CHAPTER II

HISTORY

Valproic acid.

Valproic acid (VPA; 2-propylpentanoic acid, or 2-propylvaleric acid) is an anticonvulsant agent that differs markedly from all anticonvulsant drugs in clinical use. It has a simple aliphatic molecular structure (branched chain fatty acid) in contrast to the heterocyclic ring structures of hydantoins, barbiturates, benzodiazepines, succinimides and oxazolidinediones. Valproic acid was first synthesized in the United States by Burton in 1882 and was used worldwide for 80 years as an organic solvent. Its unexpected antiepileptic activity was discovered in Grenoble, France (Chapman et al., 1982).

Pierre Eymard, a reserch student at the University of Lyon, had synthesized a series of derivatives of khellin. This was a nature product said to have a variety of pharmacological properties. After completing his thesis, Edmard arranged to have his new compounds tested in professor G. Carraz's pharmacology laboratory at the Ecole de Medecine et de pharmacie at Grenoble. When Eymard tried to prepare a solution of the first compound to be tested, he could not get it to dissolve. He then sought advice from H. Meunier and Y. Meunier of the laboratoire Berthier in Grenoble. They suggested that valproic acid might be a suitable solvent since they had used it in the past to dissolve bismuth compounds for clinical evaluation. The valproic acid did dissolve Eymard's compounds, and subsequent tests showed the khellin derivatives to be physiologically active. Professor Carraz advised that it should be put through a general screen for a variety of possible actions, and this revealed it to have anticonvulsant activity. Shortly after this, H. Meunier used valproic acid to dissolve a coumarin compound. Althrough chemically unrelated to Eymard's compounds, it too proved to have anticonvulsant properties. Meunier realized this could not be mere coincidence. He immediately tested the valproic acid and discovered it was an anticonvulsant. After detailed studies by Carraz

and his colleagues, valproic acid was subjected to extensive clinical investigation before its sodium salt was first marketed as Depakine in France in 1967 (Sneader, 1985). Since this time, valproate has been commercialised in a large number of countries ("Epilim" in the U.K.). It was authorised for used in epilepsy in the United State in 1978 (Chapman et al., 1982; Ramsay, 1984).

Several clinical studies have shown that sodium valproate has a broad spectrum of activity and the degree of its efficacy depends on the type of epilepsy treated, but sodium valproate can be considered as a drug of first choice in a large number of epileptic patients (Dulac and Arthuis, 1984; Gram and Bentsen, 1984; Ramsay, 1984).

Mechanism of action.

The mechanism of action of valproic acid has yet to be fully elucidated although much attention has focused on its effect on the inhibitory neurotransmitter gamma-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 transminase. Evidence also exists which suggests that valproic acid reduces neurotransmission mediated by the excitatory amino acid gamma-hydroxybutyric acid, which produces absence-like seizures in animals. In addition to its effect on amino acid neurotransmitters, valproic acid appears to possess a direct neuronal membrane depressant effect through its influence on sodium or potassium conductance (Johnston, 1984; Rogawski and Porter, 1990; Davis et al.,1994).

Metabolism.

At least four main metabolic pathways for valproic acid has been described in humans; glucuronidation, β-oxidation, ω-oxidation and ω-1-oxidation. Only 1 to 3% of an administered dose of valproic acid is excreted unchanged in urine. A major portion of the dose is excreted as glucuronide in urine. Although β-Oxidation, ω-oxidation and ω-1-oxidation do occur, these routes appear to be of less importance than glucuronidation (Figure 15) (Chapman et al., 1982; Granneman et al., 1984; Nau and Loscher, 1984; Davis et al., 1994).

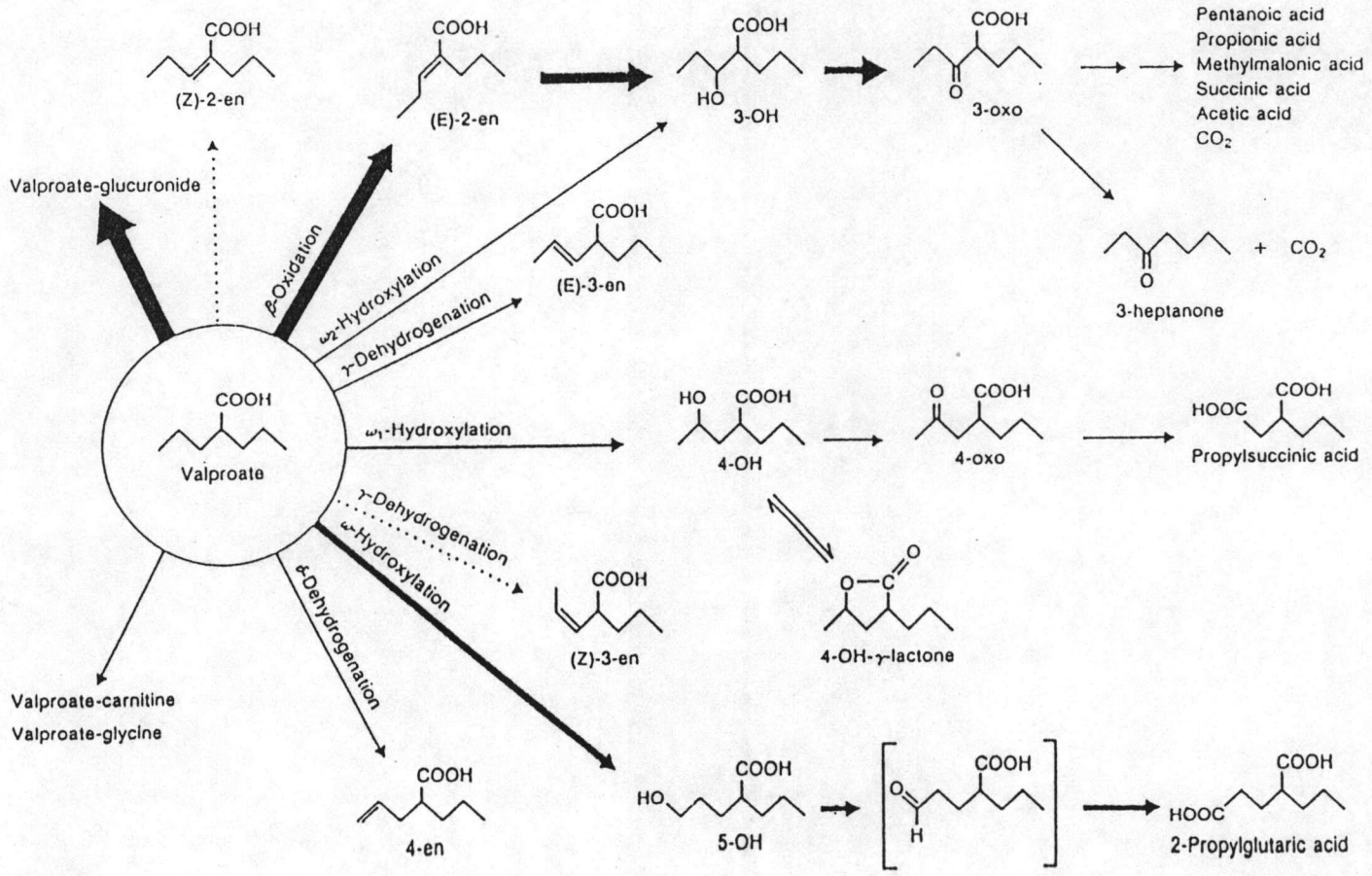


Figure 15. Metabolic pathway of valproic acid.

In animal studies, a number of metabolites possess anticonvulsant activity that weaker than that of valproate (Chapman et al., 1982). 2-Propyl-2-pentenoic acid, a β-oxidation metabolite, probably contributes to the anticonvulsant activity of valproic acid (Morre et al., 1984; Davis et al., 1994), while another metabolite, 2-propyl-4-pentenoic acid, may be involved in both hepatotoxicity and embryotoxicity (Nau and Loscher, 1984; Davis et al., 1994).

Structure-activity relationship.

Valproic acid is one of many carboxylic acids that are active in protecting experimental animals against pentylenetetrazol-induced seizures. A series of other active carboxylic acids are shown in figure 16. An accurate structure-activity analysis is difficult because these structures were reported in several sources and the activity was quantitated differently, often in different species. However, anticonvulsant activity appears with C-5 and increases to a maximun of C-8. Carboxylic acids up to C-11 have tested and found active. Branching and unsaturation do not increase the potency of the carboxylic acids, but may prolong their duration of action and produce a marked sedative effect (Kupferberg, 1980; Chapman,1982; Morre et al.,1984).

