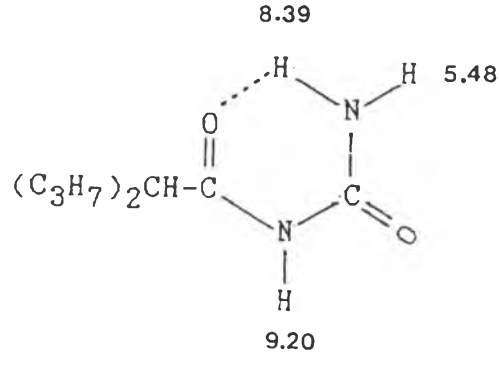


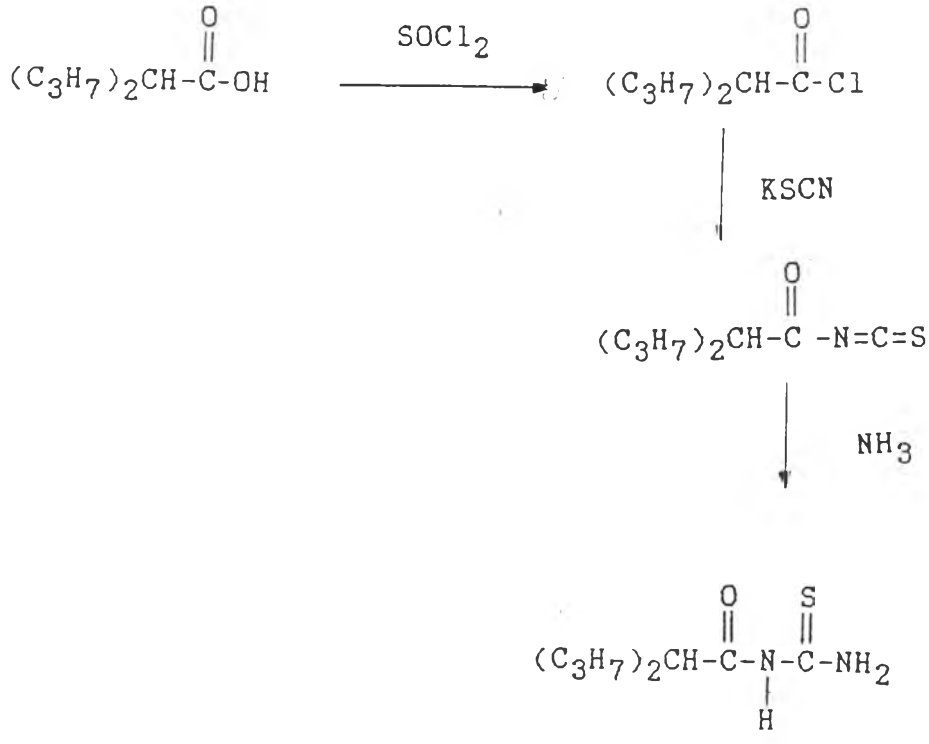
Figure 17 Proposed structure of N(2-propylpentanoyl) urea showing intramolecular hydrogen bonding.

Figure 18 showed ^{1}H-NMR chemical shifts of this compound using DMSO-d_6 as solvent. The data showed significantly characteristic extent of intermolecular hydrogen bonding that confirmed the possible formation of intramolecular hydrogen bonding as described. The polar compound, deuterated dimethyl sulfoxide oxygen (DMSO-d_6),

formed intermolecular hydrogen bond with NH protons which were deshielded, then the chemical shifts moved to more downfield. The ^{1}H-NMR spectrum (DMSO-d_6) indicated that the chemical shift of NH proton of imide shift to 9.67 ppm. The NH proton of amide shifts to 5.99 ppm and the other NH proton of amide that formed intramolecular hydrogen bonding with oxygen carbonyl showed no significant shift. Since intermolecular hydrogen bonds influenced the electron density around the free NH proton much more than that of hydrogen bonded NH proton.

N(2-propylpentanoyl) thiourea

This compound was first attempted to prepare in the same synthetic procedure as that of N(2-propylpentanoyl) urea, however, it was unsuccessful. The reaction proceeded S-acylation to give thioester compound. Therefore, an alternative method of preparation was used. In this work, 2-propylpentanoyl chloride was allowed to react with potassium thiocyanate in toluene under reflux condition, the reaction rate was speeded up by stirring, then, the acylisothiocyanate obtained underwent nucleophilic substitution with concentrated ammonia solution. Scheme V described the synthesis of this type of compound.



Scheme V Reaction of N(2-propylpentanoyl) thiourea synthesis

The reaction of acylchloride and potassium thiocyanate may result in acylthiocyanate (XLII) or the corresponding acylisothiocyanate isomer (XLIII) or mixture

(XLII) (XLIII)

of the two isomers. Thiocyanate ion possesses what has been called ambident character, since the negative charge may be located on sulfur or nitrogen atom as shown in the alternative classical structure.

