CHAPTER II

LITERATURE REVIEW

A : FREE RADICALS

A free radical is defined as any species (molecule, fragment complex, or atom) that has one or more unpaired eletrons. The unpaired electron causes the species highly reactive (25-29).

In biology, oxygen free radical such as superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($OH^{\cdot}$) are dangerous species.

Superoxide radical is formed in almost all aerobic cells, one important source being the "respiratory burt" of phagocytic cells when they contact foreign particles or immune complexes. Phagocytic cells known to produce superoxide radical include neutrophils, monocytes, macrophages and eosinophiles (1, 25, 30).

Addition of an electron to superoxide radical gives the peroxide ion (O_2^{2-}), which has no unpaired electrons and is not a radical. Peroxide ion formed at physiological pH will immediately protonate to give hydrogen peroxide (H_2O_2) (1, 25, 30). Homolytic fission of the oxygen-oxygen (O-O) bond in hydrogen peroxide produces two

hydroxyl radicals ($OH^{\cdot}$). This homolysis can be achieved by heat or ionizing radiation. In addition, a mixture of hydrogen peroxide and an iron (II) can form the hydroxyl radical via "Fenton reaction".

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \qquad \text{(Fenton reaction)}$$

An iron (III) occuring in "Fenton reaction" can react with superoxide radical to form an iron (II) that in turn reacts with hydrogen peroxide in "Fenton reaction". As a result, more hydroxyl radicals are produced (1, 25, 30).

$$Fe^{3+} + O_2^{-} \longrightarrow Fe^{2+} + O_2$$

In general, superoxide radical, hydrogen peroxide and hydroxyl radical are often called as "Reactive oxygen species" (ROS). Among reactive oxygen, hydroxyl radical is the most reactive specie (1, 25, 30).

B : LIPID PEROXIDATION AND PATHOLOGY

Lipid peroxidation is a free radical-mediated process. The peroxidation of polyunsaturated fatty acids is implicated in several

pathologic conditions including atherosclerosis, myocardial infraction, inflammation, aging, and cancer (13, 30-33).

As shown in figure 1, the first step of peroxidation process is an initiation reaction. The initiator such as hydroxyl radical abstracts hydrogen from α-methylene group of the lipid chain leading to formation of a lipid radical. The lipid radical is stabilized by a molecular rearrangement to form a conjugated diene. This then reacts rapidly with oxygen to form the peroxyl radical, which in turn attacks another α-methylene group yielding a lipid hydroperoxide and a new lipid radical which propagated the chain reaction. The hydroperoxide, which is the first product of peroxidation, is rather unstable and easily decompose into other product (25, 30, 31).

Peroxidation of low-density lipoprotein (LDL) plays a significant role in the development of atherosclerosis. Oxidized low-density lipoprotein can enter the macrophage through a scavenger receptor pathway, thereby producing lipid-rich foam cells. In addition, circulating monocyte are attracted to endothelial and smooth mucle cells by chemotatic protein 1, a chemoattractant that is augmented by the oxidatively modified lipoproteins. With continued uptake of the oxidatively modified lipoproteins by the macrophage scavenger