Derivative formation via carboxylic group has led to the interesting results methyl and ethyl esters of valproic acid (LXXVI and LXXVII, respectively) are active but no more potent than the parent compound (Kuppferberg, 1980; Chapman, 1982; Badir et al., 1991; Hadad et al., 1992; Bialer et al., 1994). Amide formation also leads to compounds with anticonvulsant activity (Kuppferberg, 1980; Chapman, 1982). Valpromide (LXVIII), a primary amide of valproic acid, has been used in several European countries as an antiepileptic and antipsychotic agent. Valnoctamide (LXXIX), an isomer of valpromide, has also been in used as an anxiolytic drug, and it also possesses anticonvulsant activity (Haj-Yehia and Bialer,1990; Bailer et al., 1994). Other derivatives that showed a good and promising anticonvulsant profile in the classical animal models for antiepileptic screening are 2-propylpentanal acetals (LXXX), the acetals of the corresponding aldehyde of valproic acid (Vicchio and Callery, 1989); the diol diester type prodrug of valproic acid (LXXXI) in which one hydroxy group of ethylene glycol is esterified by valproic acid and the other by the dihydrotrigonelline moiety (Pop et al.,1991); 1,3-dihexadecanoylamino-

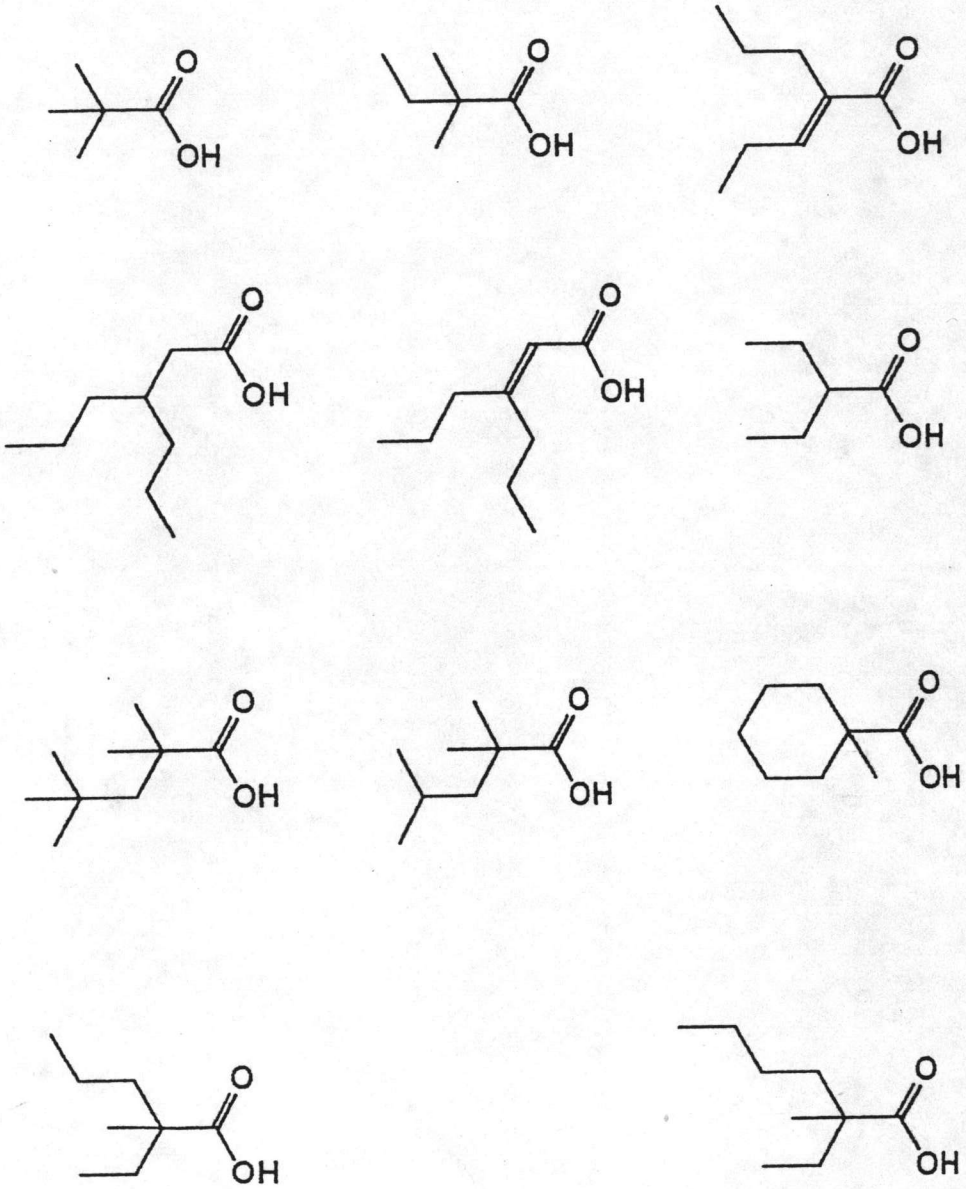
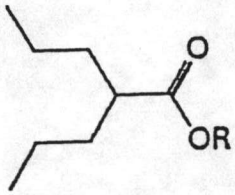
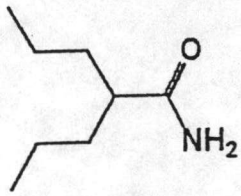


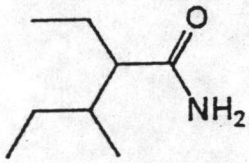
Figure 16. Some carboxylic acids found to be active against pentylenetetrazole-induced clonic seizure.



LXXVI, R = methyl



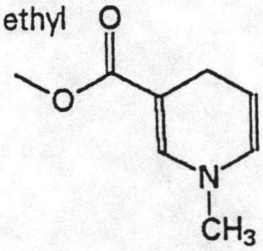
LXXVIII

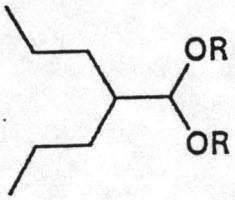


LXXIX

LXXVII, R = ethyl

LXXXI, R =

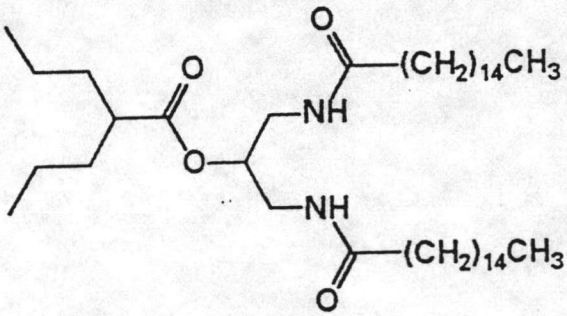




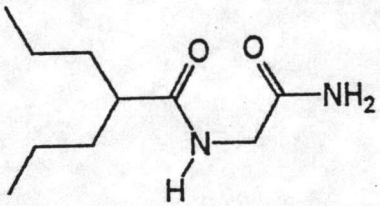
LXXX, R = methy,l

ethyl,

isopropyl,



LXXXII



LXXXIII

Figure 17. The chemical structures of derivatives of valproic acid which showed a good anticonvulsant activity.

2-valproyl-propan-2-ol (LXXXII) (Mergen et al., 1991); and valproyl glycinamide (LXXXIII)(Hadad and Bialer, 1995).

For the corresponding alcohol of valproic acid, 2-propylpentanol, it has one-half the antipentylenetetrazol activity of valproic acid (Kupferberg, 1980).

Pyridoxine (Pyridoxol).

In 1926, a U.S. physician, Joseph Goldberger, described the introduction of a pellagralike dermatitis, later called rat acrodynia, in rats fed a diet deficient in vitamin B_2. However, in 1936 Paul Gyorgy distinguished the water soluble factor whose deficiency was responsible for the dermatitis from vitamin B_2 and name it vitamin B_6 (Gyorgy, 1964; Marcus and Coulston, 1990). The structure of the vitamin was elucidated and the synthesis was completes in 1939 (Stiller et al., 1939; Harris et al., 1939; Harris and Folkers, 1939). Several related natural compounds have been shown to possess the same biological properties, and therefore all should be called vitamin B_6. The IUPAC-IUB Commission on Biological Nomenclature has recommended that the term vitamin B_6 be used in this generic sense. Pyridoxine refers specifically to 3-hydroxy-4,5-di(hydroxymethyl)-2-methylpyridine (Coffen, 1984).

