CHAPTER - I

CHAPTER - I

INTRODUCTION

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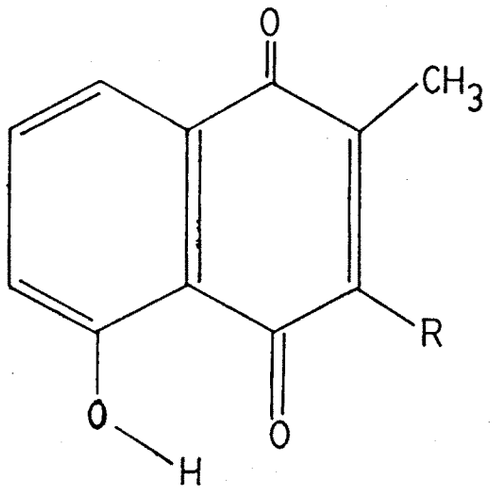
1.1 GENERAL

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) alongwith 3-chloroplumbagin have natural occurrance and are found in the whole plants of Drocera intermedia[1,2] and other Drocera spp. (Droceracease) as well as in the roots of Plumbago zeylanica[3,4] (Plumbaginaceae). These natural sources have found extensive use in the Ayurvedic system of medicine.[5,6]



R = H; Plumbagin

R = Cl; 3-Chloroplumbagin

The recent studies carried out in our laboratory have shown[7-9] that many of the hydroxynaphthoquinones and their derivatives are potent antimicrobial agents. We have selected plumbagin and 3-chloroplumbagin for the present study mainly because of the following characteristics :

(a) They can combine with almost all metal ions and form corresponding six membered chelate rings using typical oxygen-oxygen donating system.

(b) The effect of substituent at C-3 position on the chelating ability of the parent ligand can be studied systematically.

(c) The comparison of the chelating abilities of metal ions of a particular group would become interesting if the corresponding chelates are prepared using these ligands.

(d) There is a pronounced electronic and steric effect of chloro group among the electron-withdrawing groups when substitution in the quinonoid part of the ligand is studied. This seems to be important in the structural studies like hydrogen bonding and chelating abilities of the parent ligand.

(e) Although the medicinal properties of the natural sources which contain these ligands are wellknown, the study regarding the active constituent present in them is not yet reported. The comparative study of the antimicrobial properties of plumbagin and 3-chloroplumbagin would be, therefore, interesting

(f) The association of chloro group with the enhancement of antimicrobial activity of the parent compound is known.[10] It is also observed that chelation with biologically important metal ions can increase the antimicrobial activity of the parent compound.[11,12] We are, therefore, prompted to undertake the present study to varify these observations as the literature survey reveals no reports about such studies for these ligands.

1.2 REVIEW OF ANTIMICROBIAL PROPERTIES OF HYDROXY-1,4-NAPHTHOQUINONES

Hydroxy derivatives of 1,4-naphthoquinones in which hydroxyl group is situated at either peri- or ortho- position to one of the carbonyl groups are collectively known as Juglones. Among the microbiologically active 1,4-naphthoquinones, Juglones seem to have important place because their study for structure activity relationship indicates the importance of following factors :

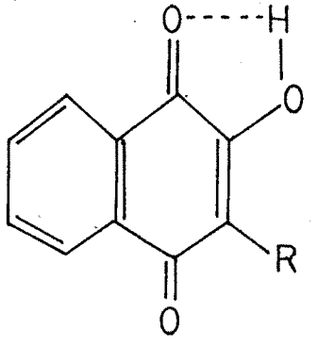
A) Effect of Hydrogen Bonding

B) Effect of Substitution in the 1,4-naphthoquinone moiety

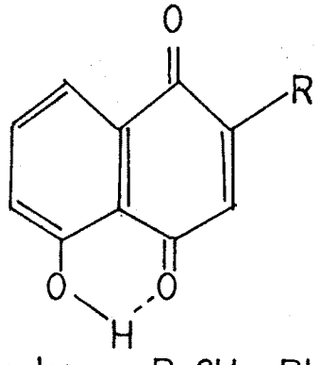
C) Effect of Metal Chelates

A) Effect of Hydrogen Bonding

In 1,4-naphthoquinones, the effect of hydrogen bonding on their antimicrobial activity was first studied by Padhye[13] Ranganatha Rao et al.[14] They showed that the activity of naphthoquinones is enhanced due to the presence of hydroxyl group, that forms hydrogen bonding. juglone and plumbagin were found to exhibit more tuberculostatic activity than lawsone and phthiocol. Thus a remarkable decrease in potency was observed due to the hydroxyl group in the quinone ring than in the benzene ring. It was also seen that increase in the number of hydroxy groups lowers the activity.



