CHAPTER - I
INTRODUCTION
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Allyl compounds are subject of interest as these occur in nature and are important as chemical intermediates. Allyl compounds derive their prefix “allyl” from the latin word allium, garlic, as these are constituents of garlic\(^1\).

Organic chemists find sulfur-sulfur bond as intriguing functional group because it is easily isolable but very labile chemically. It is, therefore, rather unique in its character\(^2\). Thiols and disulfides occur frequently in biological systems; their relation to possible intermediates of sulphenyl character is of special interest. Sulphenyl compounds have synthetic utility for biochemical and pharmaceutical industries.

\[
\text{Allicin } \left( \text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2 \right), \text{ thiosulfinate ester, is a sulphenyl derivative formed by enzymatic cleavage of alliin}
\]

\[
\text{[CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2\text{]} \text{, a sulfoxide, found in garlic}^3.
\]

Sulfoxide represents a family of sulfur compounds which has been investigated less intensely than related sulfides and sulfones. For a time, considerable attention was focussed on sulfoxide in connection with study of optical activity, geometrical isomerism and more recently because of isolation of naturally optically active sulfoxide. Alliin, \((+)\)S-allyl-L-cysteine sulfoxide, a flavour precursor of garlic, is one such compound of interest\(^3\).
Hall described the power of garlic which is much in air these days as follows:

“Take the 100-plus-year-old Delany sisters; in their best selling memoir, having our say, they attribute their extraordinary vigour, in part, to the daily consumption of a clove of raw garlic. Then there was the first world congress on the health significance of garlic and garlic constituents, which in 1990 attracted some 150 researchers, scientists and government officials to Washington, not to mention several recent studies sponsored by the US National Cancer Institute. Indeed, the purported health effects of *Allium sativum* have been a matter of record since 1500 BC, when Egyptian sages dutifully noted a papyrus medicinal applications for the smelly bulb. More recently, in 1858, Louis-Pasteur realized that garlic could kill bacteria. Then in 1983, biochemist ‘Sidney-Belman’ of the New York University Medical Centre made a dramatic discovery; painting the skin of laboratory mice with garlic oil could inhibit the development of tumours.”

Hall (1995) further cited evidence provided by recent Italian and Chinese epidemiological studies that garlic plays a role in protecting humans against cancer. The Italian study indicated that those Italians who included garlic in meals almost everyday or ate garlic once a week on an average suffered the lowest incidence of stomach cancer. The Chinese study showed that those who consumed the five members of allium family, viz., garlic, garlic stocks, scallions, Chinese chives and onions had only 40 percent the stomach cancer risk of those who rarely ate them. The person who designed both these studies, William Blot, a
former Chief Biostatistician of the US National Cancer Institute contended that “It is beginning to look like garlic really is protecting against cancer.” The results of current studies mentioned by Hall in his article show that consumption of a clove or two of garlic everyday lowers the risk of stomach, breast, prostate, colon, rectum, oesophagus and skin cancers perhaps by inhibiting bacteria in digestive tract, which convert constituents of food into nitrosamines, owing to one or more of its potent organosulfur compounds. The use of fresh garlic is reported to promote inhibition of nitrite reducing bacteria. The other way by which garlic reduces the risk of cancer is by preventing formation of free radicals which have tendency to damage DNA, cell membranes and cellular proteins. An appropriate standard medicinal dose of garlic is around three cloves a day, according to Stephen Fulder, the author of book "Garlic : Nature's Original Remedy".

Garlic is valued for its sulfur containing compounds. It has been widely used as flavouring agent. Several workers investigated its pharmacological use.

A number of ingredients in garlic have been observed to reduce the stickiness of platelets, blood elements, that clump together to form clots which lead to heart attack. Garlic cuts down on the thickening and hardening of arteries known atherosclerosis by preventing platelets from adhering to the lining of blood vessels. A minor component of garlic oil which inhibits platelet aggregation was isolated, identified and finally synthesized. MS and NMR showed the component to be methyl allyl trisulfide. Later, Morimitsu et al. found that mixing
onion with garlic results in marked increase in platelet aggregation inhibitory activity. The antithrombic compounds isolated were α-sulfinyl disulfide and thiosulfinates. A therapeutic active garlic component ajoene [CH₂CHCH₂SSCH₂CH₂S(O)CH₂CH₂] prepared by decomposition of allicin,¹⁵ is a potent antiplatelet agent. It blocks the exposure of fibrinogen receptors by modulating the function of G-proteins¹⁶.