The six pyridine derivatives that exhibit significant vitamin B_6 activity are pyridoxine, Pyridoxal, Pyridoxamine and their respective 5-phosphates. The compounds differ in the nature of the substituent on the carbon atom in the position 4 of the pyridine nucleus that can be found as the methylhydroxy (pyridoxine), the aldehyde (pyridoxal), or the methylamine (pyridoxamine). Each of these forms can also be phosphorylated at the 5 position (Figure 18). Pyridoxal-5-phophate and pyridoxamine-5-phosphate are the active coenzyme forms, with pyridoxine-5-phosphate being the primary form of biological interest (Brody 1994; Leklem, 1994).

Interconversion and Metabolism.

The liver is the primary organ responsible for metabolism of B_6 vitamers. As a result, the liver supplies the active form of vitamin B_6, pyridoxal-5-phosphate to the circulation and other tissues. Figure 18 depicts the interconversion of the B_6 vitamers and the enzyme involved.

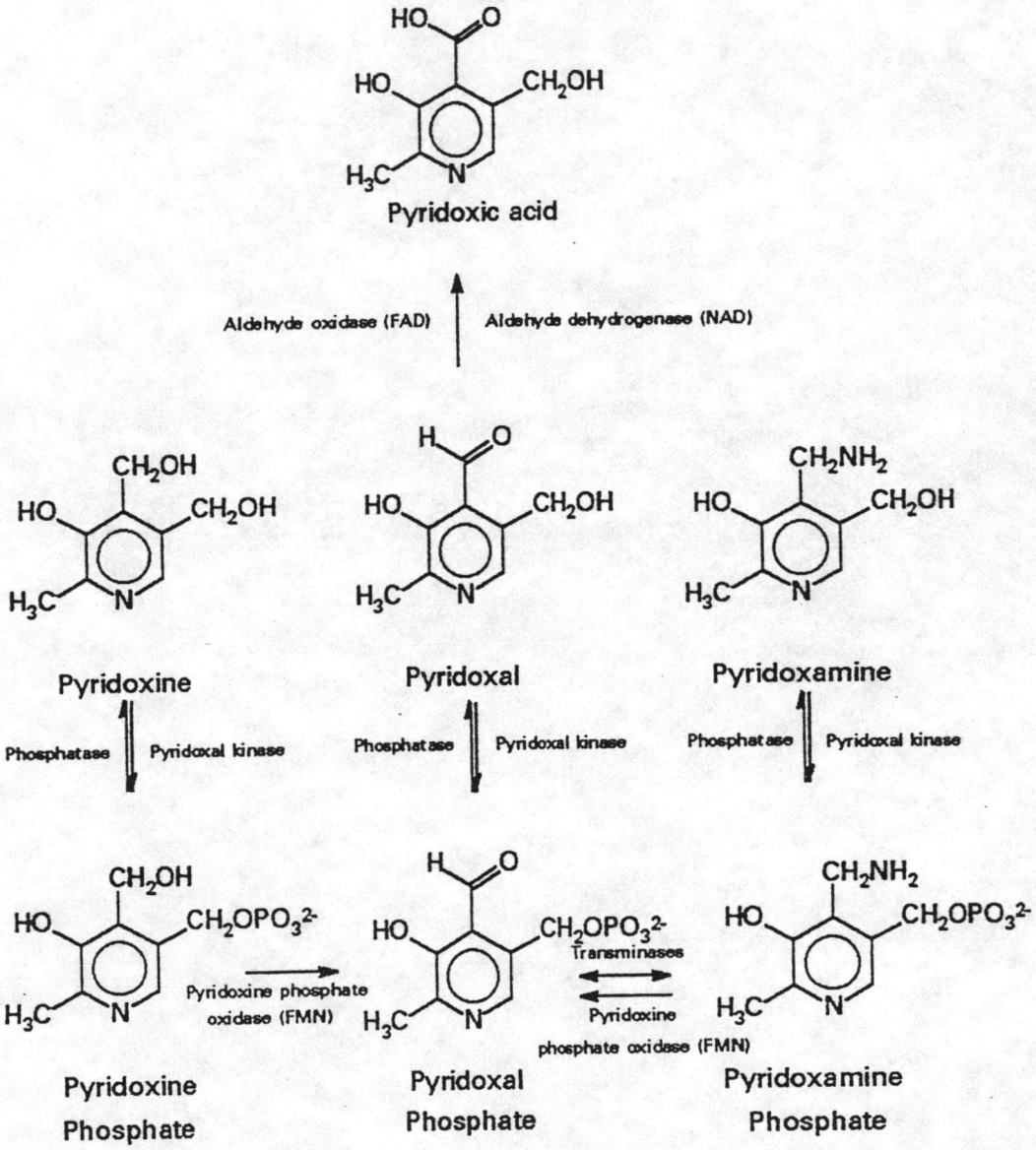


Figure 18. Forms, interconversion and metabolism of vitamin B_6.

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The three nonphosphorylated forms are converted to the respective phosphorylated form by pyridoxine kinase. Zinc and adenosine triphosphate (ATP) are cofactors for this kinase. Pyridoxine-5-phosphate and pyridoxamine-5-phosphate can then be converted to pyridoxal-5-phosphate via a flavin mononucleotide (FMN) oxidase.

Phosphorylated forms can be hydrolyzed by alkaline phosphatases. The pyridoxal that results form this dephosphorylation, as well as that which is present from dietary sources, can than be converted to 4-pyridoxic acid (4-PA) in a nonreversible reaction that involved flavin adenine dinucleotide (FAD) and an aldehyde oxidase (Leklem,1994).

Physiologic functions.

The vitamin B_6 are involved in a number of extremely important metabolic reactions of the α-amino acids including transaminations, in which pyridoxal-5-phosphate and pyridoxamine-5-phosphate are equally active and racemizations, and decarboxylations which only pyridoxal-5-phosphate is active (Dyhe, 1965).

The decarboxylation reaction is vital to any organism having a nervous system, since the neurotransmitters (biogenic amines) are produced in this way. These include dopamine, leading to norepinephrine and epinephrine, serotonin, tyramine, tryptamine, taurine, histamine and GABA. for this reason, vitamin B_6 deficiency in human is often manifested in dysfunction of the central and peripheral nervous systems (Coffen, 1984).

Pyridoxal-5-phosphate is also needed for the activity of enzymes, involved in the metabolism of protein, carbohydrate and fat. Without vitamin B_6 the non-essential amino acids can not be synthesized. Vitamin B_6 is needed for the synthesis of hemoglobin, which is the oxygen-carrying molecule in red blood cells, and the synthesis of white blood cells, which are important in the immune system (Smolin and Grosvenor, 1994).

Symptoms of deficiency.

Important features of pyridoxine deficiency involved the skin, the central nervous system, and erythropoiesis.

Skin. In man, seborrhea-like skin lesions about the eyes, nose, and mouth accompatied by glossitis and stomatitis can be produced within a few weeks by feeding a diet poor in vitamin B complex plus daily doses of the vitamin antagonist 4-deoxypyridoxine. The lesions clear rapidly after the administration of pyridoxine but do not respond to the other members of the vitamin B complex (Marcus and Coulston, 1990).

Nervous System. Infants who were fed a formula in which the vitamin B_6 was destroyed during processing showed abnormal electroencephalogram (EEG) tracings and convulsions. Both EEG abnormalities and convulsions are corrected by 30 to 100 mg of pyridoxine per day, while the anticonvulsant hydantoin may prove ineffective (Bordy, 1994; Leklem, 1994).

The induction of convulsive seizure by pyridoxine deficiency may be the result of a lowered concentration of gamma-aminobutyric acid (this inhibitory CNS neurotransmitter synthesized by glutamate decarboxylase, a pyridoxal phosphate-requiring enzyme). In addition, pyridoxine deficiency leads to decreased concentration of neurotransmitters norepinephrine and 5-hydroxy tryptamine (Marcus and Coulston, 1990; Leklem, 1994).

Erythropoiesis. A severe chronic deficiency of vitamin B_6 can lead to hypochromic microcytic anemia. In addition, some patients with sideroblastic anemia and other anemias do respond favorably to pyridoxine therapy (Leklem, 1994).

Human requirements.

Since vitamin B_6 is needed to metabolize protein, recommended intakes are based on the amount of protein in the diet. The recommended daily allowance is based on an estimated need of 0.16 mg of vitamin B_6 per gram of protein intake per day. The average adult

requirement for pyridoxine is 2 mg. per day for man and 1.6 mg. per day for woman (Smolin and Grosvenor, 1994).

