CHAPTER II

HISTORY

VALPROIC ACID (VPA; di-n-propylacetic acid, DPA; 2-propylpentanoic acid, or 2-propylvaleric acid).

The acid, a colourless slightly viscous liquid with a characteristic odor, was first synthesized by Burton (1882). No therapeutic application of drug was known until the discovery of its anticonvulsant properties by Eymard (1962). As he synthesized a series of derivatives of khelline and studied their pharmacological properties, impeded by their poor solubility in water and the usual organic solvents, he had the idea of dissolving the most active molecule of khelline derivative in 2-propylpentanoic acid. This solution exhibited a marked protection against pentylenetetrazole induced seizures. Subsequent studies indicated that the anticonvulsant action was due to the 'solvent; i.e. 2-propylpentanoic acid, and further investigations demonstrated a broad spectrum of anticonvulsant activity in a wide variety of animal seizure models (Chapman et al., 1982).

A simple aliphatic molecular structure of valproic acid (branched chain fatty acid) in contrast to the heterocyclic ring structures of hydantoins, barbiturates, benzodiazepines, succinimides and oxazolidinediones appears to differ significantly from all other anticonvulsants in terms of its spectrum of anticonvulsant activity, clinical use and its time course of action.

Several investigators have attempted to explain the mechanism of anticonvulsant action of valproic acid, however, the apparent mechanism of action of valproic acid is still unknown. Two general hypotheses have been proposed to explain the anticonvulsant activity by experimental and clinical studies of valproic acid. The first hypothesis was that valproic acid acts by enhancing GABA-mediated inhibition and relies primarily on data demonstrating that the drug increases brain GABA levels. However, the mechanism by which valproic acid increases GABA levels is not well understood. Studies on biochemical actions of valproic acid revealed that the drug inhibits several enzymes involved in GABA degradation, including gamma aminobutyric acid transaminase (GABA-T), (Fowler et al., 1975), succinic semialdehyde dehydrogenase (SSA-DH), (Van der Laan et al., 1979; Van der Laan et al., 1980) and aldehyde reductase (White and Turner, 1978). It has been reported that valproic acid inhibits at least three biological enzymes but inhibitions

of GABA-T and SSA-DH received most attention and indeed
seem to be the preferential action involved anticonvulsant
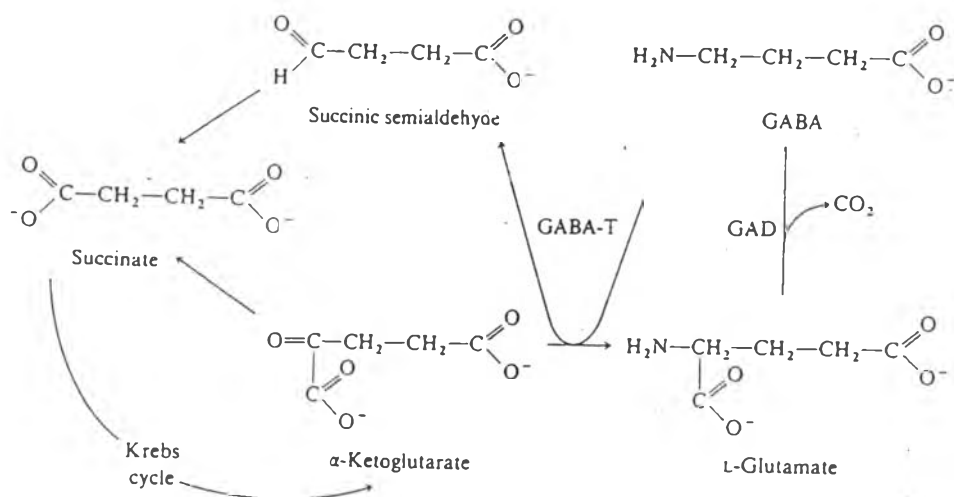activity. Figure 2 shows partial metabolic pathway of
GABA.

Figure 2   Partial metabolic pathway of GABA
synthesis and degradation.