R=H; Lawsone. R=CH_3; Phthiocol



R=H; Juglone R=CH_3; Plumbagin

The antimicrobial studies from our laboratory[7-9] also indicate that the stronger the hydrogen bonding, greater is the activity. Due to intramolecular hydrogen bonding, juglone type compounds form six membered ring structure which is more stable than five membered ring structure formed in lawsone type compounds. Lawsone type compounds have possibility of weaker intramolecular hydrogen bonding also.[13]

Chelation of trace metals is a probable mode of action responsible for activity of Juglones.[13] For juglone and juglone-type compounds the deactivation of polyphenol oxidase in such way is a possible explanation for the enhanced fungicidal activity as compared to 1,4-naphthoquinone.[15]

A chelating agent may, thus, inhibit partially or completely a metal enzyme function if it is powerful enough to compete with the enzyme for the metal. If this function is an essential one, death of the micro-organism results or otherwise it may be weakened by the inhibition of the enzyme.

Some chelating agents act as antimicrobials not by removing a metal essential to life, but by forming a lethal complex with a metal accidentally present in the medium.[16] The antibacterial and antifungal action shown by oxine derivatives seems to be of this type, as they have no activity in a media depleted of heavy metals.

B) Effect of substitution in 1,4-naphthoquinone

Like hydrogen bonding, the type and number of substituents as well as steric factor are also important in determining the activity of the compounds.[17-19] Same may be true for Juglones also e.g. the 3-alkyl derivatives of lawsone are found to be more active against malaria parasites and as coccidiostats.[14]

Holmes et al.[20] have suggested that the possible activity of 1,4-naphthoquinones against bacteria and fungi may be due to short circuiting of the cell electron transfer normally executed by quinones. It has been pointed out that halogenation in the naphthoquinone nucleus increases the activity of the compound.[21] 2-Chloro-1,4-naphthoquinone has displayed antibiotic activity towards certain organisms[22] and dichlone (2,3-dichloro-1,4-naphthoquinone) is shown to be a powerful fungicide.[23] Ambrogi et al.[24] resumed the study and found that the 1,4-naphthoquinones substituted with chlorine in the nucleus and side chain show a particularly marked antimicrobial activity. They have considered the possibility of the reaction of the naphthoquinones with the bacterial proteins. They have tried to correlate the activity of these compounds with their electronic structure and ability to form hydrogen bonds. After studying a number of substituted 1,4-naphthoquinones, they have observed the following points which are of our interest.

(a) In series of compounds tested, the microbiologically active 1,4-naphthoquinones were those substituted in the quinone moiety with electron attracting groups such as hydroxy or chloro. Although the halogenated derivatives were found to be more active, the chloro derivatives in them had higher activity than the bromo derivatives against bacteria and fungi. It was also noted that the

compounds having chloro group in the naphthoquinone nucleus were more active than those having chloro group in the side chain.

(b) In the naphthoquinones having substituent at C-2 and or C-3 position, those having chloro group were found to be more active than the derivatives having amino or nitro group. It is assumed that the nitro group prevents the conjugation due to the increased electron affecting character while the amino group which although keeps extended conjugation, shows decrease in the ability to form hydrogen bonds.

Thus the substituted 1,4-naphthoquinone derivative should have all of the following probable factors for its activity :

(i) The increase in the degree of conjugation in the quinone moiety.

(ii) Generation of the molecular forms involving a greater separation of charge

(iii) Retention of the ability to form hydrogen bonds

It is also reported that a halogen atom in the quinone ring or presence of a vacant quinonoid position of the naphthoquinone moiety is related to the fungicidal activity.[25]

The chlorohydroxy-1,4-naphthoquinones as well as their natural sources are reported to show antimicrobial activity[10,26] and therefore have therapeutic use.[27] Some synthetic halohydroxy-1,4-naphthoquinones have also been shown to act in a varying degrees as antibacterials and antifungals.[24,28,29,30]

C) Effect of metal chelates

The naturally occurring chelates in biological systems are known and the effect of metal chelates, which do not occur naturally , on the biological systems is also being studied.[16,19]

Metal chelates of L-amino acids as methionine and ethionine have much greater antibacterial activity than the free ligands, rendering the metal ion fat soluble and so capable of acting within a cell.[16] The antibacterial activity of an antibiotic appears to depend on its ability to chelate metal[31] and the metal ion that gets chelated. This is indicated from the example of tetracycline. Tetracycline complexes of copper and calcium are found to be more active than tetracycline itself.[12] However, the addition of a large amount of magnesium ions can inhibit the antibiotic effects of tetracycline. Although the nature of coordination between tetracycline molecule and magnesium ion is not fully known , this is a notable observation.[31] Ampicillin chelate of cobalt[32] also shows increased activity than the ligand itself.

It has been found that activity of some drugs is increased considerably when administered as their metal chelates.[33-37]

The antibacterial activity of metal chelates of ligands such as amodiaquine hydrochloride.[16], 5-iodo-7-chloro-8-hydroxy-quinolino-4-(p-tolyl)-sulphonamide,[11] sulphonamido and benzamido derivatives of amino benzoic acid[38] was greater than the ligands as well as metal ions.