Toxicity.

The vitamin is relatively non-toxic and excess amounts in the body are readily removed by renal clearance. The acute toxicity of vitamin B_6 is 6,000 md/kg (oral) and 700 mg/kg (intravenous) in mice, and 3,700 mg/kg (subcutaneous) in rat. No chronic toxicity was observed in dogs and rats at doses of 20 and 25 mg/kg per day, nor were teratogenic effects observed in rat at doses up to 80 mg/kg per day. Damage to the nervous system resulted in dogs fed 1,000 mg/kg per day for several days, this is equivalent to 70,000 mg/day for a man. Vitamin B_6 administration in humans, either as a diet supplement or in therapeutic doses, rarely exceeds 50 mg/day (Coffen, 1984).

Synthesis of aldehyde: 2-propylpentanal.

Since the formation of 5-hydroxymethyl-8-(1-propylbutyl)-4*H*-dioxino[4,5-*c*]pyridine can be obtained from the condensation of 2-propylpentanal and pyridoxine, 2-propylpentanal must then be prepared to be a starting material of this reaction. Aldehyde may be prepared by a wide variety of general methods, a few of which will be described here. In addition to these, a number of special reactions have been developed for special aldehyde.

1. Syntheses of aldehydes from Grignard reagents (RMgX).

There are some different substances which react with Grignard reagents leading to the formation of aldehydes, such as (Smith and Bayliss, 1941; Smith and Nichols, 1941),

1.1. Reactions of ethyl ethoxyacetate ($EtOCH_2COOEt$) with Grignard reagents. (Figure 19A)

R_2CHCHO type aldehydes can be prepared by the reaction of ethyl ethoxyacetate and Grignard reagents, the alcohol $EtOCH_2(OH)R_2$ formed then be transformed to aldehydes by the use of acid. The yields varied between 50-80% of the theoretical quantity.

1.2. Reactions of ethyl orthoformate ($CH(OEt)_3$) with Grignard reagents. (Figure 19B)

This method is also known as Bodroux-Tschitschibabin aldehyde synthesis. A Grignard reagent reacts with an ethyl orthoformate to form an acetal which is hydrolyzed to the corresponding aldehyde with diluted acid.

1.3. Reactions of N,N-disubstituted formamides (R'R"NCHO) with Grignard reagents. (Figure 19C)

This method is also known as Bouveault aldehyde synthesis. This reaction, while successful in certain cases, is very complicated and frequently produces tertiary amine as the chief product. Moreover, even when the aldehyde is the chief product, the reaction does not compare in efficiency with the one using ethyl orthoformate.

1.4. Reactions of carbon disulfide (CS_2) with Grignard reagents. (Figure 19D)

A Grignard reagent reacts with carbon disulfide to form a dithio acid while was in turn, converted into an aldehyde derivative by action of semicarbazide, phenylhydrazine or hydroxylamine. The aldehyde derivative was then hydrolyzed to the aldehyde in the usual way.

2. The oxidation of primary alcohols to aldehydes. (Figure 20A)

Aldehydes may be prepared by oxidation of the corresponding alcohol with manganese dioxide, a sulfuric acid solution of potassium dichromate, pyridinium chlorochromate or pyridinium dichromate. The reaction must be carefully controlled and/or the product should be continuously removed from the reaction mixture to avoid oxidation of the aldehyde to the carboxylic acid (Corey and Schmidt, 1979; Furniss et al, 1991).

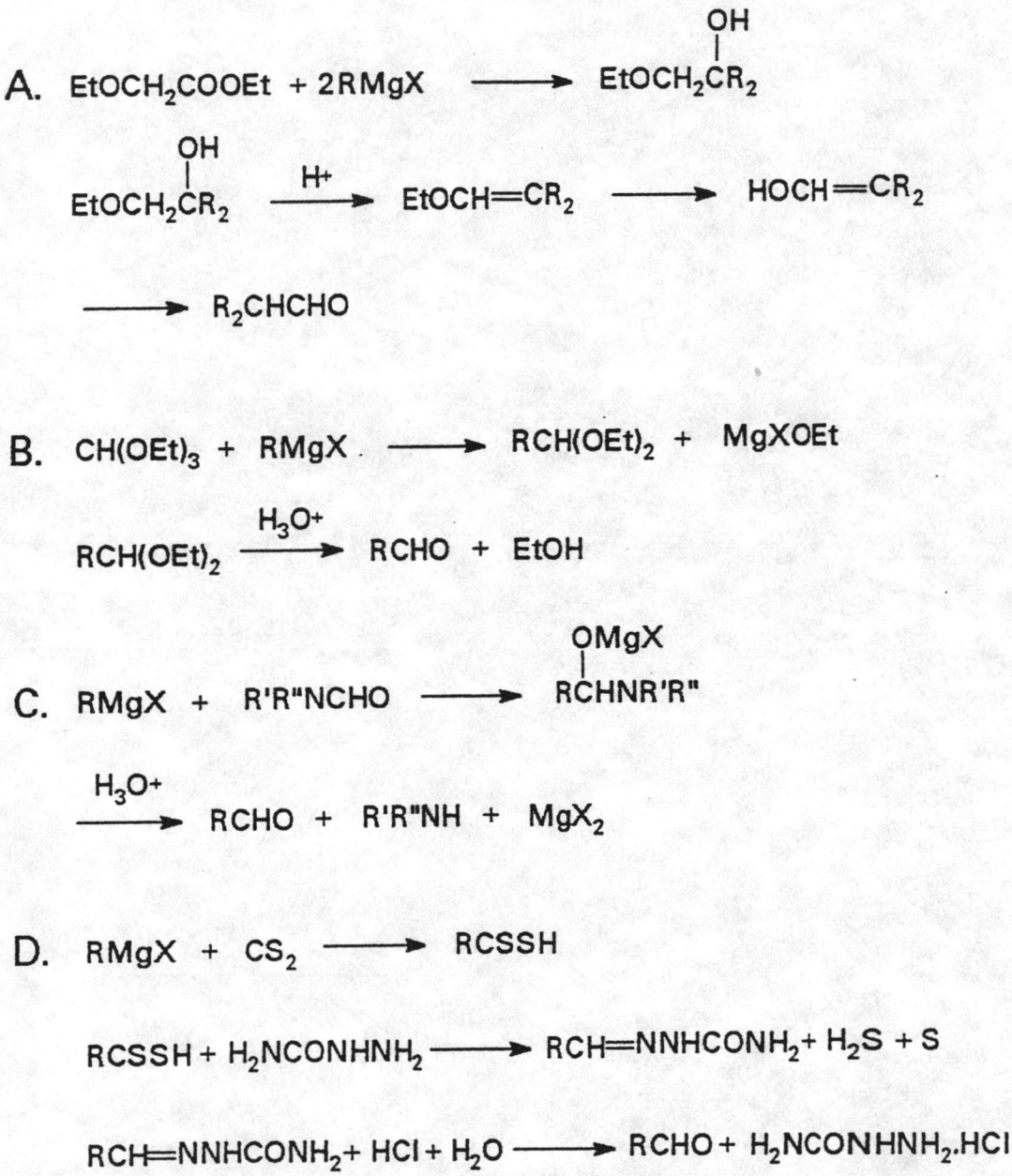

Figure 19. Syntheses of aldehydes from Grignard reagents and
A. ethyl ethoxyacetate;
B. triethyl orthoformate;
C. N,N-disubstituted formamides;
D. carbon disulfide.

3. The ozonolysis of substituted alkenes. (Figure 20B)

Oxidation of alkenes with ozone followed by cleavage of the resulting ozonides to form aldehydes. The cleavage of the ozonide is carried out by catalytic hydrogenation over palladium hydroxide-on-calcium carbonate, treatment with dimethylsulphide in methanol, or treatement with water (Furniss, 1991; Parker, 1993).

4. Chlorination of the methyl-substituted aromatic compound. (Figure 20C)

The chlorination of the methyl-substituted aromatic compound in the presence of strong light (which serves as a catalyst) proceeds stepwise to form the mono- and di-chlorination product, respectively. The resultant dichloride is converted to an aldehyde by hydrolysis with an iron catalyst (Sherman, 1980; Parker, 1993).

5. The oxidation of the methyl-substituted aromatic compound. (Figure 20D)

Partial oxidation of the methyl-substituted aromatic compound with chromic acid also leads to the aromatic aldehyde. Acetic anhydride is added to the reaction mixture to acylate the aldehyde as it is formed and prevent further oxidation. The resultant diacetate is easily hydrolyzed with aqueous acid to regenerate the aldehyde (Sherman, 1980; Parker, 1993).