Accumulation of GABA by valproic acid induction
can be explained in the role of SSA-DH inhibition. The
primary inhibition of SSA-DH will result in accumulation
of SSA, which will inhibit the forward reaction of
GABA-T preventing the degradation of GABA. A further
increase of SSA will initiate the conversion of SSA into
GABA by the reverse reaction of GABA-T. The inhibition of
GABA-T activity directly results in GABA elevation. Of
the two mechanisms, increment of GABA concentration is
predominantly caused by SSA-DH inhibition, since SSA-DH is
much more sensitive to valproic acid inhibition (Van der
Laan et al., 1979).

Some investigators demonstrated that potentiation of inhibitory responses to GABA could result from an effect on a modulator site in the GABA-receptor complex, (Gent and Normantor, 1978).

The second hypothesis involves the blockade of voltage-dependent Na^+ channels. Like phenytoin, valproic acid limits the ability of cultured CNS neurons to fire Na^+-dependent action potentials at high frequency (McLean and Macdonald, 1986). Since valproic acid has a broad spectrum of anticonvulsant activity, the clinical activity may relate to combination of mechanisms. Insufficient data are available at present to definitively establish either of these mechanisms. This is partly because the basic knowledge of neuronal receptors and responses is deficient. (Schechter et al., 1978). Nevertheless, these observations highlight the fact that the full spectrum of pharmacological actions of valproic acid are not yet well understood.

Clinical use of Valproic acid

Initial experiments indicated that valproic acid was of most value in the treatment of primary generalized seizures, especially those of the absence type (Pinder et al., 1977). However, valproic acid also appears to be effective against certain myoclonic seizures, generalized tonic-clonic seizures, infantile spasm and photo sensitive epilepsy. In addition, psychiatric disorders such as schizophrenia, manic-depressive psychosis can effectively be treated with valproic acid (Lambert et al., 1975, Lautin et al., 1980). While valproic acid was considered initially to be a very safe drug, serious adverse reactions affecting the hepatic, pancreatic, and hematologic systems have been reported recently. Of these, the greatest concern has arisen over the incident of valproic acid-induced hepatic injury (Zimmerman and Ishak, 1982). It was supposed to be the 2-n-propyl-4-pentenoic acid, a toxic metabolite of valproic acid, involved hepatotoxicity.

Extensive structural anticonvulsant activity relationships of valproic acid have been reported (Abbott and Acheampong, 1987), it was demonstrated that anticonvulsant activity correlated with lipophilicity and pKa of the acid. The carboxylic acid containing eight carbon atoms, 2-propylpentanoic acid, possessed high potency with the lowest side effects. However, increasing

the number of carbon atoms might raise the potency with increasing the side effects. On the other hand, decrement of the number of carbon atoms resulted in considerably lower activity. The pKa value might reflect different binding characteristics at an active site or involved the entry of drug into the central nervous system.

Structural Modifications of Valproic acid

Valproic acid is a good lead compound for structure-activity relationship studies because of its unique chemical structure as compared to conventional antiepileptic drugs. It is structurally related to GABA. Structural modifications of valproic acid are generally divided into two series : modification of alkyl side chain moiety and carboxyl moiety.

Numerous derivatives of valproic acid, modified on alkyl side chains, have been tested for anticonvulsant action (Carraz et al., 1965; Benoit-Guyot et al., 1973; Loscher and Nau, 1984, Abbott and Acheampong, 1987). Among the close analogues of valproic acid with branching in the 2-position, compounds in which one or both side chains were shorter compared to valproic acid, such as 2-ethylpentanoic acid, 2-ethylhexanoic acid, were considerably less active than valproic acid. Elongation of one side chain resulted in no increase of

anticonvulsant potency compared to valproic acid as shown
by 2-propyl hexanoic acid.

On the other hand, elongation of the both side-
chains increased anticonvulsant activity progressively as
shown by 2-butylhexanoic acid.

However, additional branching with methyl   group
at C2 considerably enhanced the anticonvulsant activity.
Thus, 2-ethyl-2-methylpentanoic acid was 10 times more
potent than 2-ethylpentanoic acid.

A   considerably enhancement of anticonvulsant
potency could be reached by branching in position 3.
Thus, 3-propylhexanoic acid was about 4 times more potent
than 2-propylhexanoic acid and valproic acid.

