CHAPTER II

HISTORY

Valproic acid

Valproic acid (2-propylpentanoic acid) is a branched-chain fatty acid. Its structure is unrelated to any other antiepileptic drugs. It was first synthesized by Burton in 1882 as an organic solvent. The anticonvulsant activity was fortuitously discovered in 1962 by Pierre Eynard, a research student at the University of Lyon who had synthesized a series of derivatives of Khellin and used valproic acid as a solvent in pharmacological testing. The solution was found to have anticonvulsant activity. Shortly after that, Meunier used valproic acid to dissolve his compound and found that his dissolved compound had anticonvulsant activity too. So, he immediately tested the valproic acid and discovered that it was an anticonvulsant activity. After detailed study by Carraz and his colleagues, valproic acid was subjected to extensive clinical investigation before its sodium salt, Epilim, was marketed in 1967. Since then it has been widely used in Europe and was also approved for use in USA in 1978 (Sneader, 1985).

Valproate, sodium salt of valproic acid, is indicated for use as monotherapy and add-on therapy in the treatment of simple and complex

absence seizures. It is also indicated for use as an adjunct therapy when absence seizures are an aspect of multiple seizure types. The use of valproic acid is growing as a broad spectrum antiepileptic drug for other types of primary generalized seizure , including tonic-clonic seizure, myoclonic , Lannox-Gastent syndrome, and West's syndrome.(Lloyd and Gillenwater, 1994)

Mechanism of Action

The mechanism of action of valproic acid has yet to be fully elucidated, although much attention has focused on the inhibitory neurotransmitter γ-aminobutyric acid (GABA) . Valproic acid increases synaptosomal GABA concentrations via activation of the major synthetic enzyme, glutamic acid decarboxylase. It has also demonstrated strong inhibition of the catabolic enzyme succinic semialdehyde dehydrogenase and GABA transaminase. Evidence also exists which suggests that valproic acid reduce neurotransmission mediated by the excitatory amino acid γ-hydroxybutyric acid. In addition to its effect on amino acid neurotransmitter, valproic acid appears to possess a direct neuronal membrane depressant effect through its influence on sodium or potassium conductance (David, 1994).

Pharmacokinetics

Valproic acid appears to be completely absorbed from available oral dosage forms when administered on an empty stomach. Peak

concentrations occur in 0.5 - 1 hour with syrup, 1 - 3 hours with the capsule, and 2 - 6 hours with the enteric-coated tablet. It is normally 90 % protein binding, primarily to albumin. However, this is saturable at blood concentration of 80 $\mu g/ml$. The plasma half-life of valproate is about 15 hours in monotherapy and decreases to 7-9 hours in patient receiving polytherapy. The therapeutic window for valproate plasma concentration is in the range of 30-150 $\mu g/ml$.. However, there is a lack of correlation between plasma concentrations and antiepileptic or toxic side effect. There is equilibration of valproate between blood and brain (Garnett, 1993; and David, 1994).

Valproate is almost completely metabolized before excretion. Only 1-3% of administered dose is found as unchanged drug in the urine. Four metabolic pathways have been found for valproate: glucuronidation, β-oxidation, ω-oxidation, ω-1 oxidation, as shown in figure 10.