6. The reduction of nitriles to aldehyde. (Figure 21A)

The reduction of a nitrile is achieved with anhydrous tin(II) chloride dissolved in ether or ethyl acetate saturated with dry hydrogen chloride (the Stephen aldehyde synthesis). The resulting aldimine hydrochloride is then hydrolyzed in warm water.

The method is used mainly for the synthesis of aromatic aldehyde but reduction of the higher aliphatic nitriles normally gives good yields (Stephen, 1925, Furniss et al., 1991).

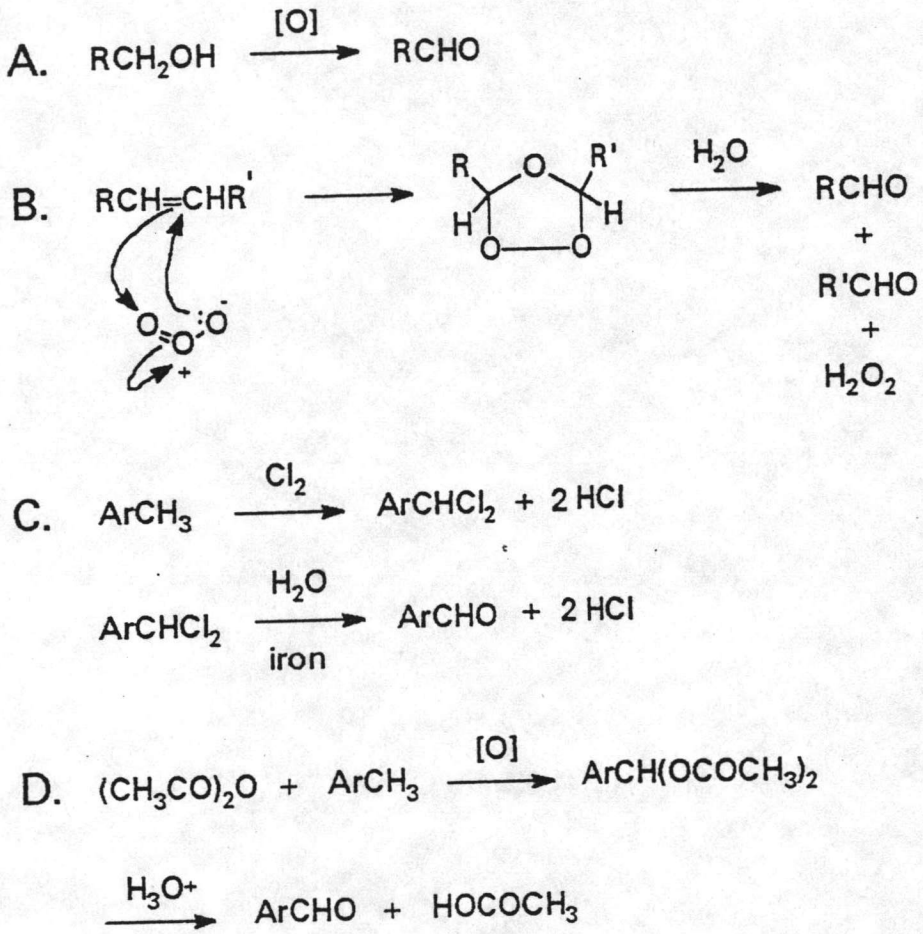

Figure 20. Syntheses of aldehydes from

A. The oxidation of primary alcohols;
B. The ozonolysis of substituted alkenes;
C. The chlorination of the methyl-substituted aromatic compounds;
D. The oxidation of the methyl-substituted aromatic compounds.

7. The reduction of acid chlorides (ROCl) to aldehydes. (Figure 21B)

Acid chlorides react with hydrogen in the presence of an appropriate catalyst to form an aldehyde and hydrogen chloride. It is usefully for the transformation of a carboxylic acid to the corresponding aldehyde. This reaction is known as the Resenmund synthesis.

The catalyst used in the hydrogenation of acid chloride is a palladium catalyst which has been poisoned with barium sulfate or a metal hydride, such as lithium tri-*tert*-butoxy aluminium hydride (Brown and McFarlin, 1958; Sherman, 1980).

8. The reduction of carboxylic acid to aldehydes. (Figure 21C,D)

A most convenient procedure for the conversion of carboxylic acids to aldehydes results from their initial treatment with borane-dimethyl sulphide to give first, the triacyloxyborane, which is then reduced further to the intermediates, trialkyloxyboroxine. Oxidation of trialkyloxyboroxine with pyridinium chlorochromate(PCC) then yields the aldehyde (Furniss, 1991).

The carboxylic acids were also reduced to aldehydes by lithium in methylamine. One reduction intermediate is thought to be a carbinolamine salt which is converted to the imine during the isolation procedure. The imine is then hydrolyzed to the aldehyde (Bedenbaugh et al., 1970).

9. The reduction of acid amides to aldehydes. (Figure 21E)

Lithium aluminium hydride reacts with acid amide at low temperature to form aldehyde. The formation of aldehydes probably proceeds through the complex formed as the result of a usual nucleophilic substitution, under the attacks of the hydride in aluminium hydride ion. Upon hydrolysis, this complex would give an unstable amino alcohol which would decompose, by intermolecular displacement, to aldehyde and the starting amine (Micovic and Mihailovic, 1953).

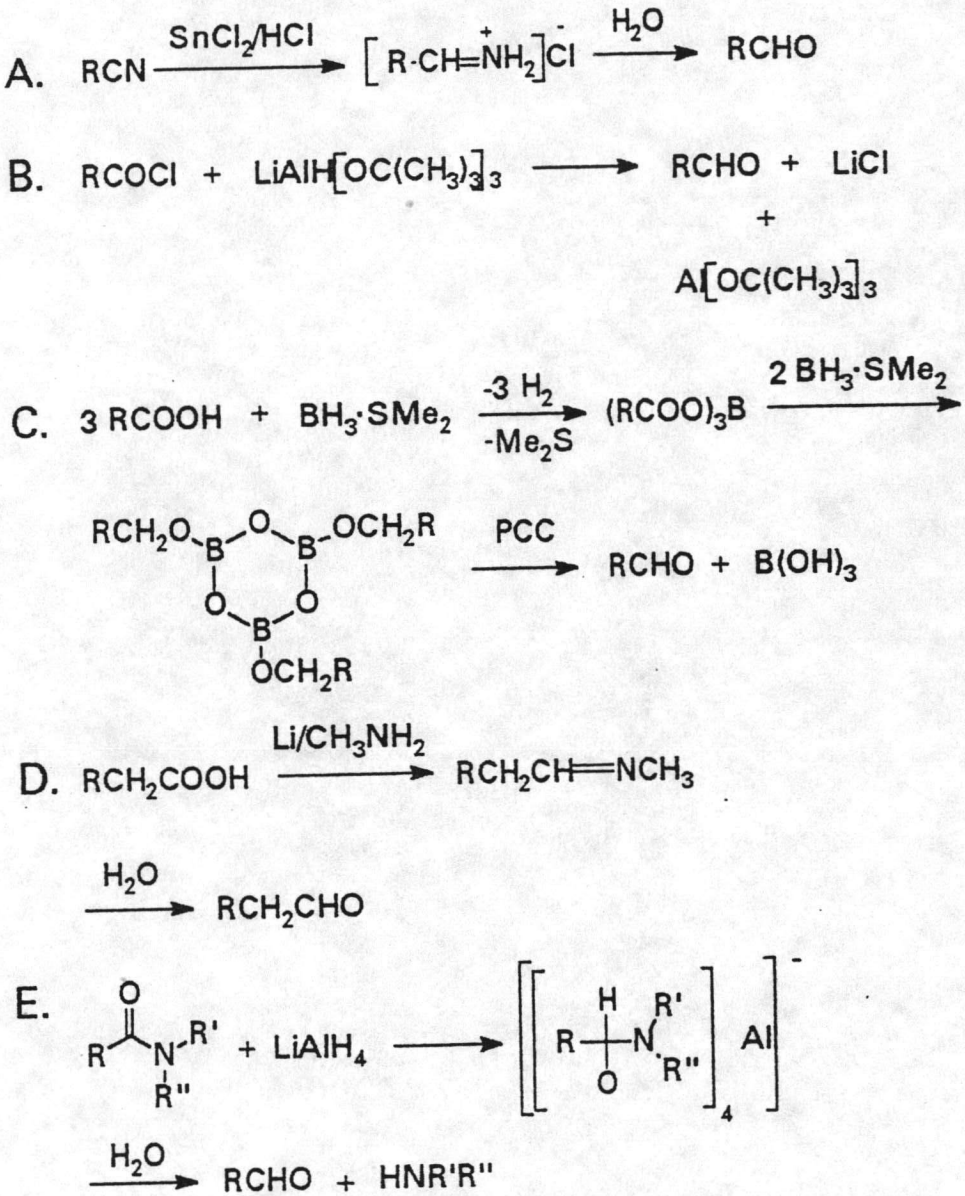

Figure 21. Syntheses of aldehydes from the reduction of
A. nitriles;
B. acid chlorides;
C, D. carboxylic acids;
E. acid amides.