Although it cannot be predicted that the reaction of acylhalide with metal salt of thiocyanic acid will result in the formation of a normal thiocyanate or isothiocyanate isomer, the ease of formation of isothiocyanate increases from primary to secondary to tertiary carbon derivatives, and is favored by the presence of aryl, ethylenic or carbonyl groups on the carbon atom at which substitution occurs. Whether a reaction product is a thiocyanate or an isothiocyanate can readily be ascertained by infrared spectrophotometric analysis, thiocyanate exhibit a strong, sharp band, due to the C≡N stretching vibration, while isothiocyanate exhibit a very strong and broad band at about 2273 - 2000 cm^{-1}. The IR spectrum (Figure 20) showing a very strong broad band at 1985 cm^{-1} and C=O stretching vibration at 1720 cm^{-1} indicated that this compound was acylisothiocyanate. The C=O stretching vibration showed absorbance at higher frequency due to the predominantly inductive effect.

The infrared spectrum of N(2-propylpentanoyl) thiourea (Figure 21) showed the peaks of NH stretching vibration at 3350 cm^{-1} for secondary amide the peaks at 3300 cm^{-1} and 3250 cm^{-1} for primary thioamide, the C=O stretching vibration at 1700 cm^{-1}, the peak at 1625 cm^{-1} for NH bending. The peak at 1250 cm^{-1} represented C=S stretching vibration. The ^{1}H-NMR of N(2-propylpentanoyl) thiourea (Figure 22) showed the peaks of two methyl protons at 0.91 - 0.98 ppm (multiplet, 6H) peaks at 1.20 -

1.83 ppm (multiplet, 8H) for two ethylene protons and the peak at 2.25 ppm (multiplet, 1H) for the methine proton. The broad peaks at about 7.35 ppm (s, 1H), 9.20 ppm (s, 1H) and 10.02 ppm (s, 1H) represented NH protons. The chemical shift assignment of each NH proton was similar to that of N(2-propylpentanoyl) urea. Thus, the peak at 10.02 ppm which was the most deshielded should be the NH proton of thioimide. The chemical shift at 7.35 ppm and 9.20 ppm should be the NH protons of thioamide, the two signals were much different chemical shifts due to intramolecular hydrogen bonding. The NH proton formed intramolecular hydrogen bonding being more deshielded should be the chemical shift at 9.20 ppm. Figure 23 showed the proposed structure of N(2-propylpentanoyl) thiourea showing intramolecular hydrogen bonding. The mass spectrum (Figure 24) showed m/e 202 for molecular ion peak, m/e 203, 204 for $(M + 1)^+$ and $(M + 2)^+$ respectively.

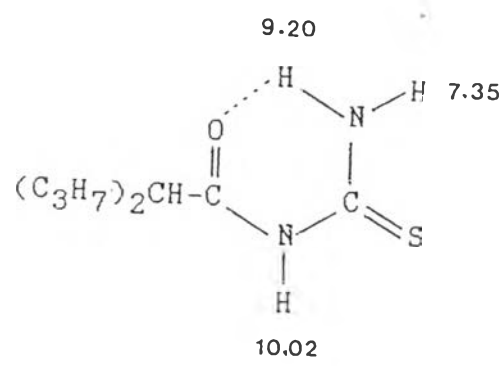
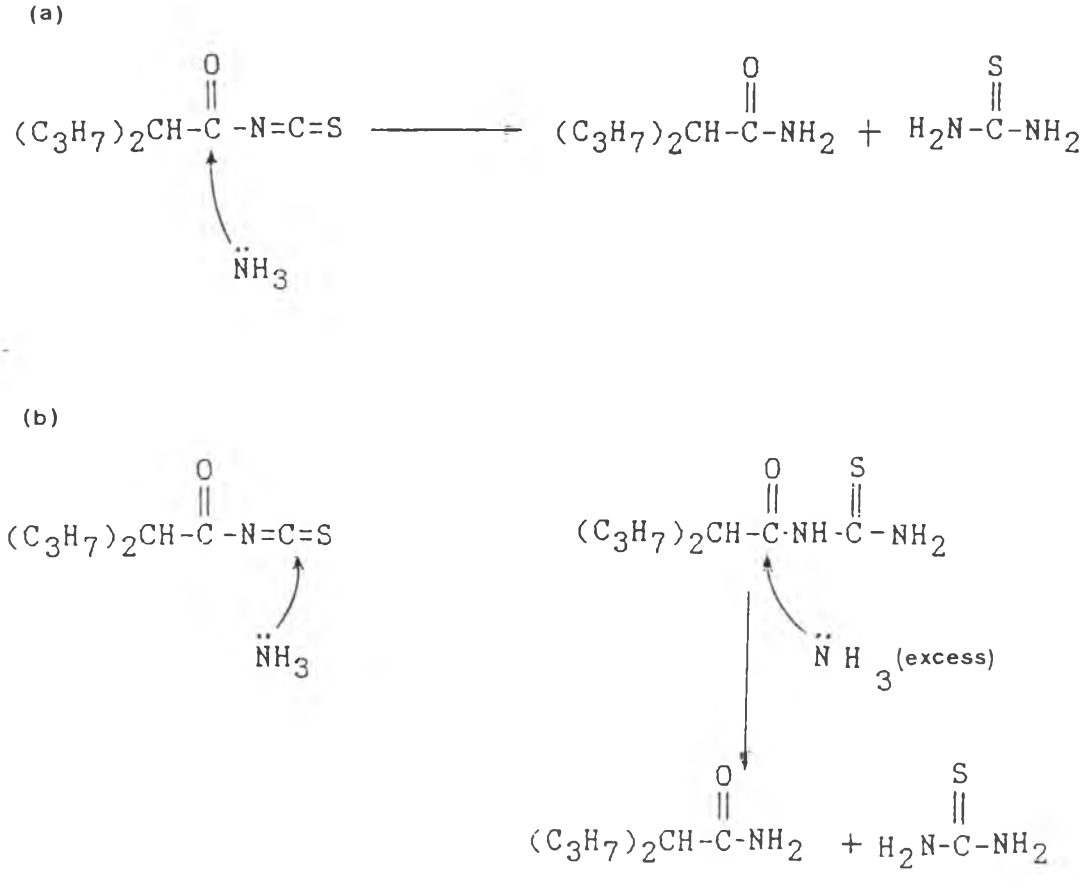