Most of valproic acid is converted to the conjugated ester of glucuronic acid, while mitochondrial metabolism (both β-oxidation and ω-oxidation) accounts for the remainder. Some of these metabolites, notably 2-propyl-2-pentenoic acid, and 2-propyl-4-pentenoic acid are nearly potent antiseizure agent as the parent compound. However, only the former (2-propyl-2-pentenoic acid) accumulates in the plasma and brain to a potentially significant extent. The half-life of valproate is approximately 15 hours, but it is reduced in patients taking other antiepileptic drugs. (Chapman, 1982; McNamara, 1996)
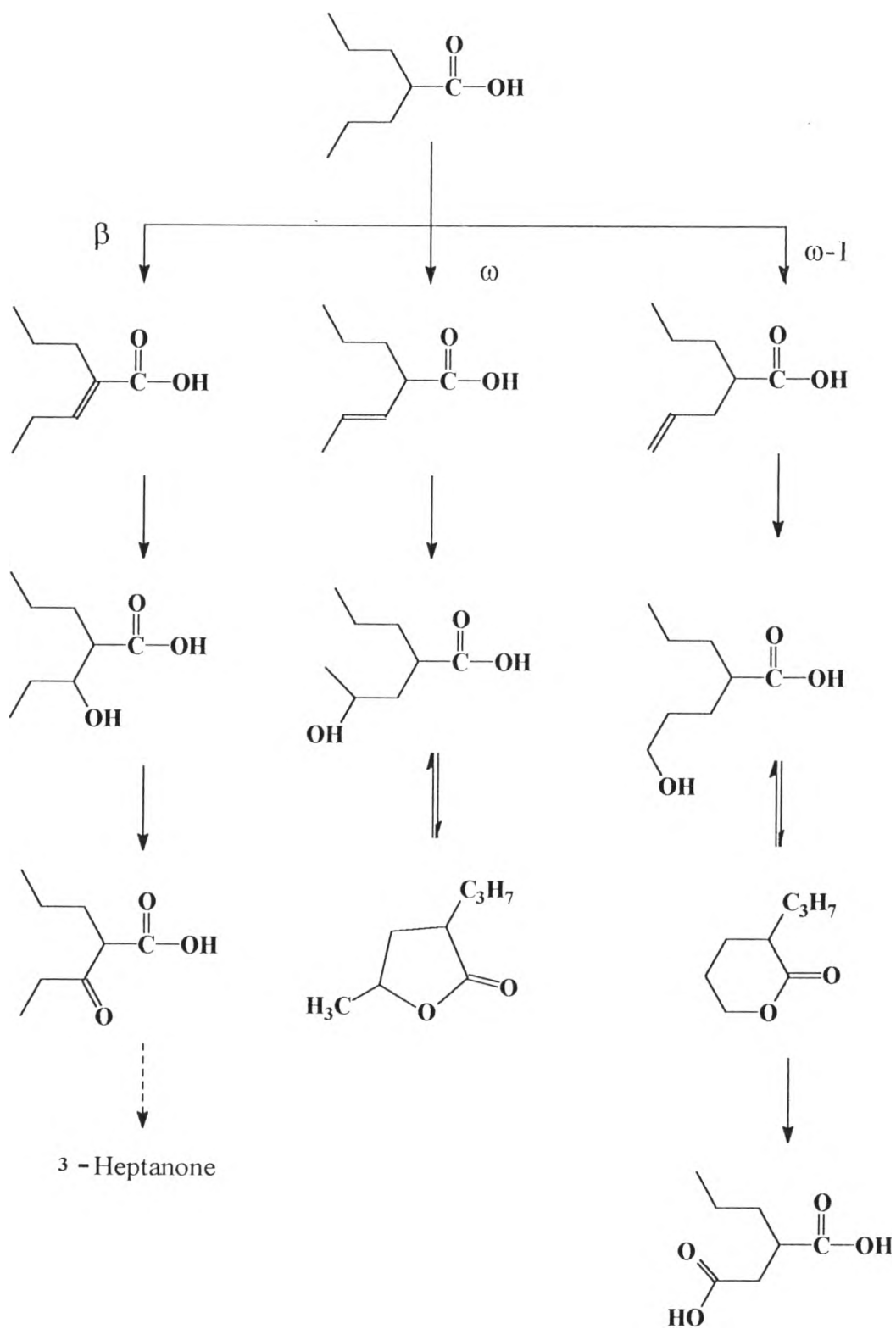
Figure 10   Metabolic pathway of valproic acid

Toxicity

The most common side effect of valproic acid may cause mild patient discomfort, but are not life threatening. The most frequently reported side effects are gastrointestinal complaints (up to 20%) including nausea, vomiting, anorexia, and weight gain. Effects on the CNS, including sedation, ataxia, and tremor occur infrequently and usually respond to a decrease in dosage. Rash, alopecia , and stimulation of appetite have been observed occasionally. Valproic acid has several effects on hepatic function. Elevation of hepatic enzymes in plasma is observed in up to 40% of patients and often occurs asymptomatically during the first several months of therapy. A rare case of complication is hepatitis that is frequently fatal (McNamara, 1996).

Phenacemide

Phenacemide (Phenylacetylurea) was introduced in 1949 for the treatment of intractable complex partial seizures. Initially, the drug was thought to represent a breakthrough in epilepsy treatment. It was found to be effective against generalized tonic-clonic and absence seizures and most effective against complex partial seizures. Later evaluations disclosed its clinical value as severely limited by its potential for serious toxicity (Uthaman and Wilder, 1993).

Mechanism of action

In laboratory animals, phenacemide was shown to elevate the seizure threshold for electroshock-induced convulsions and to abolish the tonic phase of electroshock-induced seizures. Phenacemide prevents or mitigates pentylenetetrazol-induced seizures. In comparative tests, phenacemide is as effective or more effective than other anticonvulsants in the treatment of complex partial seizures induced by low frequency stimulation of the cerebral cortex in mice.