Syntheses of carboxylic acid ester.

The reaction of valproyl chloride and pyridoxine to form the valproate esters of pyridoxine and the direct esterification of chloroacetic acid and ethanol to produce ethyl chloroacetate in the steps of the preparation of 2-propylpentanal are the esterification reaction used in this research. General methods for the production of carboxylic acid esters (esterification) are described as below. (Zey, 1980; Furniss et al., 1991).

1. **Direct esterification.** (Figure 22A)

The interaction of a carboxylic acid and an alcohol is fundamental organic reaction. This reaction is the reverse of the hydrolytic procedure. To drive the reaction forward, the alcohol is usually used in large excess, and it may also be necessary to remove water as it is formed. This can be done by azeotropic distillation in some cases.

The equlibrium constant for this reaction is ordinarily favorable, but the reaction is rather slow. Equlibrium is only attained after refluxing for several days. If, however, about 3 percents (of the weight of the alcohol) of either concentrated sulfuric acid or of dry hydrogen chloride is added to the mixture, the same point of equlibrium can be reached after a few hours.

This method of esterification, in general gives good yields with primary alcohols and fairly good yield with secondary alcohols. The method is unsatisfactory for use with tertiary alcohols owing to competing alkene formation from an acid catalysed dehydration.

2. **The use of acid anhydride.** (Figure 22B)

Carboxylic acid anhydride, an acylating agents that is more reactive than carboxylic acid reacts rapidly with most unhindered hydroxy to give ester. The general mechanisms are well known. The nucleophilic hydroxy group undergoes addition at the carbonyl group, followed by the elimination of the carboxylate group. Acid anhydride is reactive acylating reagent because of a combination of the inductive effect of the oxygen substituent on the reactivity of the carbonyl group

and the ease with which the tetrahedral intermediate can expel such relatively good leaving groups.

The acid produced from an acid anhydride can not hydrolyze the ester, and hence this reaction goes to completion. However, this method is applied only when esterification can not be effected by the usual means because of the higher cost of the acid anhydrides.

3. **The use of acid chloride.** (Figure 22C)

Acid chlorides are generally more reactive than the corresponding acid anhydrides. They react readily with primary and secondary alcohols to form esters in very good yield. Because of the hydrogen chloride liberated from the reaction, the acid chlorides are not used with alcohols susceptible to rearrangement.

4. **The use of acid amides.** (Figure 22D)

Amides may be converted to ester by reaction with alcohols. The reaction is the reversible process and produces ammonia as by product. In order to produce high yields of ester it is necessary to remove the ammonia produced, either by heating or by combining with a mineral acid, e.g., sulfuric acid or hydrogen chloride.

5. **The use of nitrile.** (Figure 22E)

Alcoholysis of nitrile offers a convenient way to produce esters without isolating the acid. The reaction produces ammonia as by product, so acids are used to combine with the ammonia formed. A large excess of alcohol is used but the amount of water is generally kept small. Catalysts such as hydrogen chloride, hydrogen bromide, and sulfuric acid have been employed.

6. **Ester interchange.** (Figure 22F)

Ester interchange (transesterification) is a reaction between an ester and another compound, characterized by an exchange of alkoxy groups or of acyl groups, and resulting in the formation of a different ester.

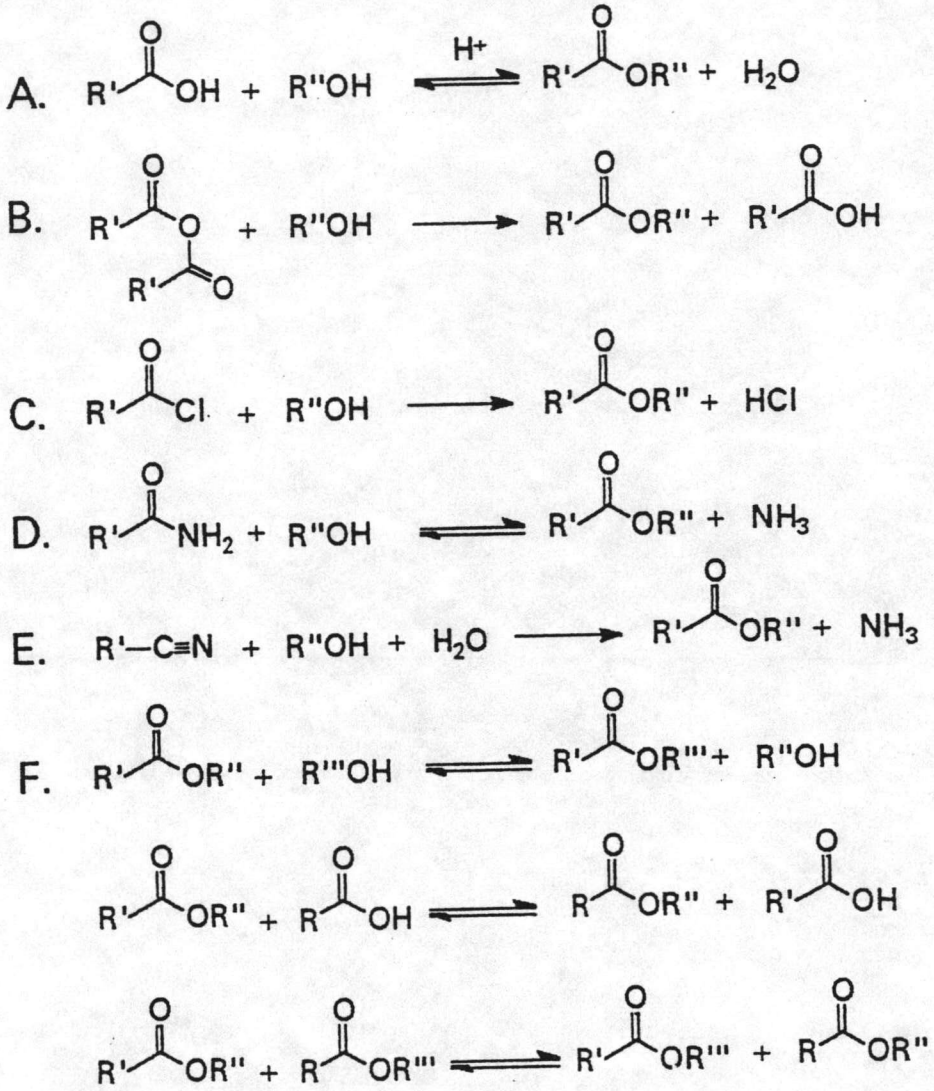

Figure 22. Syntheses of esters by
A. Direct esterification;
B. The use of acid anhydrides;
C. The use of acid chlorides;
D. The use of acid amides;
E. The use of nitriles;
F. Ester interchange.

In the best-known types of ester interchange, the compound with which the ester reacts is an alcohol, an acid or another ester. These ester interchanges may be called, more specifically, ester-alcohol interchange or alcoholysis, ester-acid interchange or acidolysis, and ester-ester interchange, respectively. These reactions are reversible and ordinarily do not involve large energy changes.

Synthesis of acetals.

Acetals and ketals are characterized by presence of two alkoxy group (-OR) attached to a carbon atom. Acetals differ from ketals in that they always have at least one hydrogen atom attached to the central carbon atom involved in C-O bond formation. Because of the similarity of acetals and ketals, it is common to find both categorized as acetals. These compounds are prepared by reversible addition reactions of aldehydes or ketones with alcohols.

