CHAPTER - IV
Introduction
Salinity of water and soil are two factors generally unfavourable for plant growth. However, not all group of plants show their inability to grow under such extreme conditions. The mangrove plants are best adapted to such inhospitable environment of high salt conditions of the soil and water which is totally unsuitable for other plants.

The word "mangrove" has been used to refer to the constituent plants of tropical intertidal forest communities or to the community itself. MacNae (1968) proposed "mangal" as a term for the community, leaving "mangrove" for the constituent plant species, and this usage is increasingly adopted. Mangroves in the more limited sense may thus be defined as tropical trees restricted to the intertidal and adjacent communities.

Although mangroves may grow throughout the tropics in suitable areas, with the exception of the Central Pacific, particular regions are noted for the broad extent of mangal. Typically these are estuaries of large rivers that run over a shallow continental shelf. Examples are the mouth of the Ganges and Brahmaputra rivers (the Sunderbans) mainly in Bangladesh, the Fly and Purari rivers in Papua New Guinea and the Mekong Delta in Vietnam. The Florida Everglades is a drainage basin that gradually changes from fresh water to an extensive mangal at its seaward margin. The two largest tropical rivers, the Amazon and the Congo, do not develop extensive estuarine mangal for physiographic reasons.

The history of mangroves revealed that they were certainly known to the ancients (MacNae, 1968), but significant study began with the European colonization in the sixteenth and seventeenth centuries. Since mangroves are common coastal plants that are readily observed and collected, many of them especially in the East Indies, were familiar to the early European naturalist. The earliest published account of them is found in the *Hortus indicus*.
*malabaricus* of H. van Rheede tot Drakenstein (Rheede 1678-1703) but especially the *Herbarium amboinense* of Georg Everhard Rumph (Rumphius 1741-1755). However, the names used by Rheede and Rumphius are of no scientific significance because they pre-date the publication of Linnaeus's *Species Plantarum* (1753), which is the starting point for names of higher plants according to the International Rules of Botanical Nomenclature. The descriptions and identifications are important, however, because they served as a basis for many validly published names by Linnaeus and subsequent authors (Tomlinson, 1986).
Review of Literature
The mangrove plants thus form an interesting vegetation on saline area whose internal factors relating to growth habit are not clearly understood. In order to find out a reasonable explanation to this growth, extensive work was done in this laboratory on isolation and identification of growth regulators from mangrove plants. A number of gibberellins isolated from the mangrove plants (Ganguly et al., 1970; Gaskin et al., 1971; Ganguly and Sircar, 1974) have been reported from this laboratory. Gibberellins may not be the only factor which allow the plants to grow vigorously under such conditions. Keeping this in mind, it was felt worthwhile to include one plant, *Bruguiera gymnorrhiza*, collected from the mangrove forests of the Sunderban area (Gangetic Delta) in West Bengal, for isolation and characterization of endogenous growth substances.

In the subsequent paragraphs, a brief review of various biologically active compounds isolated from the mangrove, *B. gymnorrhiza*, during the past fifteen years is presented.

A chemical investigation of the stem and bark of *B. conjugata* led to the isolation of brugierol (cis-I) and isobrugierol (trans-I) (Kato and Hashimoto, 1979). Bactericidal and insecticidal screening tests were carried out with I derivatives. The highest insecticidal activity against several species was shown by 5-N,N-dimethylamino-1,2,3-trithiane hydrochloride. Yaga et al. (1991) also examined the termiticidal substances from five species of mangrove trees, including *B. gymnorrhiza*. From the chloroform extract they isolated 0.045% brugierol and
0.015% isobrugierol. These two compounds accounted for the entire termiticidal activity of the wood.