Figure 23 Proposed structure of N(2-propylpentanoyl) thiourea showing intramolecular hydrogen bonding.

The target product, N(2-propylpentanoyl) thiourea, was successfully prepared by this method, however, the disadvantage of this method was an evitable side reaction occuring during the ammonolysis step. Carbonyl carbon and isothiocyanate carbon are competitive nucleophilic sites of attack by ammonia. Scheme VI represented the possible reactions of acylisothiocyanate and ammonia.



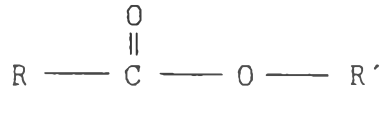
Scheme VI Possible reaction of acylisothiocyanate and ammonia. (a) nucleophilic attack at carbonyl carbon. (b) nucleophilic attack at isothiocyanate carbon.

Attempted Synthesis of N(2-propylpentanoyl) guanidine

Attempted to synthesize the monoureide analogue N(2-propylpentanoyl) guanidine were performed in several ways. First, the method of preparation used was similar to that of Greenhalgh and Bannard (1959), the reaction involved nucleophilic substitution of 2-propylpentanoyl chloride and guanidine hydrochloride under reflux condition. Although the reaction time used was upto 48 hours, no target product could be traced. It might be the low nucleophilicity of guanidine salt rendered the nucleophilic reaction difficult. Therefore, in order to increase the nucleophilicity, the guanidine base was used. The products obtained from this method were at least 3 compounds. The purifications of these compounds were difficult. One of these compounds was successfully isolated and analysed by mass spectrometer. The spectrum showed m/e 312 (Figure 25) which was suspected to be diacylguanidine compound. Another method supposed to be the most satisfactory method used for preparation of unsubstituted and monosubstituted acylguanidines was the condensation of the appropriate ester with guanidine base (Bream et al., 1975). Thus, this reaction was performed using the starting material, ethyl ester of valproic acid allowed to react with guanidine base which was prepared by treating guanidine hydrochloride with the equimolar of sodium ethoxide solution. The reaction was performed in ethanol at room temperature for several days, however, no

target product formed. It is well known that methyl ester of the same acid is more reactive than ethyl ester to the same nucleophile. Therefore, methyl ester of valproic acid was used to react with guanidine base in methanol. Also, no target product was detected. It was believed that the solvent used, another important factor, played role in this reaction rate. In the case of ammonolysis of esters, the use of hydroxylated organic solvents intensively promoted the reaction rate due to the activation of nucleophile.

Here, 10% glycerine in methanol was used as solvent in the condensation reaction of methyl ester of valproic acid and guanidine base in the hope of promotion of the reaction rate. However, no acylguanidine was detected. It was supposed that the alkyl esters of valproic acid were unreactive, which caused the reaction fail. The fact that the reactivity of ester depends on the polarization of the carboxyl group in the ester which then provides the center for nucleophilic attack at the carbon of the carboxyl group. From this representative it may be seen that the greater the electron release of R or R′ the slower should be the rate of nucleophilic attack of ester since the positive charge on the carbonyl carbon would be reduced in magnitude.



In the case of ester of valproic acid, the alkyl group attached to carbonyl carbon is secondary carbon which is a good electron donating group and the additional effect of steric hindrance due to the 2-propyl branch-chain render the nucleophilic substitution reaction difficult. In view of the difficulties encountered, the synthesis was abandoned at this stage.