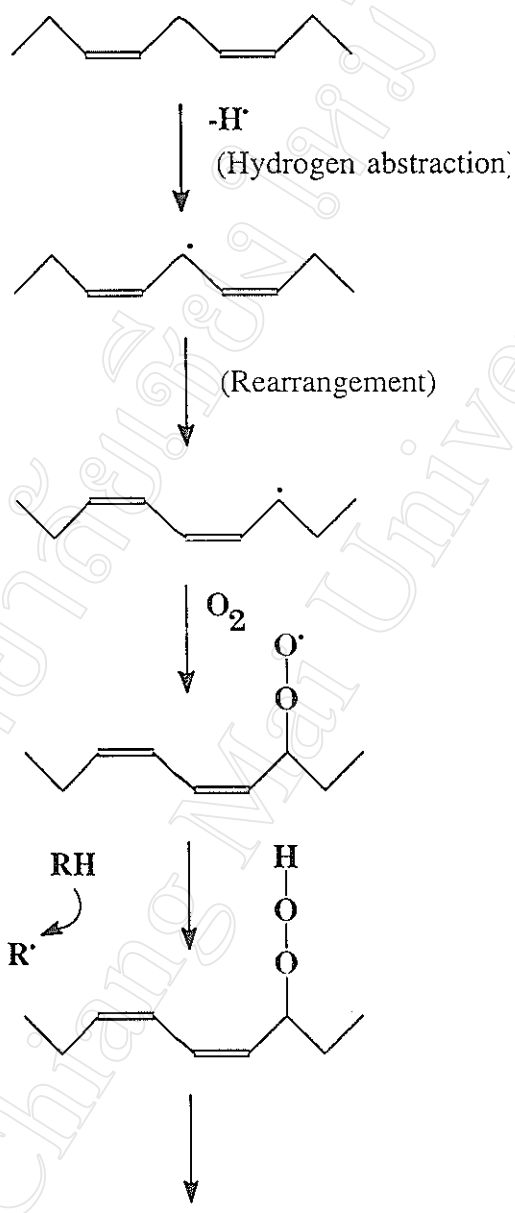
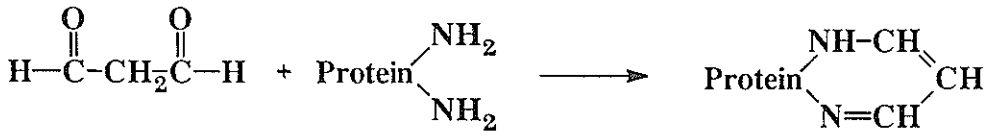


Figure 1 : Schemetic representation of the initiation and propagation reaction of lipid peroxidation

receptor, foam cells form and progress to the next phase of atherosclerosis, development of the fatty streak. Simultaneously, smooth muscle cells migrate into the subendothelial space and begin proliferating within the intima. During the next phase of atherogenesis, lesions continue to grow by increases in both smooth muscle cell proliferation and collagen synthesis. Then, necrosis of the foam cell and formation of an extracellular lipid core occur and progress as long as plasma low-density lipoprotein is elevated. Thus, atherosclerosis leads to the blockage of an essential artery. Moreover, severe restriction of blood flow leads to myocardial infraction. Finally, An autoimmune inflammatory is occurred (34).

Moreover, malodialdehyde which reacts with DNA, is the major mutagenic and carcinogenic products generated by lipid peroxidation. It can attack amino groups on protein molecule to form both intramolecular cross links and intermolecular cross link (1, 30, 31, 35).

Intramolecular cross link



Intermolecular cross link

C : ENDOGENOUS ANTIOXIDANT DEFENCE MECHANISMS

There are defence mechanisms presenting in cells and extracellular fluid for controlled over production of free radicals and reactive oxygen species. The important defence mechanisms are enzymatic and non-enzymatic mechanism.

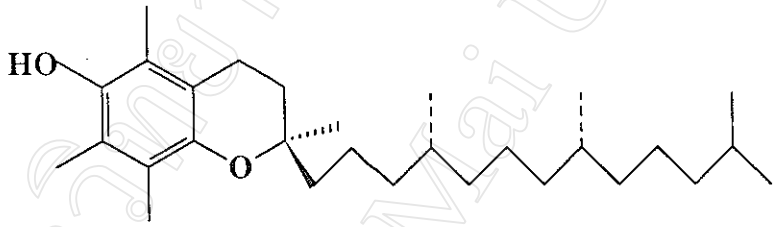
1. Enzymatic Mechanisms

The enzymes, superoxide dismutase, catalase and glutathione peroxidase are the antioxidants within the cells. Superoxide dismutase acts as a scavenger of superoxide anions by catalysing their dismutation reaction into hydrogen peroxide and molecular oxygen. Catalase and glutathione peroxidase reduce hydrogen peroxide as well as alkyl hydroperoxide into water and alcohols, respectively (36).

2. Non-Enzymatic Mechanisms

2.1 Hydrophobic protection mechanism

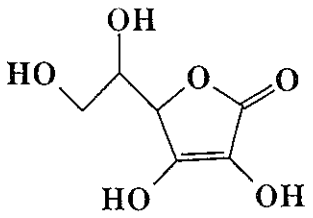
Hydrophobic protection mechanism controls free radicals in the hydrophobic regions within the cell. Vitamin E, mainly (R,R,R)-α-tocopherol, is the major lipid-soluble chain-breaking antioxidant in this mechanism(1).