There is increasing evidence that garlic lowers cholesterol level. A recent study at New York Medical College showed that people with elevated cholesterol levels who took the equivalent in extract form of up to a clove of garlic a day showed their levels dropped by about 9 percent, which was significant as every percent reduction in cholesterol translate into a 2 percent reduction of cardiac risk. Bhushan et al.¹⁷ reported cholesterol lowering effect of garlic even in individuals with normal cholesterol level. Di-, tri-, polyallyl sulfides formed as decomposition products of allicin also have medicinal importance. Injections or oral compositions containing diallyl trisulfides are used for treatment of heart, brain and blood vessel diseases¹⁸.

Ayurvedic and Unani systems consider garlic as carminative and gastric stimulant that aids in digestion and absorption of food. As a result, garlic finds importance in culinary and medicinal applications.

Garlic has been used as an antiseptic during world wars I and II, when infantry men carried cloves of garlic to rub on wounds suffered in the field.
Garlic oil inhibits many strains of fungi including those which cause vaginitis and cryptococcal meningitis that invades nervous system. The allicin was patented by Winthrop's-Stearn Inc. in 1951 in USA as a potent antibiotic. Inhibitory effect of allicin on acetyl Co-enzyme A has also been reported. Several workers have reported antibacterial, antimicrobial, antifungal compounds in garlic which also act against gram +ve and gram-ve bacteria including food poisoning bacteria. Diallyl trisulfide and other polysulfides have been demonstrated to possess potent in vitro fungicidal property. Further, their activity is synergistic with amphotericin-B. Garlic oil has also been shown to possess larvicidal action against mosquitoes. Soon and When demonstrated detoxification effect of garlic in phenyl mercury poisoning of rats. Alliin has strong antioxidant activity. It also inhibits diabetes due to peroxide effect as found in alloxan rats. Allicin has also been demonstrated to lower the ocular pressure and modify sulfhydryl enzymes.

Garlic has dilatory qualities because of which it is an excellent asthma preventive and ideal treatment of relating breathing problems. It also prevents calcification of bone which causes osteoporosis and selectively kills bacteria that causes diarrhoea, preventing loss of minerals and vitamins during stomach upset. The garlic, thus, finds its importance in serving not only as a condiment but also as a popular remedy for various ailments and physiological disorders.

The chemical constituents of garlic are reported in several reviews which include sugars, pectic substances, vitamins, proteins, lipids, pigments, aroma and flavour compounds, and pharmacologically
active principles. The carbohydrates in garlic are glucose, fructose, lactose, sucrose, raffinose, rhamnose, fructosans, starch, pectin and mucilage. The garlic skin contains high level of pectins that account for 27-28% of its weight. The garlic lipids are comprised of neutral lipids (62.6%), glycolipids (14%) and phospholipids (23.4%); the major saturated and unsaturated fatty acids being palmitic and linoleic acid respectively. The predominant pigments are carotenoids, viz., α & β-carotenes, xanthophyll, anthocyanin and chlorophyll. Garlic is rich in vitamin A, vitamin C, niacin, riboflavin, thiamine, nicotinic acid and biotin. Garlic is reported to contain several trace elements, viz., Zn, Cu, Cr, Cd, Co, Ni, Mg, V, Mo, Ti, Ce, Li & Se. The proteins contain all essential amino acids and account for roughly one-half of its nitrogen content. The remaining half non-protein nitrogen is in the form of free α- and γ-amino acids, amides and peptides including glutamyl-S-allyl-L-cysteine. The basic, aromatic and sulfur containing amino acids predominate in garlic. Alliin, the active principle of garlic, has been regarded as a sulfur amino acid.

Organic sulfur containing compounds naturally present or formed by enzymatic reactions are the major volatile compounds responsible for aroma of alliums\textsuperscript{35}. The kind and amount of these compounds present or formed depend on species, variety, maturity, and environmental, storage and processing conditions.