Cyclic analogues of valproic acid, i.e.
cyclopentanoic acid, cyclohexanoic acid, were also
inactive.

However, addition of methyl group in position 1 at
the ring i.e. 1-methyl-1-cyclohexanoic acid, resulted in a
pronounced enhancement of anticonvulsant activity (Loscher
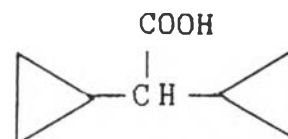and Nau, 1984).

Brana et al., (1983) studied on anticonvulsant activity of valproic acid rigid homologues and some of them were shown as following.
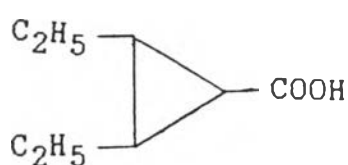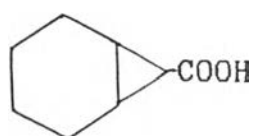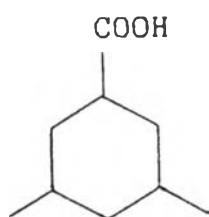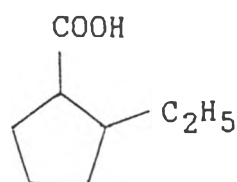

XXX	XXXI	XXXII

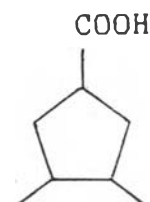|		
XXXIII	XXXIV	XXXV

This study showed that (±)-(E)-2,3-diethyl cyclopropanecarboxylic acid (XXX) and dicyclopropylacetic acid (XXXII) were as active as valproic acid.

Modifications of carboxyl moiety of valproic acid have been investigated. Benoit-Guyot et al. (1971) studied various amides and esters of valproic acid. No attractive anticonvulsant agents were reported.

Later, derivatives of 2-propyl pentylamine have been studied on anticonvulsant activity. Nevertheless, no compounds showed significant anticonvulsant action.

In 1983, Kraus designed some new valproic acid analogues based on the bioisosteric concept. The abservation showed bio-equivalences between the 5-substituted tetrazoles and the carboxyl group on one hand, and between the heterocycle 3,5-dioxo 1,2,4-oxadiazolidine and the carboxyl group on the other hand.

Two compounds, N-2 (4-heptyl) 3,5-dioxo 1,2,4 oxadiazolidine (XXXVI) and (4-heptyl)-5-tetrazole (XXXVII) were synthesized and tested in vitro inhibiting properties towards the enzyme SSA-DH.
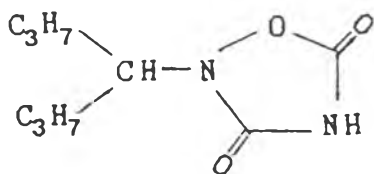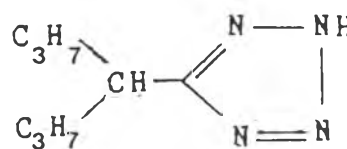
XXXVI   XXXVII

The results revealed that N-2 (4-heptyl) 3,5-dioxo 1,2,4-oxadiazolidine was the most potent inhibitor, while valproic acid and its tetrazole analogue appeared to be equipotent SSA-DH inhibitors.

Recent study reported of 2-propylpentanal acetals as prodrugs of valproic acid, Vicchio and Collery (1989) using the acetal as a functional group in prodrug design, demonstrated that acetals of 2-propylpentanal are metabolically converted through an aldehyde intermediate to the anticonvulsant, valproic acid.

I. Synthesis of potential prodrugs of valproic acid

Several types of bioreversible derivatives have been exploited for utilization in prodrug designs. Here, three prodrug forms were synthesized.

1. Ester

2. Amide

3. N-Mannich base

Synthesis of esters

Esters are usually prepared by the reaction of alcohol with acid or acid derivatives. The most common methods are described as following.