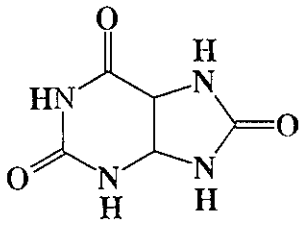
R,R,R-α-tocopherol

2.2 Hydrophilic protective mechanism

Hydrophilic mechanism controls free radicals in the hydrophilic regions within the cell. Ascorbic acid and uric acid are the major antioxidants in this mechanism (1).



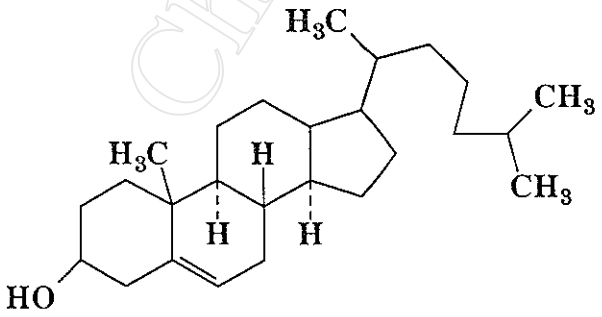
Ascorbic acid



Uric acid

2.3 Structural mechanism

The protective mechanisms are associated with the structural integreity of living cells. Disruption of this structural integrity leads very quickly to the peroxidative free radical induced reaction within polyunsaturated lipids. Cholesterol, by its structural and size, protect living cell from peroxidative injury (37).



Cholesterol

D : STRATEGIES FOR ANTIOXIDANT THERAPY

When endogenous antioxidative defence machanisms can not work or imbalance, the excess free radicals cause pathophysiological process. Thus, antioxidant therapy is useful for diseases relating to lipid peroxidation (1).

The most commonly strategies for antioxidant therapy must include the following:

1. Enhancement of tissue endogenous antioxidant levels through administration of antioxidant vitamins or glutathione precursors.

2. Prevention of initiation events by scavenging (commonly discussed for hydroxyl radical scavengers).

3. Chelation of metal ion by introducing multidentate complexing agents.

4. Decomposition of peroxide and superoxide into non-radical products (using antioxidant enzyme or synthetic superoxide dismutase or glutathione peroxidase mimics).

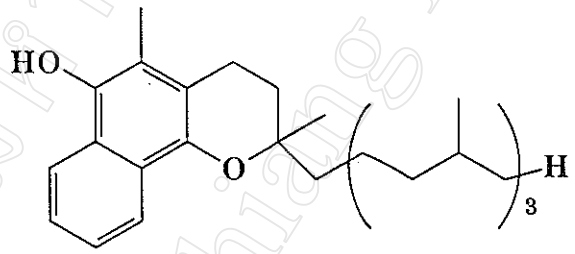
5. Termination of free radical peroxidation (through administration of generally lipophilic chain-breaking antioxidants).

A large number of compounds included semisynthesis analogues of endogenous antioxidant and natural products are widely developed for antioxidant therapy.

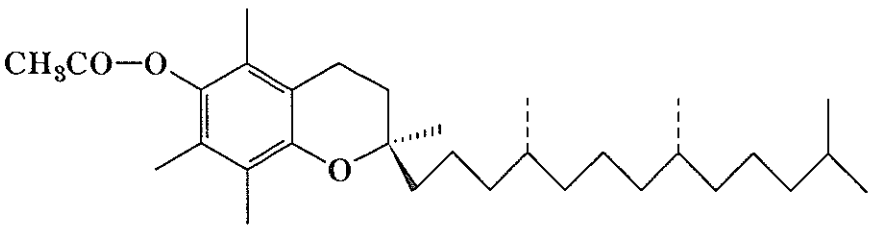
1. Semisynthesis analogues of endogenous antioxidant

1.1 Tocopherol analogues

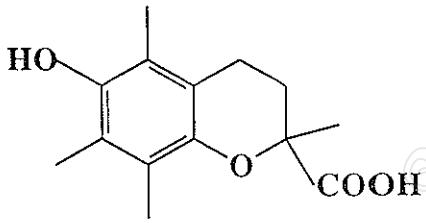
To improve antioxidant effects, lipophilic and hydrophilic tocopherol analogues are developed (1, 38). Examples are vitamin K_1-chromanol, vitamin E acetate and trolox.