Allicin is the major constituent responsible for fresh garlic flavour. The amount of allicin in garlic has been quantitated by estimating volatile sulphur compounds or pyruvic acid\textsuperscript{36,37} using gravimetric,
spectrophotometric and chromatographic techniques. Di- and tri-
Sulfides contribute to the odour of cooked garlic. Flavour and odour
precursors in garlic are (+)S-allyl-L-cysteine sulfoxide, (+) S-
methyl-L-cysteine sulfoxide, and (+)S-propyl-L-cysteine sulfoxide.

The food chemists have concentrated attention on constituents
of steam distillate of garlic for flavouring the processed food. The
steam distillates contain garlic components formed by fascinating
enzymatic transformations, spontaneous chemical rearrangements and
thermal degradation of sulfoxide precursors. The mechanisms of these
chemical transformations are summarized in Schemes 1-4.

Scheme 1 explains the selective enzymatic hydrolysis of odourless
alliin (I), catalyzed by enzyme alliin-lyase (alliinase), to yield allicin (II),
which is responsible for the characteristic odour of crushed garlic cloves.
Since S-propyl and S-methyl cysteine sulfoxides are also found in garlic,
other minor symmetrical and asymmetrical analogues of allicin are
formed by enzymatic reaction. The reaction is specific for derivatives of
L-cysteine and rate of reaction is considerably faster with (+) form of
the sulfoxide. β-elimination from allicin forms two highly reactive
intermediates, 2-propene sulfenic acid (III) and thioacrolein (IV). Allicin
may also hydrolyse to form 2-propene thiol (V) and 2-propene sulfenic
acid (VI). Reduction of allicin with 2-propene thiol forms diallyl disulfide
and 2-propene sulfenic acid. Desulfonation of 2-propene sulfenic acid
gives propylene.
Scheme 1: Enzymatic and spontaneous chemical transformation of allin to highly reactive intermediate.
Scheme 2: Possible modes of rearrangements of thioacrolein on heating and steam distillation.
Where $R \Rightarrow \text{CH}_2 = \text{CH} - \text{CH}_2$.

Scheme 3: Reactions leading to the formation of ajoene from allicin.
Where $R \Rightarrow CH_2 = CH - CH_2$.

Scheme 4: Reactions of diallyl sulfide on heating
Various rearrangement products of thioacrolein are represented in Scheme 2. The flavour precursors of steam-distilled garlic oil arise from thermal decomposition of allicin to broad range of symmetrical and asymmetrical sulfides formed from thioacrolein (IV) as intermediate\textsuperscript{46-49}.

The mechanism for the chemical transformation that leads to formation of ajoene from allicin is summarized in Scheme 3. This is due to the ability of allicin to form two highly reactive intermediates for rearrangement, disproportionation and reactivity towards different functional groups. S-thioallylation of allicin (II) could give sulfonium ion which could undergo β-elimination followed by γ-addition to 2-propene sulfenic acid to give ajoene. Allicin may also undergo hydrolysis in presence of 2-propene sulfenic acid to give thioallyl alcohol and diallyl disulfide.

Scheme 4 illustrates heating of diallyl disulfide which undergoes free radical mechanism. The disulfide can disproportionate to mono- and trisulfides. Tetra-, penta- and hexasulfides have also been detected in the mixtures formed by heating disulfides at 100°C. Such complex reactions could form the basis for substantially what goes on during heating\textsuperscript{50} and steam distillation, giving rise to the characteristic aroma of cooked garlic.

The major flavour precursor of garlic is S-allyl-L-cysteine sulfoxide, an allyl compound with trivial name alliin. Alliin is crystalline, odourless solid. Natural alliin is the sulfoxide of S-allyl-L-cysteine in which both α-C and S atoms are asymmetric\textsuperscript{51}. It decomposes at 164-166°C. It has a specific rotation\textsuperscript{52} $[\alpha]_D^{21}$ of +62.8°. Isolation and
identification of S-allyl-L-cysteine sulfoxide, purified and quantitated by HPLC and colorimetry. has been done by IR, NMR and MS. Alliin can also be extracted through metal co-ordination from unwanted impurities present in crude mixture. Alliin is reported to undergo cyclization during isolation from garlic. It is unstable to heat under cooking conditions.