A. Reaction of acids and alcohols

$$R - \overset{\overset{\text{O}}{\|}}{C} - OH + R' - OH \xrightarrow{\quad H^+ \quad} R - \overset{\overset{\text{O}}{\|}}{C} - OR' + H_2O$$
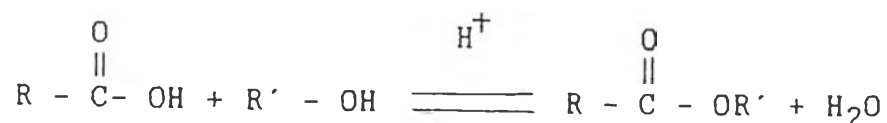
A carboxylic acid is converted directly into an ester when heated with an alcohol in the presence of a little mineral acid, usually concentrated sulfuric acid. This reaction is reversible and generally reaches equilibrium when there are appreciable quantities of both reactants and products present. However, this disadvantage can be overcomed by removing one of the products off.

B. Reaction of acid chlorides or anhydrides with alcohols.

$$R - \overset{\overset{\displaystyle O}{\|}}{C} - Cl + R' - OH \longrightarrow R - \overset{\overset{\displaystyle O}{\|}}{C} - OR' + HCl$$

$$\begin{matrix} R - \overset{\overset{\displaystyle O}{\|}}{C} \\ \diagdown \\ O + R' - OH \longrightarrow R - \overset{\overset{\displaystyle O}{\|}}{C} - OR' + R - \overset{\overset{\displaystyle O}{\|}}{C}OH \\ \diagup \\ R - \underset{\underset{\displaystyle O}{\|}}{C} \end{matrix}$$
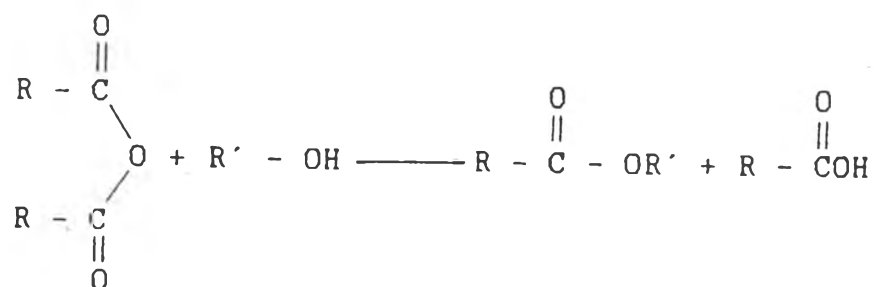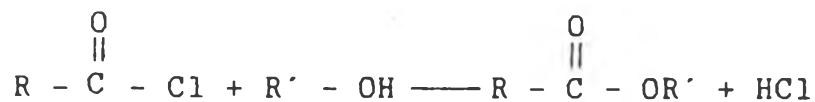
Acid chlorides typically undergo nucleophilic substition. Esters are usually prepared from acid chloride rather than from the acid itself. The reaction is irreversible and goes to completion. On the other hand, preparations of esters from acid anhydrides undergo the same reaction as acid chlorides but a little more slowly and obtain different by-products.

C. Transesterification (Ester Interchange)

$$RCOOCH_3 + C_2H_5OH \xrightleftharpoons{H^+} RCOOC_2H_5 + CH_3OH$$
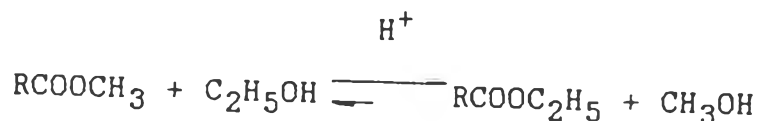
When the methyl ester of an acid is refluxed with excess ethanol containing a few percent of sulfuric acid, it is converted into ethylester. Rapid interchange of

alkyl groups can be brought about with a catalytic amount of sodium alkoxide. Sodium borohydride also catalyses transesterification (Fieser and Fieser, 1961).