Vitamin K_1 - chromanol



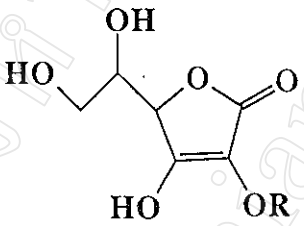
Vitamin E acetate



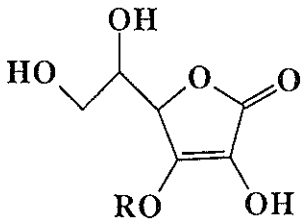
Trolox

1.2 Ascorbic acid analogues

Ascorbic acid was rapidly oxidized with loss of activity in time, and very limited transcellular potency owing to its being hydrophilic. In order to solve these problems, ascorbic acid analogues were synthesized (39-43). Examples are 2-O-Alkylascorbic acids and 3-O-Alkylascorbic acids.



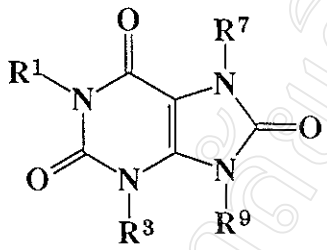
2-O-Alkylascorbic acids



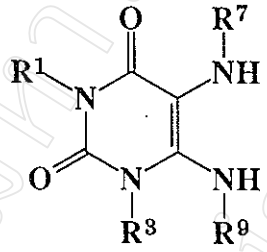
3-O-Alkylascorbic acids

1.3 Uric acid analogues

A new series of N-alkylated uric acids (2,6,8-purinetrione and 5,6-diaminouracils (5,6-diamino-2,4-pyrimidinedione) were synthesized, and their activities against free radicals were evaluated (44).



N-Alkylated uric acids

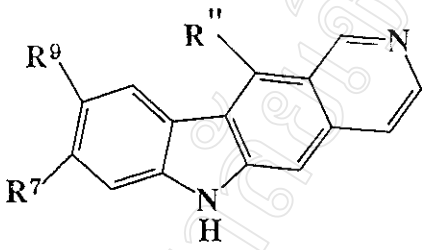


N-Alkylated diaminouracils

2. Natural products

2.1 Alkaloids

Some alkaloids have antioxidant activity. Structure-activity relationship in a series of ellipticine analogues, were investigated with respect to antioxidant activity (1).



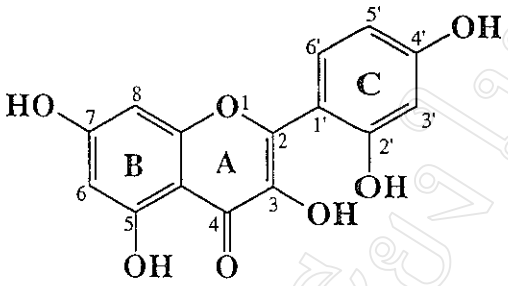
Ellipticine analogues

2.2 Flavonoids

Several flavonoids have been reported to inhibit either enzymatic or non-enzymatic lipid peroxidation (13, 45-49).

In addition, the studies have shown that morin has the important pharmacological activities such as anti-inflammatory, anti-atherosclerosis and anticarcinogenic activity (13, 49).

Structure-activity relationships of morin as antioxidant are demonstrated below (2, 17-20, 50, 51).



1. Two hydroxy group on C-ring and one hydroxy group at 3-position increase antioxidative activity.

2. C_2-C_3 unsaturated bond (C=C) increases antioxidative activity.

Moreover, the antioxidative mechanisms of morin are considered. First, morin acts as free radical scavenger. Second, morin can chelate some metal ions such as iron(II) ion in Fenton reaction (18).