Pharmacokinetic

Phemacemide is rapidly and completely absorbed from gastrointestinal tract , with peak plasma levels occuring 3 to 5 hours after a single dose. Extent of protein binding is not known. Phenacemide is metabolized by means of hepatic microsomal enzymes by p-hydroxylation of the phenyl group and conjugated to glucuronic acid. Although a metabolite of phenacemide has been detected in patients receiving the drug, the identity of the metabolite and its contribution to efficacy have not been established. Studies in rabbits have shown hydroxylation of the benzene nucleus of phenacemide followed by methylation of the 3-hydroxyl group and hydrolysis of the uride group. Arene oxide intermediates have been proposed as responsible for the serious toxicity of the phenacemide since they bind covalently to macromolecules.

The metabolite and its glucuronide are excreted through the kidney, with little or no free drug found in urine. Half-life is 5 to 12 hours.

Toxicity

A significant proportion of patients may experience behavioral aberrations, including personality changes, aggressive behavior, paranoid and depressive reaction, and acute psychosis with mania. Anorexia, weight loss, sedation, insomnia, paresthesia, vertigo, headaches, rash and drowsiness may occur. Aplastic anemia, hepatitis and nephritis also have been reported.

Structure-Activity Relationship of acylureas (Mereier, 1973)

In aliphatic series:

The acid which possesses about 7 carbon atoms confers the maximum anticonvulsant. This is reduced progressively as the molecular weight is increased, while conversely, hypnotic activity develops. The most active compound is 2-ethyl-isovalerylurea.

In aromatic series :

$$R_1 \diagdown \atop R_2 \diagup CH-\overset{\overset{O}{\|}}{C}-\underset{\underset{R_3}{|}}{N}-\overset{\overset{O}{\|}}{C}-NH-R_4$$
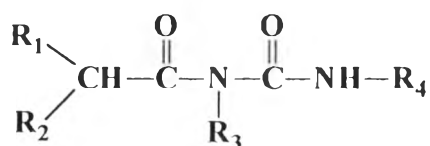
If R_1 is phenyl group, SAR can be shown as follows.

R_1	R_2	R_3	R_4	Activities
C_6H_5	H	H	H	Phenacemide, which possesses maximum activity toward all types of experimental epileptic seizures.
C_6H_5	H	CH_3	H	antipentetrazole activity is increased.
C_6H_5	H	H	CH_3	sedative property is increased , but not anticonvulsant activity.
C_6H_5	H	CH_3	CH_3	anticonvulsant activity is reduced.
C_6H_5	H	CH_3	CH_3	antipentetrazole activity disappears.
C_6H_5	C_2H_5	H	H	sedative activity increases; this compound is active in relation to electrical seizures but less active with respect to pentetrazole.
C_6H_5	C_6H_5	H	H	all activities disappear.

Moreover, if R1 is the phenyl group substituted by CH_3 or Cl , or if it is replaced by a cyclohexane or a thienyl group,then activity again disappears.

General Method of Synthesis

$\underline{N}$-acyl-$\underline{N}'$-arylurea derivatives can be obtained via 2 ways. The first way is via isothiocyanate intermediate, thiourea and then oxidized to urea derivatives, respectively. The other way is a direct way, via isocyanate intermediate.

Synthesis of acyl chloride (March, 1968; Morrison and Boyd, 1973; Furniss, 1991)

1. From carboxylic acid

A carboxylic acid can be converted to acyl chloride with several reagents such as $SOCl_2$, PCl_5, PCl_3 and $POCl_3$. This reaction is the best and the most common method for the preparation of acyl chloride.

Thionyl chloride is the best reagent, since the by-products are gases, which do not contaminate the product, and excess thionyl chloride is generally separated by fractional distillation. But this reagent cannot be employed for the preparation of acetyl chloride because of separation problem.

$$RCOOH + SOCl_2 \rightarrow ROCl + HCl + SO_2$$

Phosphorous pentachloride and phosphorous trichloride are also suitable reagents for acid chloride formation, but their use is a largely restriced to aromatic acids.

$$RCOOH + PCl_5 \rightarrow RCOCl + POCl_3 + HCl$$

$$RCOOH + PCl_3 \rightarrow RCOCl + H_3PO_3$$

Moreover, very pure acid chloride may be obtained by the reaction between anhydrous sodium salt of the acid and phosphorous oxychloride ($POCl_3$).