D. Reaction of alkylhalide and the salt of an acid.

$$CH_3I + AgOC\overset{\overset{\displaystyle O}{\parallel}}{} - CH_3 \longrightarrow AgI + CH_3 - \overset{\overset{\displaystyle O}{\parallel}}{C} - OCH_3$$
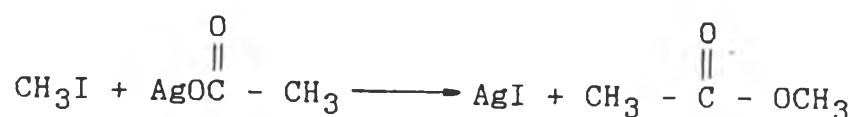
Esters may also be made by using alkyl halide and the silver salt of carboxylic acid. The reaction proceeds through simple metathesis involving fission of the O-Ag bond. However, this method has the objection of being expensive (Fieser and Fieser, 1961).

E. Reaction of an alcohol with amide

$$CH_3CONH_2 + C_2H_5OH \rightleftharpoons CH_3CO_2C_2H_5 + NH_3$$

This reaction is reversible, it is carried to completion by the use of an acid to take up the ammonia (Wertheim, 1951).

Above the five synthetic approachs, the reaction of acid chloride and alcohol seemed to be the most preferable in the preparation of target ester.

Synthesis of amides

The carboxylic acid derivatives, amides, are formed by several distinct methods. Following are some well known methods for preparation of amide-type compounds.

1. Heating the ammonium salt of a carboxylic acid

$$CH_3 - \underset{\underset{O}{\|}}{C} - ONH_4 \xrightarrow{100-200^o} H_2O + CH_3 - \underset{\underset{O}{\|}}{C} - NH_2$$
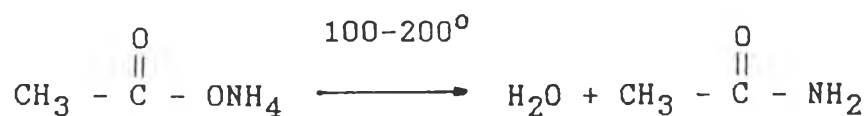
This method is useful in preparing large-scale amides. It is supposed that ammonium salt breaks down on heating to give free ammonia and acid. Then, the acid is attacked by the nucleophilic ammonia to give amide and H_2O. (Wertheim, 1951).

2. Hydrolysis of nitriles

$$CH_3 - CN + H_2O \longrightarrow CH_3 - \underset{\underset{O}{\|}}{C} - NH_2$$
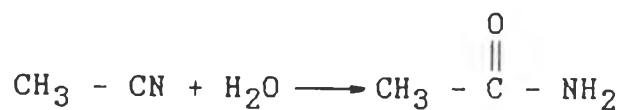
The hydrolysis of nitriles to give amides, frequently, may be accomplished by the use of a solution of hydrogen peroxide and sodium hydroxide or with

concentrated sulfuric acid (Wertheim, 1951; Turner and Harris, 1952).

3. Reaction of acid chlorides, acid anhydrides or esters with amines

$$RCOCl + NH_3 \longrightarrow RCONH_2 + HCl \ (NH_4Cl)$$

$$RCO\text{-}O\text{-}COR' + NH_3 \longrightarrow RCONH_2 + R'COOH$$

$$RCOOR + NH_3 \longrightarrow RCONH_2 + ROH$$

The typical method for preparation of amides is the reaction of acid chlorides and amines. Although the treatment of acid anhydrides with amines possess the same manner reaction, half of acyl is lost as the acid. Esters seldom constitute convenient source of amide, the structural factors which influence formation are similar to those operating in the hydrolysis of esters. For example, ethyltrichloroacetate reacts rapidly with aqueous ammonia whilst ethyltrimethyl acetate hardly reacts at all (Turner and Harris, 1952).

4. Miscellaneous

Several methods for preparing amide-type compounds have been investigated. In general, the special reagents were used to accomplished the reaction. For example, aminolysis of esters requires high temperature and long reaction times, when using dimethylaluminium amide as a

source of amide, the reaction proceeds to completion with mild condition in a short reaction time (Busha, et al. 1977).

$$(CH_3)_2AlNR_1R_2 + R\text{-}COO\text{-}R_3 \xrightarrow[25\text{-}41^\circ]{CH_2Cl_2} RCONR_1R_2$$
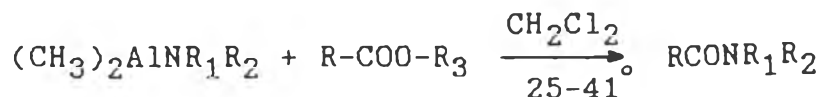
Collum, Chen, and Ganem (1978) using catecholborane as essential carbonyl-activated moiety for the synthesis of amides.