E : MORIN AND ITS DERIVATIVES

MORIN :$C_{15}H_{10}O_7$

Chemical name

2-(2,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one or $2'$,3,$4'$,5,7,-pentahydroxyflavone (14)

Molecular weight

302.24

Description

Yellow crystal, crystallizes with 1 or 2 moles water, anhydrous needles from absolute alcohol

Melting point

285-290 °C

Solubility

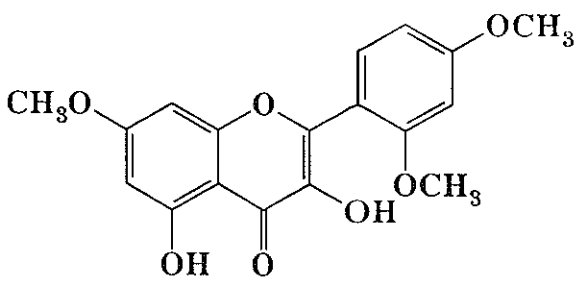
One gram dissolves in 4 liters water at 20 °C, in 1060mililiters boiling water, freely soluble in alcohol, soluble in aqueous alkaline with intense yellow color which turns brown on exposure to air.

Storage : Under argon atmosphere (52)

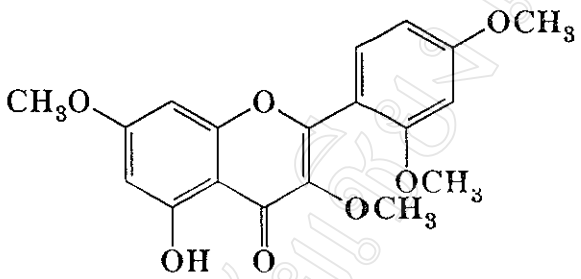
Some derivatives of morin were synthesized (53-57).