Alliin is enzymatically cleaved by alliin lyase-a phosphopyridoxal enzyme, commonly known as alliinase (alliin alkyl sulphenate lyase EC [4.4.1.4]). It has been extracted from garlic juice by gel chromatography, purified on electrophoresis in polyacrylamide gel, by affinity chromatography or combination of liquid chromatography, anion exchanger and chromatofocussing medium. Alliinase requires an aliphatic substituent on the sulfur of L-cysteine sulfoxide as well as free and unsubstituted amino group on the substrate for its activity. It also requires pyridoxal phosphate.

Enzymes are biochemical catalysts and play an important role during chemical transformations. Their catalytic activity which results from precise highly ordered tertiary structures, juxtaposes R groups of specific amino acid(s) in such a way as to form stereo-specific substrate binding sites and catalytic centres. The tertiary structure of enzyme is maintained primarily by large number of weak non-covalent bonds. Practically, enzyme molecules are very delicate and fragile structures. If the tertiary structure of enzyme disrupts owing to absorption of excessive energy, it is denatured, i.e., it loses its solubility and catalytic activity.
The mechanism of reaction catalyzed chemically or enzymatically is known to vary depending upon its catalysis. Information on enzyme catalysed reaction can be obtained concerning the mechanism by studying the effect of pH, temperature, and concentration of substrate, inhibitors and activators on velocity of reaction. Alliin undergoes enzymic hydrolysis with alliinase to form ammonia, pyruvic acid and allicin as follows:

$$2RSOCH_2CH(NH_2)COOH + H_2O \rightarrow 2NH_3 + 2CH_3COCOOH + RSSOR$$

(alliin) (ammonia) (pyruvic acid) (allicin)

where, R represents allyl group, viz., $CH_2 = CH-CH_2$.

The allicin resulting from cleavage of alliin by alliinase is major constituent of garlic extract. It is unstable and further undergoes non-enzymatic decomposition to either disulfides and thiosulfonate or disulfide, monosulfide and sulfur-dioxide as follows:

$$RSSR + RSSO_2R$$  
(disulfide thiosulfonate)

$$2RSSOR$$  
(disallylthiosulfinate)

$$RSSR + RSR + SO_2$$  
(disulfide monosulfide)

Disulfide so formed, decomposes and rearranges to trisulfide and monosulfide as follows:

$$2RSSR \rightarrow RSSSR + RSR$$  
(disulfide trisulfide monosulfide)
Finally, a complex mixture of mono-, di-, tri- and polysulfides are formed.

Further studies on stability of alliin and allicin as a function of pH at different temperature and/or in presence of solvents will be useful in understanding chemical nature and activity of alliin and allicin. The rate of conversion of alliin to allicin and its further decomposition under aforesaid conditions will be helpful in elucidating the mechanism and conditions required to stabilize these compounds to the maximum extent possible for retaining their necessary activity.

The main object of the present study is to investigate the stability of alliin and allicin under various conditions. The studies have been divided into two parts as follows:

Part I: The Conversion of Alliin to Allicin

The rate of conversion of alliin to allicin has been studied spectrophotometrically. Pyruvic acid, as one of the product formed during enzymatic cleavage of alliin has been estimated by spectrophotometer at different time intervals and rate constants for the formation of pyruvic acid i.e. decomposition of alliin to allicin has been obtained at various initial reactants concentrations. The effect of pH, solvent, temperature, etc. has been studied on the basis of observed rate constant. The results are reported and discussed in Chapter III.

Part II: Decomposition of Allicin

Volatile oil contains the decomposition products of allicin. Therefore, the rate of decomposition of alliin (at fixed steam distillation time) and allicin (at fixed incubation period) has been studied by
estimating volatile oil at different time intervals by chloramine-T method as described in Chapter II. The effect of various factors, viz., solvent, temperature, pH etc. on decomposition of alliin and allicin has been studied. The results are reported and discussed in Chapter IV.

Finally the optimum conditions for the stability of alliin and allicin have been discussed on the basis of results.
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