And in 1990, Hossain and Borthakur prepared some amides from carboxylic acids and amines by heating the acid with a mixture of an amine and 0,0-diethyl dithiophosphoric acid (DDTPA) in boily toluene in the present of phase transfer catalyst.

$$RCOOH + R_1R_2NH \xrightarrow[\text{Toluene, } \triangle]{DDTPA,\ PTC} \overset{O}{\underset{}{R\text{-}\overset{\|}{C}\text{-}NR_1R_2}}$$
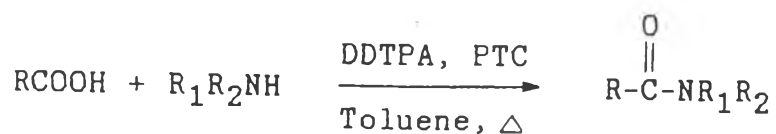
Synthesis of N-Mannich base

The Mannich reaction consists in the condensation of ammonia or a primary or secondary amine, usually as the hydrochloride, with formaldehyde and a compound containing at least one hydrogen atom of pronounced reactivity. The essential feature of the reaction is the replacement of the active hydrogen atom by an aminomethyl or substituted aminomethyl group. The compounds containing active

hydrogen atom may be ketones, aldehydes, acids, esters, amides, acetylenes, phenols, etc. Formaldehyde is used in the form of a 20-40% aqueous solution or as paraformaldehyde, however, aldehydes other than formaldehyde may be used in certain condensations of Mannich type.

$$-\overset{|}{\underset{|}{C}}-H + RCHO + HNR_2 \longrightarrow -\overset{|}{\underset{|}{C}}-\overset{\overset{H}{|}}{\underset{\underset{R}{|}}{C}}-NR_2 + H_2O$$
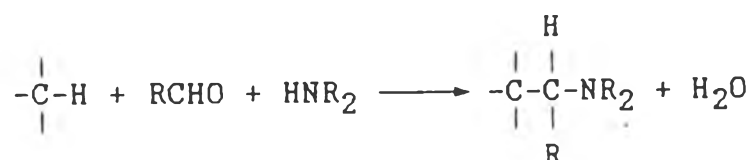
Several applications of the Mannich reaction in synthesis have been summerized (Blicke, 1942). Nowadays, Mannich reaction is also useful to prodrug design. N-Mannich bases have been proposed as potentially useful prodrug candidates for NH- acidic compounds such as various amides, imides, carbonates and urea derivatives. The process can be considered as following.

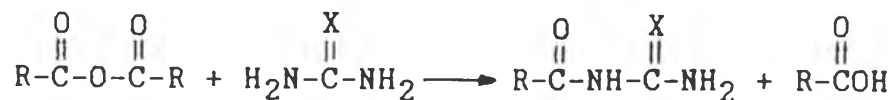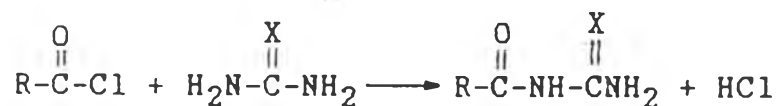$$R-CONH_2 + CH_2O + R_1R_2NH \rightleftharpoons R-CONH-CH_2-NR_1R_2 + H_2O$$

Kinetic studies of decomposition of N-Mannich base indicated that the breakdown of the N-Mannich bases does not rely on enzymatic catalysis but apparently occurs spontaneous decomposition.