(1) Morin methyl ether

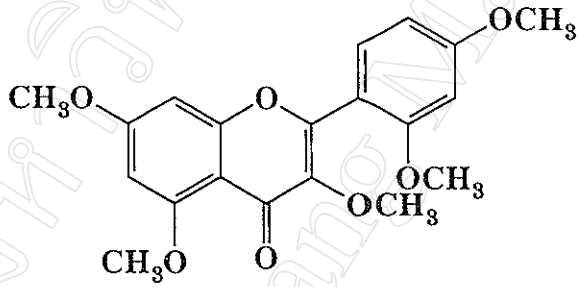
1.1 Morin-7,2′,4′-trimethyl ether



1.2 Morin-3,7,2′,4′-tetramethyl ether

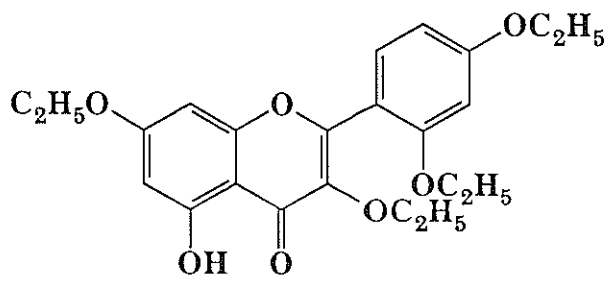


1.3 Morin-3,5,7,2′,4′-pentamethyl ether



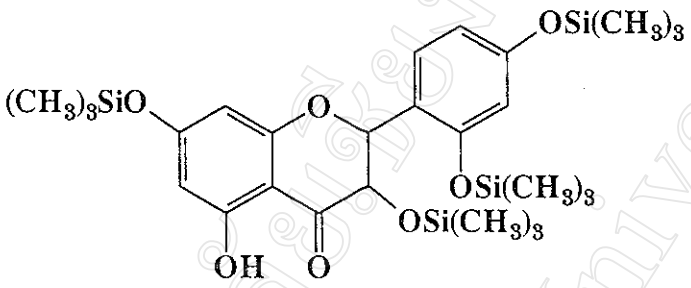
(2) Morin ethyl ether

2.1 Morin-3,7,2′,4′-tetraethyl ether

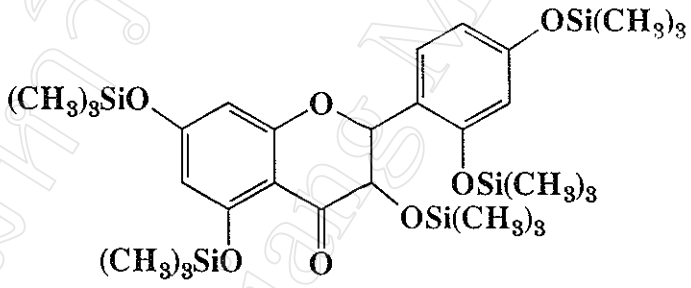


(3) Morin trimethylsilyl ether

3.1 Morin-3,7,2′,4′-tetratrimethylsilyl ether

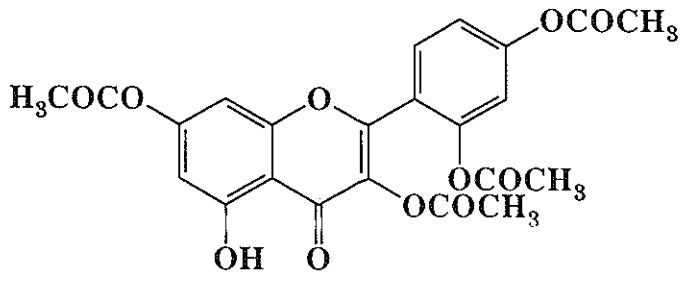


3.2 Morin-3,5,7,2′,4′-pentatrimethylsilyl ether

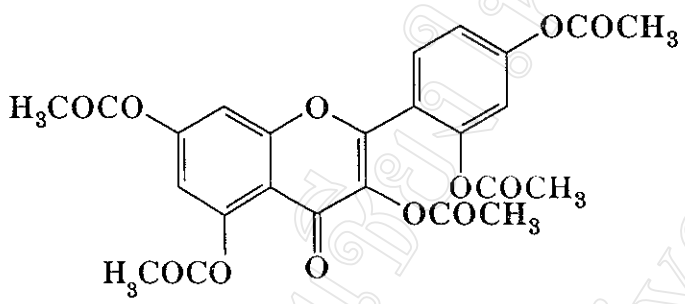


(4) Morin acetate

4.1 Morin-3,7,2′,4′-tetraacetate



4.2 Morin-3,5,7,2′,4′-pentaacetate



(According to literature review in 1929-1996, only two reports mention to Morin-3,5,7,2′,4′-pentaacetate.)

F : PHOTOREACTION OF MORIN

Morin can undergo photoreaction to give degradation products as [7] and [8]. The 3-hydroxyl group of morin is essential for the reaction to occur. The mechanism shown in Figure 2, involves addition of photoexcited singlet oxygen to the ene-ol system of morin to form the hydroperoxide [2]. Then, hydroperoxide forms two cyclic peroxide, one with a 5-membered ring [3] and another with a 4-membered ring [4]. Decarbonylation of [3] leads to [5]. And ring opening of [4] leads to [6] that prone to photo oxidative decarboxylation to [5]. Finally, hydrolysis of [5] leads to [7] and [8] (58).