The transition metal chelates of juglones and their 3-substituted derivatives also show more antimicrobial activity than the ligands.[8,39,40] The copper(II) chelates of lawsone and 3-halolawsones have also shown to be useful as disinfectants and fungicides.[39,40]

The mechanism of action of a metal chelate is not clear. It has been observed that generally kinetically labile complexes act more rapidly than their inert analogues. This suggests that a complex or a metal chelate

acts as an efficient transporter of the ligand to the site of action in the cell, where it dissociates into the free ligand which is actually an active species.[16]

1.3 THERAPEUTIC IMPORTANCE OF PLUMBAGIN AND 3-CHLOROPLUMBAGIN

The chlorohydroxynaphthoquinones have found applications in the fields of medicine and microbiology. The use of extract of the roots of Plumbago zeylanica (Called 'Chitrakamula' or simply 'Chitraka') which contains plumbagin and 3-chloroplumbagin has been prescribed in the Indian system of medicine i.e. 'Ayurveda' since ancient times. The earliest reference to the use of the extract of 'Chitrakamula' is, thus,found in Charak Samhita[5] in the following versus :

कटुक: कटुक: पाके वीर्योष्णश्चित्रको मत: ।

'Chitraka is pungent by taste and very heat producing by virya'

चित्रकमूलं दीपनीय पाचनीय गुदशोथार्श: शुलहराणाम ।

'The roots of Chitraka is the best among the drugs used as the appetisers and digestion promoting. It removes diseases of the swelling of the anus (curative of proctitis), piles and abdominal pains (colic pains)'.

One also finds a reference to the medicinal properties of the extracts of 'Chitrakamula' in Vagbhata[6] as ;

चित्रको ग्निसम: पाके शोफार्श: कृमिकुष्ठाहा ।

'Chitraka is like abdominal fire with respect to digestion and can also be used as a cure for swellings, piles and skin diseases.

It is interesting to note that these properties are almost identical with the ones attributed to the properties of 'Chitraka extract' in the modern pharmacopo eia prepared at a much later date. Thus the pharmacopoeia

considers 'Chitraka extract' as a drug which increases the digestive power, promotes the appetite and is useful in the treatment of dyspepsia, piles, anasarca, diarrhoea and skin diseases.[41-43]

In Malaya chewing of roots of Plumbago zeylanica is said to be effective for producing abortion[44,45] a property which requires further study in the light of the link between estrogens and coagulation mechanism.

Pendse and Iyengar[46] have investigated the medicinal properties of the root powder of Plumbago zeylanica and compared with plumbagin. Surprisingly the root powder was observed to be more effective than pure plumbagin suggesting the presence of another active constituent in the root powder besides plumbagin. Sidhu et al.[3] and Padhye and Kulkarni[4] have shown the presence of 3-chloroplumbagin in the root powder of Plumbago zeylanica The drug activity, thus, seems to be more due to the presence of the 3-chloroplumbagin than plumbagin in it.

The extracts of Drosera Spp. containing both plumbagin and 3-chloroplumbagin are still used in the treatment of whooping cough.[47,48] U.S. Dispensatory mentions its use in relieving tooth ache.[49] The vesicant properties of Drosera peltata ("Mukhajali") have been mentioned in the Indian System of Medicine since long[50] and its extract has also been used in the therapeutic preparations of mineral origin, particularly those involving gold containing minerals.

1.4 PRESENT INVESTIGATIONS

Lawsone and juglone are the two parent isomeric ligands that form the basis of coordination chemistry of hydroxy-1,4-naphthoquinones and their

derivatives. Lawsone and its derivatives form five membered while juglone and its derivatives form six membered chelate ring systems. In fact both these series provide ideal compounds to investigate the relative stabilities and other structural features of isomerism involving five membered versus six membered ring chelates.

The structural pecularities of some of the transition metal chelates and rare earth chelates of lawsone, 3-halolawsone, juglone,3-halojuglones alongwith some other derivatives of them have been reported.[51-53] Analytical and antimicrobial studies of these ligands as well as their chelates have been exhaustively carried out in order to investigate structure activity relationship.

The literature survey reveals that there are no reports on the metal chelates of plumbagin and 3-chloroplumbagin except by Rane[54] who has carried out the structural characterisation of some transition metal chelates of plumbagin. We have, therefore, prepared for the first time the transition metal chelates of 3-chloroplumbagin. Partial characterisation of these chelates is discussed in Chapter-II of the present thesis on the basis of their elemental analyses, infra-red and far infra-red spectra, magnetic susceptibilities and the thermogravimetric data.

Alkaline earth metal chelates of plumbagin and 3-chloroplumbagin, which are also not reported so far, have been prepared and studied on the basis of their elemental analyses, infra-red and far infra-red spectra and thermogravimetric data. The comparative study is described in Chapter-III.

The medicinal importance of the natural sources which contain these ligands has already been explained. The comparative antimicrobial study

has been carried out for detecting the active constituent present which is responsible for the drug activity. It is wellknown that chelation with biologically important metals can increase the antimicrobial properties of the parent ligand. We have selected magnesium(II), calcium(II), copper(II) and zinc(II) chelates of plumbagin and 3-chloroplumbagin for the comparative antimicrobial studies with the view to find out a chelate having more medicinal properties than either plumbagin or 3-chloroplumbagin. This discussion is included in Chapter-IV of the present thesis.

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