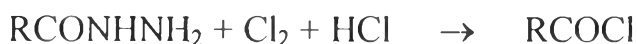$$RCOONa + POCl_3 \rightarrow RCOCl + Na_3PO_4$$
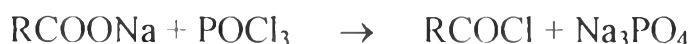
2. From esters

Acyl chloride may be prepared from esters with similar reagents used in carboxylic acids. This method is less often employed. Acid anhydrides may also serve as substrate.

$$RCOOR' + PCl_5 \rightarrow RCOCl$$

3. From hydrazides

Treatment of acyl hydrazides with chlorine and HCl will give acyl chloride.

$$RCONHNH_2 + Cl_2 + HCl \rightarrow RCOCl$$

Synthesis of acyl isothiocyanate

1. From acyl chloride

The reaction between an acyl halide and an inorganic thiocyanate is standard preparation method for an acyl isothiocyanate. Preferred reagent is lead (II) thiocyanate in refluxing solvents such as benzene, toluene, or xylene (Gilmore and Gallagher, 1995).

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-Cl \quad \xrightarrow{\text{Pb(SCN)}_2} \quad R-\overset{\overset{\displaystyle O}{\|}}{C}-N=C=S$$
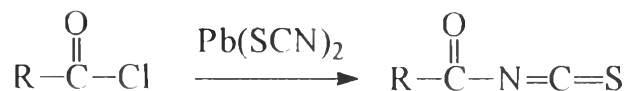
Other inorganic thiocyanates such as potassium thiocyanate, ammonium thiocyanate, magnesium thiocyanate and sodium thiocyanate similarly appear to react well with a variety of acid chlorides in a number of different solvents (Frank and Smith, 1955; Oba and Nishiyama, 1994).

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-Cl \quad \xrightarrow{\text{XSCN}} \quad R-\overset{\overset{\displaystyle O}{\|}}{C}-N=C=S$$

$$X = \text{Na, Mg, NH}_4\text{, K}$$

Using tributylammonium bromide (TBAB) as a catalyst in the reaction can help in avoiding the need for longer period of time and for anhydrous reaction condition. This has been employed for aroyl and long chain alkanoyl isothiocyanate. However, this approach is also successful with lower alkyl homologues due to competing hydrolysis of acid chloride.

2. From carboxylic acid

Carboxylic acids can be converted into their acyl isothiocyanates by using organophosphorous bis(isothiocyanates) such as triphenylphosphine isothiocyanate, $PPh_3(NCS)_2$), phosphoryl isothiocyanate , $P(O)(NCS)_3$ (Gilmore and Gallagher, 1995).

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-OH \quad \xrightarrow{\text{Ph}_3\text{P(NCS)}_2} \quad R-\overset{\overset{\displaystyle O}{\|}}{C}-N=C=S$$
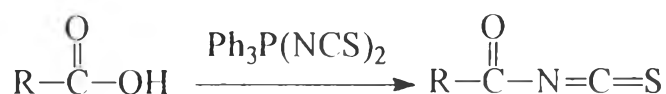
This method can be applied to a wide range of substrates and avoid the need to synthesize the intermediates acyl chloride. It provides a good general method for the synthesis of acyl isothiocyanates.
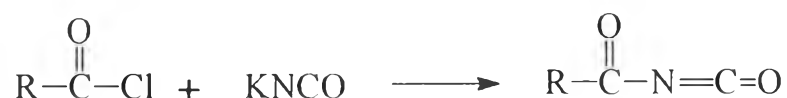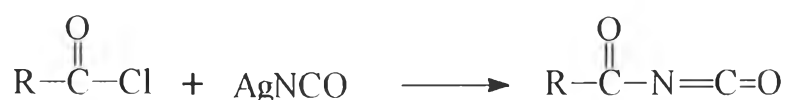
Synthesis of acyl isocyanate

1. From acyl chloride

Acyl chloride can react with isocyanic acid, which is obtained by thermal decomposition of cyanic acid, in the present of proton receptor B (pyridine, triethylamine, or N-methylmorpholine) at low temperature to give corresponding isocyanate. The best result were achieved by using THF as solvent and pyridine as base (Molina and Tarraga, 1995).

$$R-\overset{O}{\overset{\|}{C}}-Cl + HNCO + B \longrightarrow R-\overset{O}{\overset{\|}{C}}-N=C=O + BH^{+}Cl^{-}$$