Several sterols and fatty acids were isolated from three species of mangroves, Acanthus ilicifolius, B. gymnorrhiza and Rhizophora mucronata leaves (Misra et al., 1984). The major sterol isolated was sitosterol, along with cholesterol, campesterol, stigmasterol and 28-isofucosterol. Stigmast-7-en-3β-ol was isolated from R. mucronata leaves. The component fatty acids found in all three species were 16:0, 18:0, 18:1, 18:2 and 18:3. The relative proportion of sterols and fatty acids were found to be significantly different chemotaxonomically. In continuation with their work, Ghosh et al. (1985) further analysed seven species of mangroves including Bruguiera for the presence of sterols and triterpenoids. Several sterols were found which included cholesterol, campesterol, stigmasterol, sitosterol and stigmst-7-en-3β-ol. The triterpenoids isolated included α-amyrin, β-amyrin, lupeol, oleanolic acid and ursolic acid. Further work led to the isolation of several fatty acids like palmitic, stearic, oleic and linolenic acid.

The compositions of B. gymnorrhiza and R. stylosa bark were examined by Weissmann (1985). The content of tannins (polyphenols) in the hot water extract were approximately 80% and 75% for B. gymnorrhiza and R. stylosa respectively. Upon hydrolysis, tannins yielded cyanidin and delphinidin in nearly equal amounts. Holocellulose and klason lignin contents of B. gymnorrhiza bark were 35.5% and 14.1% respectively. Corresponding amounts in R. stylosa were 39.0% and 15.9%. The major sugar constituents were rhamnose, arabinose, glucose and galactose; the minor sugar constituents were xylose, mannose and galacturonic acid.
Bagchi et al. (1988) studied the lipid and waxes in leaves of some mangrove plants of the Sunderbans, India. They found that mangrove leaves in general have characteristic low triglycerides and simple fatty acids and concluded that fatty acids probably control water economy and help adaptation of mangroves in the physiologically dry soil.

Richter et al. (1990) isolated 1D-1-O-methyl-muco-inositol from the various members of the family Rhizophoraceae. The compound was suggested to be a stress metabolite.

Ultraviolet (UV) absorbing phenolic compounds were found in the leaves of Bruguiera species. The phenolic compounds have been shown to be protective against the damaging effects of UV radiation (Lovelock et al., 1992).
Experimental
DETECTION, ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY
OF CHEMICAL CONSTITUENTS ISOLATED FROM *Bruguiera gymnorhiza*

**Note:** All m.p.'s are uncorrected. Petroleum ether refers to the fraction b.p. 60-80°C unless otherwise stated. Samples for analysis were dried over P₂O₅ in vacuum for 24 hours at 80°C.
5 kg of immature leaves of *B. gymnorhiza* was extracted with methanol. The methanolic extract was concentrated and extracted with petroleum ether. After extraction, the residue was washed with water and pH was adjusted to approx. 3 using phosphoric acid. It was then absorbed in a column of Dowex-50 (H\(^+\) form, 50-100 mesh). The column was successively eluted with 70% ethanol, water and 4N ammonium hydroxide. Each of the fraction collected was concentrated and tested for cytokinins. Only the last fraction showed the presence of cytokinin. Then the fraction containing cytokinin was subjected to paper chromatography using Whatman No.1 filter paper. The chromatogram was developed in isopropanol:ammonia:water::10:1:1. The developed chromatogram was sprayed with 2% aqueous AgNO\(_3\) solution followed by treatment with 0.5% Na\(_2\)Cr\(_2\)O\(_7\) solution. Subsequently, the paper was treated with 0.5N HNO\(_3\) and the paper was finally washed with water. Two brick red colour spots developed on spraying with AgNO\(_3\) confirming the presence of two cytokinins in the fraction.

**TLC:**

The cytokinin fraction was subjected to TLC in the solvent system, n-butanol:ammonia:water::6:1:3. The plate was then observed under UV light. Two spots were observed, one at Rf. 0.76 and the other at Rf. 0.82.

**Rf. 0.76:**

The spot corresponding to Rf. 0.76 was identified as zeatin based on Co-TLC with authentic sample.
IR spectrum of 2-hydroxy-1'-methyl dihydro zeatin
Rf. 0.82:

The spot corresponding to Rf. 0.82 was next taken up for study.

TLC-CIMS:

The compound was subjected to TLC-CIMS. Based on this the molecular weight of the compound was established as 251 (M⁺).