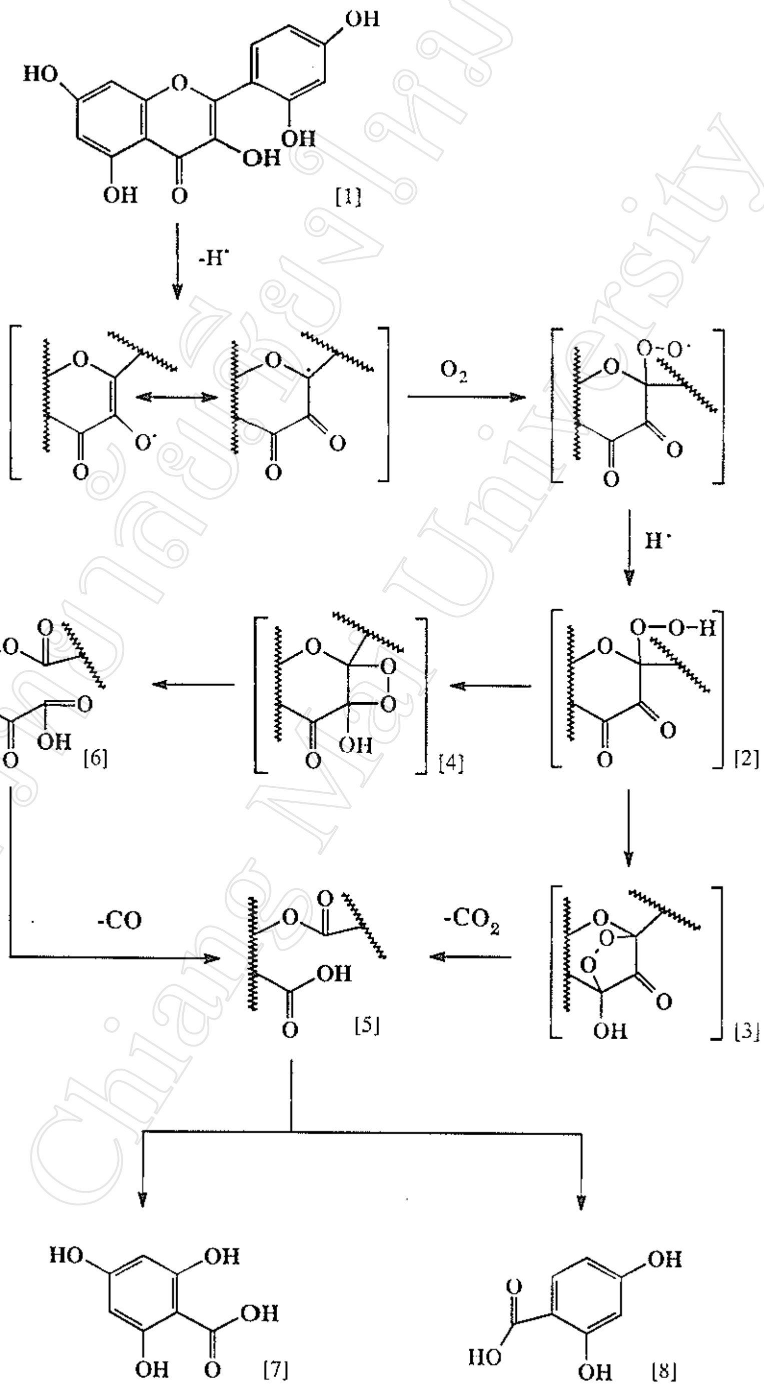


Figure 2 : Photoreaction of morin

G : PRODRUG

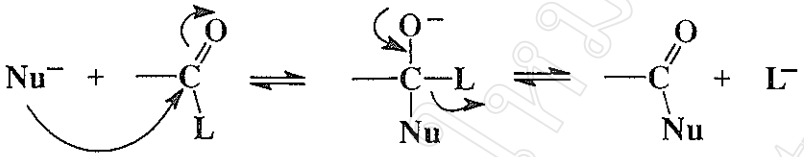
Prodrug is a structural inactive derivative of a parent drug molecule that requires spontaneous chemical or enzymatic transformation within the body in order to release the active drug. It is used to solve the problems such as instability and incomplete absorption of parent drug molecule across biological membrane which caused the limitation of the clinical usefulness of the parent drug.

A prodrug as an ester form is normally used, because of the simplicity of synthesis and facile enzymatic hydrolysis in the body (23, 59).

H : SYNTHESIS OF ESTER

MECHANISM

Ester can be synthesized via the nucleophilic substitution on the carbonyl group of carboxylic acid and its derivatives. The mechanism can classify in two steps, (i) nucleophilic addition on the carbonyl carbon atom, and (ii) elimination of leaving group (60-64).



Nu^- = Nucleophile

L^- = Leaving group

Among the carboxylic acid derivatives, acid chloride is the most reactive toward nucleophilic substitution. The reactivity of them is showed below.

High reactivity

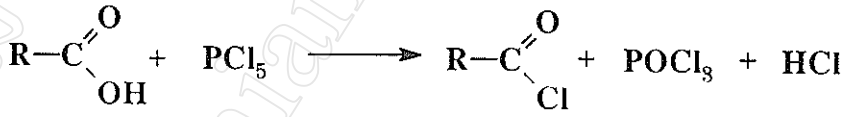
R-CO-Cl	Acid chloride
R-CO-SR'	Thiol ester
R-CO-OCOR'	Acid anhydride
R-CO-OR'	Ester
R-CO-NH_2	Amide

Low reactivity

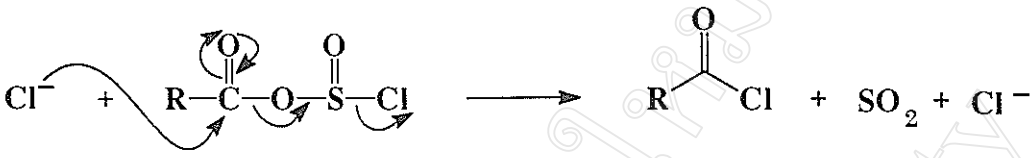
PREPARATION OF ACID CHLORIDE

Acid chloride is prepared from the reaction between carboxylic acid and the reagents such as thionyl chloride ($SOCl_2$), phosphorous trichloride (PCl_3) and phosphorous pentachloride (PCl_5).