The product of addition of one mole of alcohol to an aldehyde or ketone is refered to as a hemiacetal or hemiketal, respectively. Dehydration followed by addition of a second molecule of alcohol give an acetal or ketal (Carey and Sundberg, 1990). Diols react with aldehydes and ketones to give cyclic acetals and ketals and, as with simple alcohols, the intermediate hemiacetal or hemiketal is not usually isolated. (Figure 23A,B)

The formation of acetals is generally catalyzed by protic acids (Brown et al., 1964; Sterzycki, 1979; Patney, 1991; Lu et al., 1995). Numerous catalysts are also reported in the literature for acetalization include the use of lewis acids (Anderson and Uh, 1973; Swenton et al., 1975), ion-exchange resins (Dann et al., 1979), rhodium complexes (Ott et al., 1989), and polystyryldiphenyliodo phosphonium-iodide complex (Caputo et al., 1987).

Because the position of the equilibrium does not strongly favor product, the formation of acetals and ketals must be carried out in such a way as to drive the reaction to completion. One approach is to use a dehydrating reagent or azeotropic distillation so that the water that is released is irreversibly removed from the system (Carey and Sundberg, 1993).

There are three compounds represent acetal or ketal type in this research, the 2-(1-propylbutyl)-1,3-dioxep-5-ene, the α^4,3-O-(2-propylpentanylidene)pyridoxine, and the α^4,3-O-isopropylidene pyridoxine. The first compound is 2-alkyl-1,3-dioxep-5-ene that was synthesized by acetalization of 2-propylpentanal and *cis*-2-butene-1,4-diol, the other two are cyclic acetal and cyclic ketal derivatives of pyridoxine obtained by acetalization pyridoxine with 2-propylpentanal and acetone, respectively.

Synthesis of 2-alkyl-1,3-dioxep-5-enes.

1,3-dioxep-5-ene is obtained by the reaction of *cis*-2-butene-1,4-diol with formaldehyde in the presence of an acidic catalyst (Brannock and Lappin, 1956). Reactions of other aldehydes with *cis*-2-butene-1,4-diol to give the corresponding 2-alkyl-1,3-dioxep-5-enes was also reports. The catalysts employed are *p*-toluene sulfonic acid (Brannock and Lappin, 1956; Pattison, 1957), and dimethyl formamide-dimethylsulfate adduct (Kantlchner and Gutbrod, 1979).

Synthesis of cyclic ketal of pyridoxine.

Acetonation of pyridoxine can conceivably give rise to two ketal, the six-membered cyclic ketal and the seven-membered cyclic ketal.

The six-membered cyclic ketal of pyridoxine is well known as α^4,3-O-isopropylidenepyridoxine. The compound served as the key intermediate in the synthesis of pyridoxal phosphate and a number of potential antimetabolites of pyridoxine. It has been obtained by reaction of pyridoxine hydrochloride and anhydrous acetone in the presense of a catalyst. The catalyst used in this reaction may be concentrated sulfuric acid (Cohen and Hughes, 1952), zinc chloride (Baddiley and Mathias, 1952), and anhydrous hydrogen chloride (Korytnyk and Wiedeman, 1962) (Figure 23C).

Anyway, it has never been reported in the literature about cyclic acetals of pyridoxine, preprared by the simple acetalization. There is a report about this type of compound as the seven-membered ring cylic acetal of pyridoxine which was synthesized via the Diels-Alder reaction, as described below.

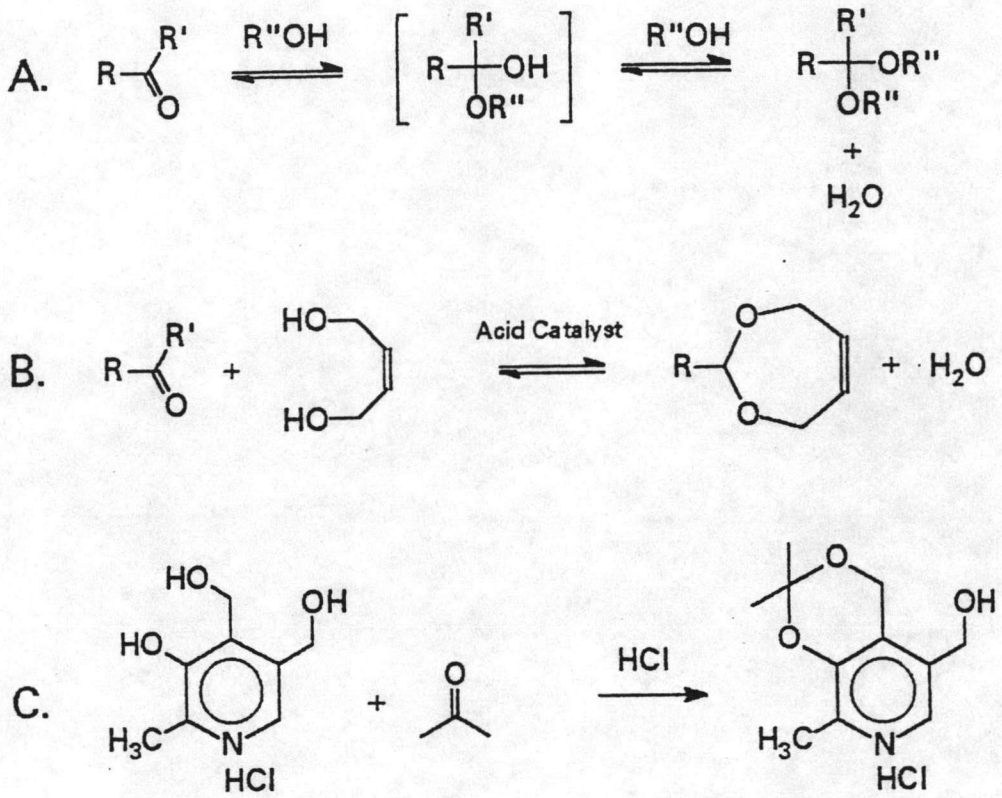

Figure 23. A. Synthesis of acetals of ketals.
B. Synthesis of 2-alkyl-1,3-dioxep-5-enes.
C. Synthesis of six-membered cyclic ketal of pyridoxine.

Synthesis of pyridoxine analogues by condensation of oxazoles with dienophiles.

Fundamental to pyridoxine production technology are processes based on the Diels-Alder reaction of oxazoles. There are two requirements for a technically feasible synthesis.

First is that oxazole has a leaving group substituent at the 5 position to ensure that bridging oxygen atom is retained in the collapse of the bicyclic adduct to the pyridine ring (certainly, the 4 position must be methyl or corresponding groups). Second, the substituents introduced with the dienophile must correspond in oxidation state to the hydroxymethyl group.

On studying, the prefered substituents at the 5-position of the oxazole ring appear to be alkoxy and cyano (compound LXXXIV and LXXXV, respectively). The prefered dienophiles are,

1. *cis*-2-Butene-1,4-diol (LXXXVI) (Pfister et al., 1966; Firestone et al., 1967) and derivatives such as,
   1.1. 1,4-Diacetoxy-2-butene (LXXXVII) (Pfister et al., 1966; Morita et al., 1973).
   1.2. The ether derivatives of *cis*-butene-1,4-diol.
      1.2.1. 1,4-Dimethyl-2-butene (LXXXVIII) (Firestone et al., 1967).
      1.2.2. 2,5-Dihydrofuran (LXXXIX) (Harris et al., 1962; Firestone et al., 1967).
      1.2.3. 2,5-Dimethoxy-2,5-dihydrofuran (LXL) (Harris et al., 1968; Naito et al., 1968).
   1.3. The cyclic ketals and acetals of *cis*-2-butene-1,4-diol.
      1.3.1. 4,7-Dihydro-2,2-dimethyl-1,3-dioxepine (LXLI) (F. Hoffman-La Roche & Co., A., 1964).
      1.3.2. 4,7-Dihydro-1,3-dioxepine (LXLII) (F. Hoffman-La Roche & Co., A., 1965, Kondrat'va, 1968).
      1.3.3. 2-Isopropyl-4,7-dihydro-1,3-dioxepine (LXLIII) (F. Hoffman-La Roche & Co., A., 1965).
2. Derivatives of maleic acid, that are
   2.1. Maleic anhydride (LXLIV) (Kondrat'va and Huang., 1961; Pfister et al., 1966; Firestone et al., 1967).
   2.2. Dimethyl maleate (LXLV) (Balyakince et al., 1968;

Doktorova et al., 1969).