Preparative TLC:

The entire fraction was then subjected to preparative TLC using a silica gel G plate (thickness 5mm) using the solvent system: n-butanol:ammonia:water::6:1:3. A viscous mass was obtained.

IR spectrum of the compound:

The IR spectrum of the compound was studied in Hitachi 260-10 infrared spectrophotometer, using nujol phase. The spectral data are tabulated in table-22.

<table>
<thead>
<tr>
<th>WAVELENGTH (cm⁻¹)</th>
<th>NATURE OF THE BAND</th>
<th>FUNCTIONAL GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>3350</td>
<td>Medium</td>
<td>-OH group</td>
</tr>
<tr>
<td>3155</td>
<td>Medium</td>
<td>-NH group</td>
</tr>
<tr>
<td>1645</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td>1515</td>
<td>Weak</td>
<td>aromatic residue</td>
</tr>
<tr>
<td>1500</td>
<td>Weak</td>
<td></td>
</tr>
</tbody>
</table>

NMR spectrum of the compound:

The NMR spectrum D₆-DmSO showed signals at d8.50 (2H, for H-9 and H-10), d7.98 (1H, H-8), d5.22 (1H, H-1') besides signals for methyls and no signals of olefinic protons.
Mass spectrum of the compound:

High resolution mass spectroscopy revealed the compound had a molecular formula \( \text{C}_{11}\text{H}_{17}\text{N}_{3}\text{O}_{2} \).

\[ \text{m}^+ \ 251 \]

Based on the above data the structure of the compound, viz., 2-hydroxy,1'-methyl dihydrozeatin was established as,

Bioassay:

Tobacco callus bioassay:

\textit{In-vitro} bioassay was carried out to test the activity of the purine derivative isolated from leaves of \( B. \text{gymnorhiza} \). Tobacco (\textit{Nicotiana tabacum}) stem pith callus maintained at 25°C 2°C, 78% relative humidity and 16h photoperiod on MS media (Murashige and Skoog, 196) supplemented with coconut milk (15% v/v) and naphthalene acetic acid (NAA) (0.5mg/l) were used for bioassay. Results were recorded after an interval of 21 days (Bottomley \textit{et al.}, 1963).

Pure crystalline solid of 2-hydroxy-1'methyl dihydrozeatin obtained by chromatography
Tobacco Callus Bioassay

Figure 1: Callus of *Nicotiana tabacum* grown in MS medium supplemented

A: Without test cytokinin (Control)

B: With 75 μL test cytokinin

C: With 50 μL test cytokinin

D: With 25 μL test cytokinin
methanolic extract of leaves of *B. gymnorhiza* was used for bioassay. A stock solution of the cytokinin was prepared (2mg/mL). For this 2mg of the cytokinin was dissolved in minimum quantity of alcohol and finally the volume was made to 1mL by adding distilled water. From this stock solution three different concentrations (25μL, 50μL and 75μL) of the cytokinin solution was prepared. In subsequent experiments coconut milk was omitted from MS medium and was replaced by these three different concentrations of cytokinin solution. Thus, three different sets of media were prepared. Each set was supplemented with 0.5mg/L NAA. One set of the MS medium acted as control which was supplemented with coconut milk and NAA (as mentioned above). 40mg of fresh callus tissue was used as inocula in each treatment, having five replicate samples (Figure 1).

Results of tobacco callus bioassay may be tabulated as follows:

Table - 23

<table>
<thead>
<tr>
<th>CONCENTRATION OF CYTOKININ</th>
<th>MEAN WEIGHT (mg)</th>
<th>PERCENT OF CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>25μL</td>
<td>966</td>
<td>125.13</td>
</tr>
<tr>
<td>50μL</td>
<td>898</td>
<td>116.32</td>
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<tr>
<td>75μL</td>
<td>600</td>
<td>77.72</td>
</tr>
<tr>
<td>Control</td>
<td>772</td>
<td>100.00</td>
</tr>
</tbody>
</table>

(For analysis of variance, c.f. Appendix-III, Table-12)

S.E. 127.31

c.d. at 5% 381.63 49.42

c.d. at 1% 525.83 68.09

* Significant at 5% level
** Significant at 1% level

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