General Reaction



Thyonyl chloride reacts with a carboxylic acid in the following way:



METHODS FOR THE SYNTHESIS OF ESTERS

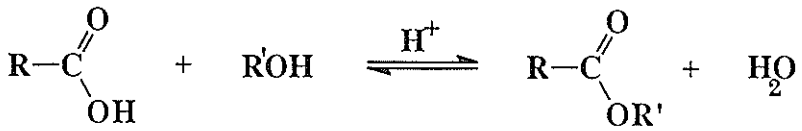
There are three methods to synthesize esters, (i) reversible processes, (ii) irreversible processes and (iii) transesterification.

1. Reversible process

1.1 The use of carboxylic acid (Fischer esterification)

In Fischer esterification, a carboxylic acid reacts with an alcohol in the presence of an acid-catalyst to form an ester.

General reaction



This process is reversible by acid-catalyzed hydrolysis, as a result, percentage yield is low. In order to increase percentage yield , an

excess of the alcohol is used, and if possible removing the water as ester is formed.

1.2 The use of amide

An amide may be converted to an ester by reaction with an alcohol. The reaction is the reversible process and produces an ammonia as by product. In order to produce high yield of ester, it is necessary to remove the ammonia, either by heating or by combining with a mineral acid (e.g., sulfuric acid or hydrogen chloride).

General Reaction

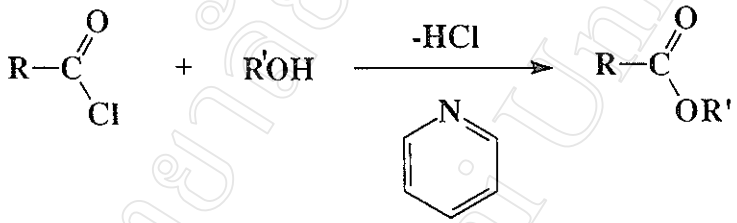
2. Irreversible process

2.1 The use of acid chloride

An ester can be synthesized by the reaction of an acid chloride with an alcohol. Since an acid chloride is much more reactive toward nucleophilic substitution than a carboxylic acid, the reaction of an acid

chloride with an alcohol occurs rapidly and does not require an acid-catalyst. Pyridine is usually added to the reaction mixture to react with hydrochloric acid which is generated in the ester-forming reaction.

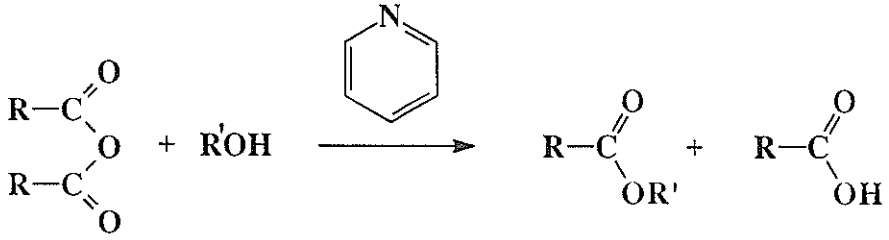
General reaction



2.2 The use of acid anhydride

An acid anhydride also reacts with an alcohol to form an ester in the absence of an acid-catalyst. Pyridine is usually employed as catalyst.

General reaction



The reaction of an alcohol with an acid chloride or an acid anhydride that is irreversible process, is often the best method for ester synthesis.

3. Transesterification (Ester interchange)

An ester can also be synthesized by transesterification. This reaction occurs when an ester is heated with an alcohol in the presence of an acid. This reaction is useful for converting one ester to another. p-Toluene sulphonic acid is usually employed as acid-catalyst.

General reaction