2.3. Diethyl maleate (LXLVI) (Harris et al., 1962; Pfister et al., 1966; Firestone et al., 1967).

3. Fumaronitrile (LXLVII) (Harris et el, 1962; Pfister et al., 1966).

The primary Diels-Alder products from these reactions (Figure 24 and 25) were 2-methyl-3-hydroxy-4,5-disubstituted-pyridines which could be converted to pyridoxine *via* a different methods. The solvent used for the Diels-Alder reaction must be high boiling because of the low reactivity of the 1,4-butenediol-based dienophiles. An excess of the dienophile is used as solvent in many procedures. In most of the published examples, the temperatures used for the Diels-Alder reaction are 110-180 °c. Reaction time of more than 20 hours are used.

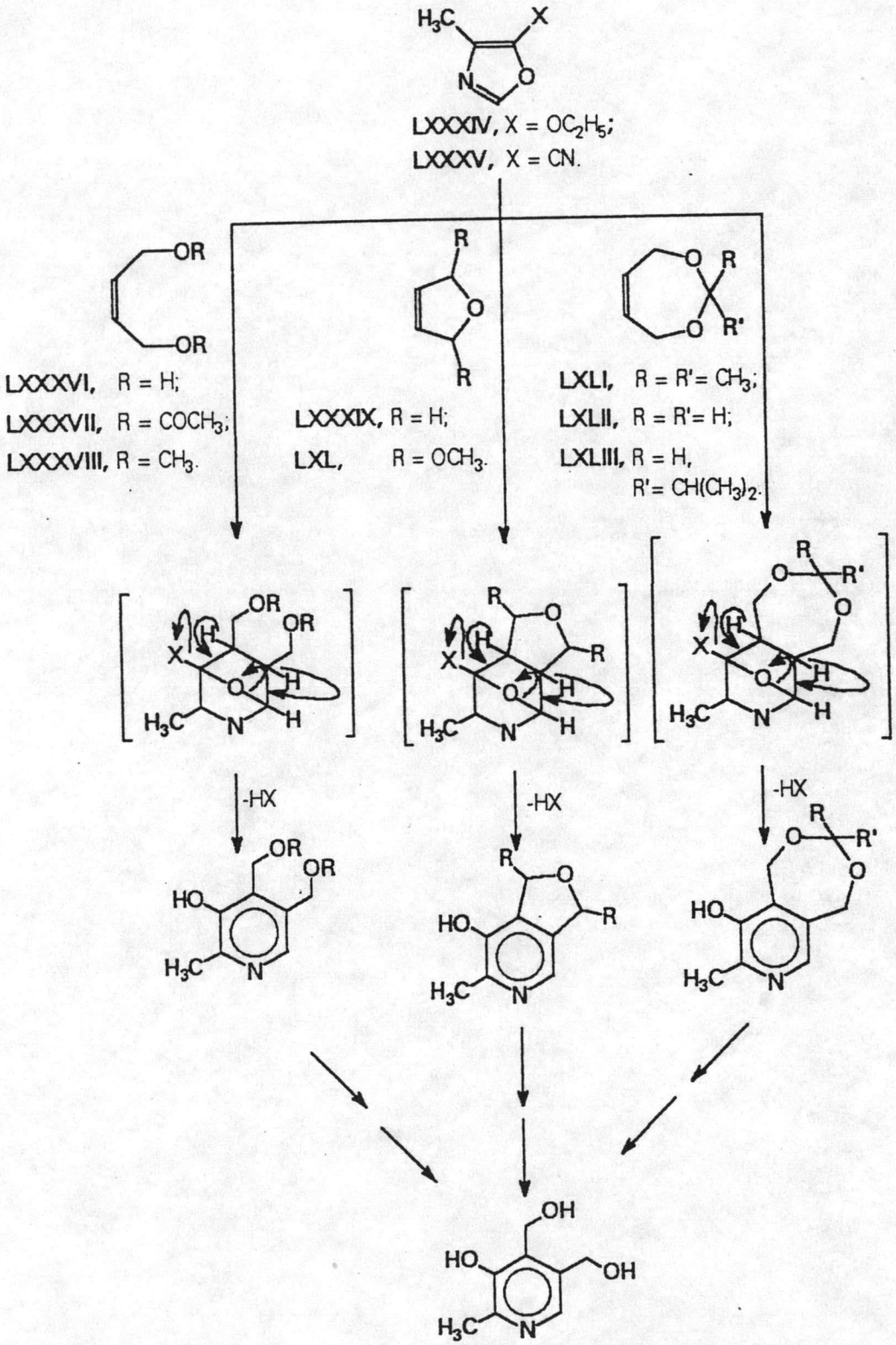

Figure 24. Syntheses of pyridoxine analogues by Diels-Alder reaction of oxazoles with *cis*-2-butene-1,4-diol and derivatives.

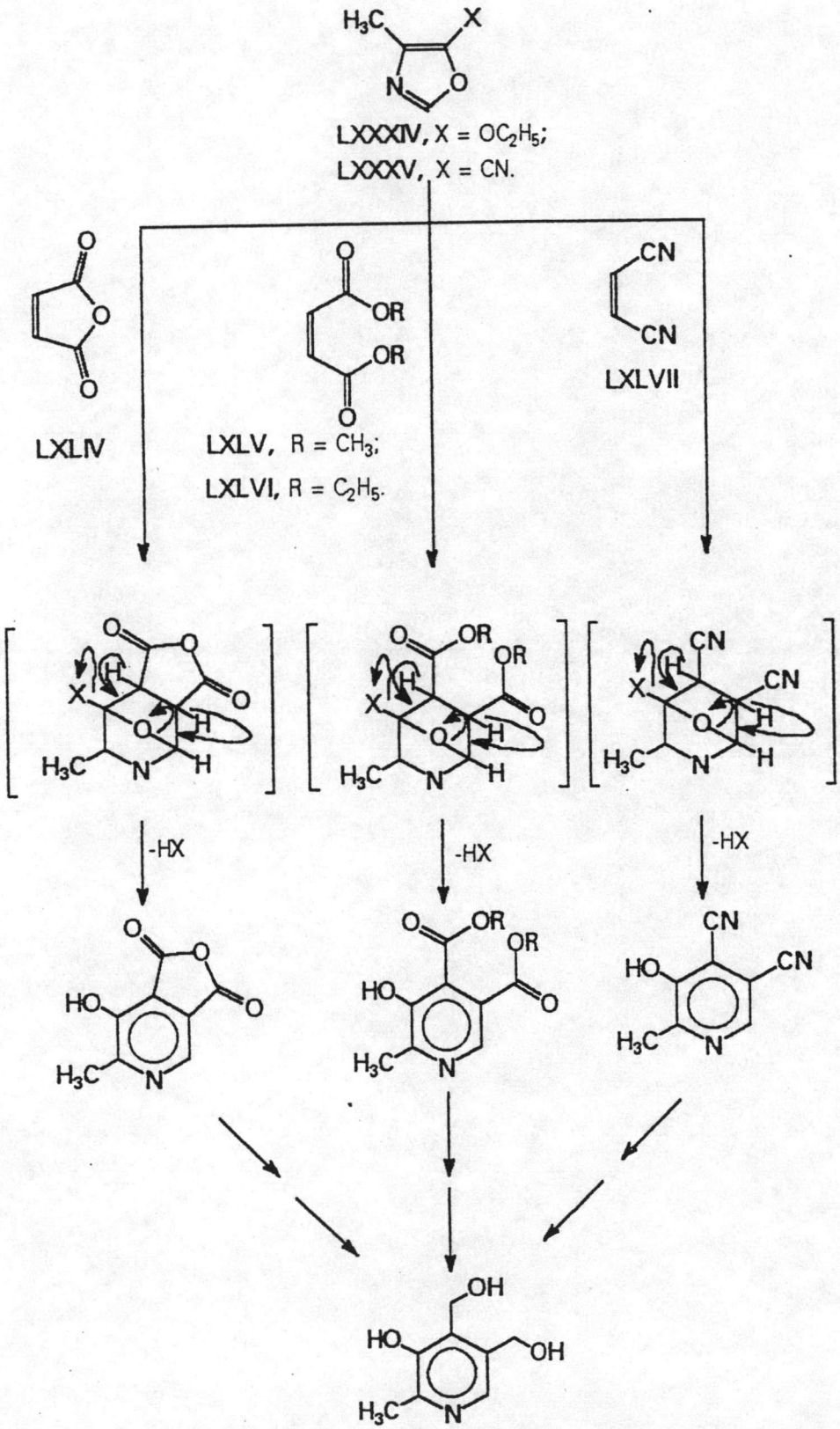

Figure 25. Syntheses of pyridoxine analogues by Diels-Alder reaction of oxazoles with maleic acid and fumaronitrile.