Other salts of cyanate such as silver cyanate and potassium cyanate can be used in this reaction.

$$R-\overset{O}{\overset{\|}{C}}-Cl + AgNCO \longrightarrow R-\overset{O}{\overset{\|}{C}}-N=C=O$$

$$R-\overset{O}{\overset{\|}{C}}-Cl + KNCO \longrightarrow R-\overset{O}{\overset{\|}{C}}-N=C=O$$

2. From amides

2.1 Reaction with oxalyl chloride

In 1962, Speziale and Smith has reported the reaction of amide and oxalyl chloride as a very convenient and general method for the preparation of acyl isocyanate. Formation of the acyl isocyanate proceeds via acyl oxamic acid chlorides and cyclic intermediate (Speziale and Smith, 1962).
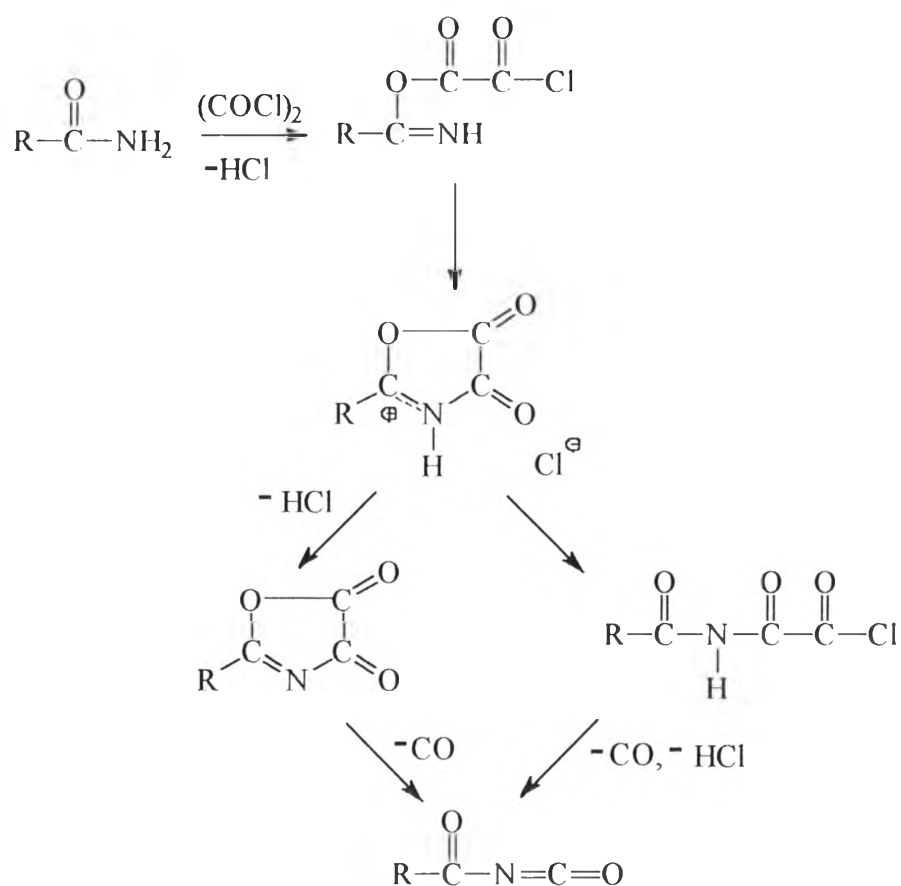
2.2 Reaction with phosgene