In the preparation of Mannich products, when aqueous formaldehyde is used, the condensation is ordinarily carried out by shaking or stirring the reactants in the absence of an organic solvent; when paraformaldehyde is used an organic solvent is required. However, in many cases absolute ethanol or methanol were added as the solvent. The condensations proceed much faster in the higher-boiling solvent, and the formation of certain by products is avoided, but the disadvantage of using higher-boiling solvent is subject to side reactions associated with instability of some products at the higher temperature. The use of aqueous solution formaldehyde or as paraformaldehyde depends on nature of reactants. In some cases, it is necessary to add enough concentrated hydrochloric acid to the mixture (Vida and Hooker, 1973). The time required for a Mannich reaction depends upon the nature of the reactants and upon the boiling point of the solvent employed. It is common practice to use 1.0 molar equivalent of the carbonyl compound, 1.0 - 1.1 molar equivalents of the amine salt, and 1.5 - 2.0 molar equivalents of formaldehyde.

II. Synthesis of Monoureide Analogues

Three forms of monoureide analogues (N(2-propylpentanoyl) guanidine, N(2-propylpentanoyl) thiourea and N(2-propylpentanoyl) urea) were attempted to synthesize. The possible synthetic approach were described as following.

1. Acylation

$$\underset{\substack{\|\\ \text{O}}}{\text{R-C-Cl}} + \underset{\substack{\|\\ \text{X}}}{\text{H}_2\text{N-C-NH}_2} \longrightarrow \underset{\substack{\|\quad\|\\ \text{O}\quad\text{X}}}{\text{R-C-NH-CNH}_2} + \text{HCl}$$

$$\underset{\substack{\|\quad\|\\ \text{O}\quad\text{O}}}{\text{R-C-O-C-R}} + \underset{\substack{\|\\ \text{X}}}{\text{H}_2\text{N-C-NH}_2} \longrightarrow \underset{\substack{\|\quad\|\\ \text{O}\quad\text{X}}}{\text{R-C-NH-C-NH}_2} + \underset{\substack{\|\\ \text{O}}}{\text{R-COH}}$$

$$X = O, S, -NH$$

This reaction is the most common method for synthesis of acylureas. The reaction was carried out by heating the appropriate acid chloride or acid anhydride with urea, either alone or in a non-polar solvent such as benzene (Spinks and Waring, 1963). Acetylation of thiourea is more easily than that of urea (Connor, 1958). In addition, thiourea reacted with acyl halide, S-acylation may occur first and upon heating, or sometimes merely upon standing at room temperature, the acyl group transfered to nitrogen (Dixon and Taylor, 1920). Acetylation of guanidine salts may result in mono-, di-, and tri- acetyl guanidine depending on the reaction conditions used and the method of working up. Although, relatively mild conditions yielded diacetyl guanidine, while treatment with excess acetic anhydride at reflux temperatures gave 2,4-diacetamido-6-methyl-1,3,5-triazine (XXXVIII) (Cockburn and Bannard, 1957).

$$CH_3 \overset{N}{\underset{N}{\diagdown}} NHCOCH_3$$

NHCOCH$_3$
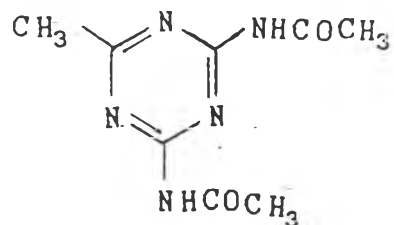
XXXVIII

Greenhalgh and Bannard (1959) reported of successful preparation of monoacetyl guanidine by deacetylation of a-di- or triacetyl guanidine in absolute ethanol.
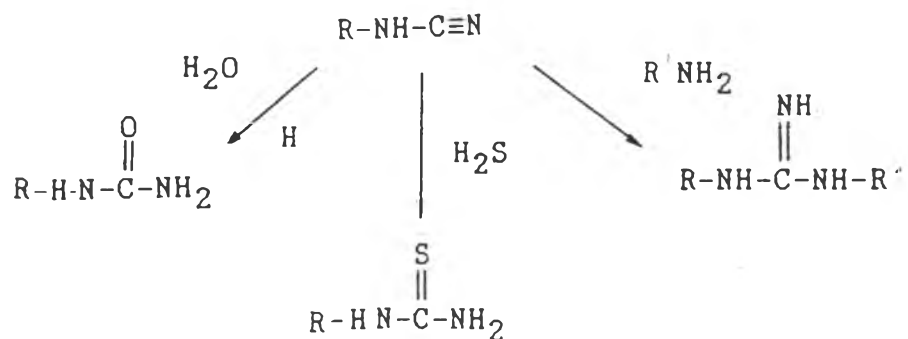
2. Replacement of Esters by Appropriate Nucleophiles

$$\underset{R-\overset{\displaystyle O}{\overset{\|}{C}}-OR' + Nu - H}{} \quad \rightleftharpoons \quad R-\overset{\displaystyle O}{\overset{\|}{C}}-Nu + HOR'$$