Although the reaction of an amide and phosgene is widely used for the preparation of alkyl and aryl isocyanate, it is unsuitable for the synthesis of acyl isocyanate, it was found that bis(trimethylsilyl)amide of carboxylic acids can produce the corresponding acyl isocynates in a monomeric form in almost quantitative yields. This method can be used for lower members of the aliphatic series which are formed via reaction with oxalyl chloride in extremely low yields (Molina and Tarraga, 1995).
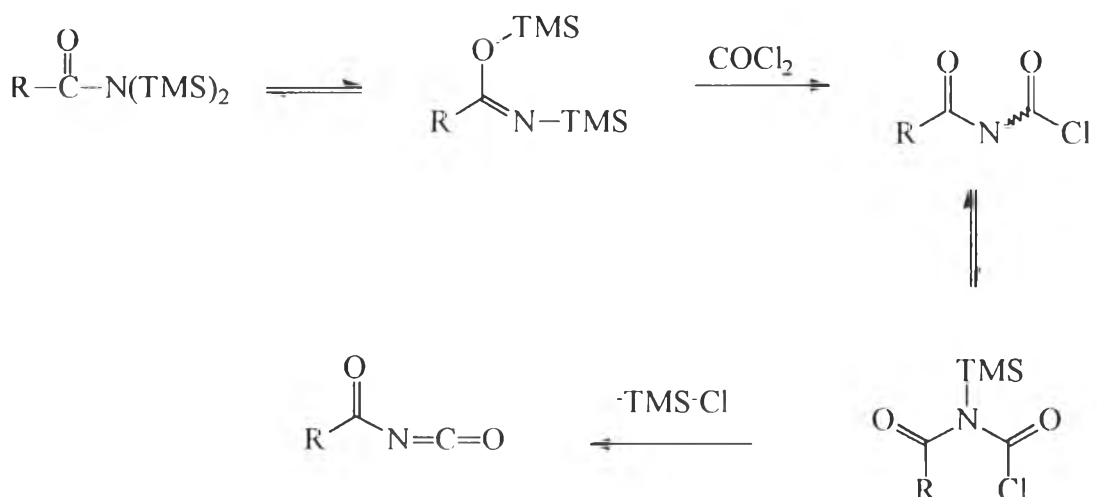
Moreover, there is a report of reaction between amide, phosgene and lime to form acylisocyanate (Saunder and Slocome, 1948).
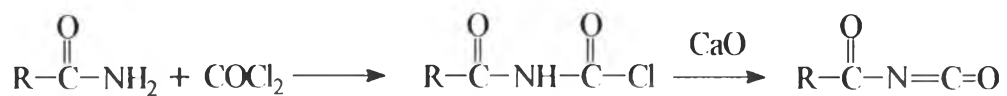
3. From carboxylic acid anhydrides
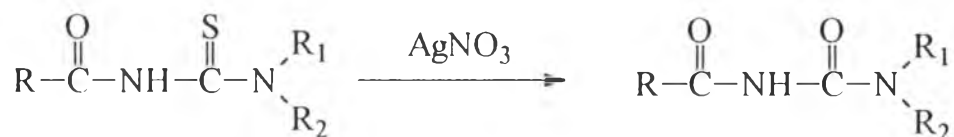
Reaction with isocyanatosilanes

With a catalyst, stannic chloride, and at low temperature, carboxylic acid anhydrides can react with isocyanatosilanes leading to the corresponding acyl isocyanate in high yield (Molina and Tarraga, 1995).

$$(RCO)_2O + TMS-N=C=O \xrightarrow[-20\ C]{SnCl_4} R-\overset{\overset{O}{\|}}{C}-N=C=O + RCO_2 + TMS$$
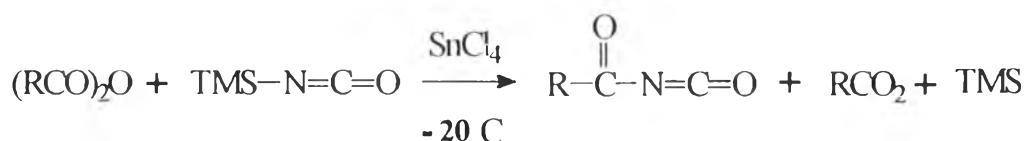
Conversion of thiocarbonyl to carbonyl

1. By silver nitrate

In 1896, Dixon had reported that a thiourea can be converted to a urea by silver nitrate in hot alcoholic solution (Dixon, 1896).

$$R-\overset{\overset{O}{\|}}{C}-NH-\overset{\overset{S}{\|}}{C}-N\overset{R_1}{\underset{R_2}{\diagdown}} \xrightarrow{AgNO_3} R-\overset{\overset{O}{\|}}{C}-NH-\overset{\overset{O}{\|}}{C}-N\overset{R_1}{\underset{R_2}{\diagdown}}$$

2. By potassium permanganate

In 1890, Maly had shown that a urea was formed when thiourea was oxidised by potassium permanganate in a neutral solution, and all of the sulfur was oxidized to sulphuric acid (Werner, 1919).

3. By peroxy acid

In 1984, Looney-Dean had shown that thioamide can be converted to amide by oxidation with alkaline hydrogen peroxide with yield of more than 80% (Looney-Dean, 1984).

$$
\underset{\substack{\|\\ R-C-NH-R'}}{S} \quad \xrightarrow{H_2O_2/\ HO^{\ominus}} \quad \underset{\substack{\|\\ R-C-NH-R'}}{O}
$$