This reaction involves condensation of appropriate esters with nucleophiles. The ease of reaction depends on nature of esters and nucleophilicity of nucleophiles, the appropriate solvent accelerates the reaction rate. However, the reaction times vary considerably, in the cases where the reaction is slow, heating did not increase the reaction rate (Bream et al., 1975). Urea and thiourea possess low nucleophilicity in contrast to guanidine, therefore, this reaction is more suitable in the

preparation of acylguanidine than that of acylurea and acylthiourea. The successful preparation acylguanidine by this method were reported (Burtner, 1956; Bream et al., 1975).

3. Preparation via Cyanamide Intermediate

$$R-NH-C\equiv N$$

$$R-H\text{-}N-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2 \quad\xleftarrow{\;H\;}\quad \overset{H_2O}{} \qquad\qquad \Big| H_2S \qquad\qquad \overset{R'\,NH_2}{}\quad R-NH-\overset{\overset{\displaystyle NH}{\|}}{C}-NH-R'$$

$$R-H\,N-\overset{\overset{\displaystyle S}{\|}}{C}-NH_2$$

Cyanamide has the structure of carbarmic acid nitrile. It undergoes smooth addition of water to form urea in the presence of acid or alkaline. Similar reaction with hydrogen sulfide gives thiourea and reaction of cyanamide with ammonia give guanidine (Turner and Harris, 1952).

4. Reaction of Isothiocyanates with Amines

$$RNCS + HN\Big\langle{\overset{\textstyle R'}{\;R''}} \longrightarrow RNH\overset{\overset{\displaystyle S}{\|}}{C}N\Big\langle{\overset{\textstyle R'}{\;R''}}$$
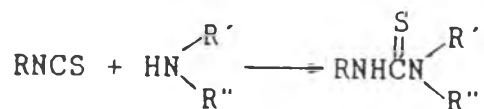
This reaction is useful in the preparation of substituted thiourea. The reactions of amines or ammonia with isothiocyanates result in substituted thiourea

(Mohsen et al., 1984). Generally, there are several methods for synthesizing isothiocyanate derivatives. Following are the synthetic method of isothiocyanates

4.1 Reaction of alkylhalides and metal thiocyanates

Although this reaction is one of the oldest methods of preparing isothiocyanates, it cannot be predicted a prior whether the reaction of an alkyl halide with potassium or ammonium salt of thiocyanic acid will result in the formation of a normal thiocyanate or the corresponding isothiocyanate. Frequently, the experimental conditions employed determine which isomer will be formed and mixtures of both isomers often result (Bacan, 1961).

4.2 Reactions of primary amine with thiophosgene

$$RNH_2 + CSCl_2 \longrightarrow [RNH\overset{\overset{\displaystyle S}{\parallel}}{C}Cl] + HCl$$
$$RNCS + HCl$$
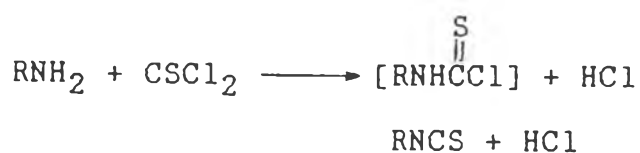
The reaction of thiophosgene with primary amines proceeds smoothly in most cases. The reaction carries on via the thiocarbamyl chloride, which eliminates hydrogen chloride to form the isothiocyanate. Preparations of some isothiocyanate derivatives were accomplished by the this method (Haugwitz et al., 1982; Brewer et al., 1987).

4.3 Preparation of acylisothiocyanates

Acylisothiocyanates   possess   much   higher
reactivities than do the isothiocyanates themselves and
are usually prepared from acid chlorides and metal salts
of isothiocyanic acid (Assong, 1961).