CHAPTER I

REVIEW OF LITERATURE

1. Medicinal importance of steroids with special refererecs to dioagenin.

2. Historical events in connection with the importance in phytochemical Industry.

3. Commercially important species of Dioscorea and Costus yielding diosgenin.

4. Chemical examination of commercially important species of Dioscorea and Costus available in North Bengal.

5. Production of Diosgenin in Costus sp.and Dioscorea sp. under various treatments and conditions.

6. Isolation, purification and quantitative estimation of diosgenin in Costus and Dioscorea spp.

7. Bio - synthesis of diosgenin and related saponins in plants.

8. Effect of physical factors on growth and development of plant with special emphasis on the production of diosgenin in Costus speciosus and Dioscorea spp.

   a. Quantity of light.
   b. Qulity of light.

9. Effect of chemical on growth and development of plant with special emphasis on production of diosgenin and other bio-Chemical parameter in Costus speciosus and Dioscorea spp.

   a. Growth regulators and growth hormones.
   b. Respiratory regulators / substrates.
   c. Phenoxy acetic acids.
   d. Inorganic nutrients.
   e. Bacterial fertilizers.

10. Production of diosgenin in Costus speciosus and Dioscorea spp. under various treatments and conditions.

11. Cultural practice of Costus speciosus.

12. Observation on pharmacognosy in Costus speciosus and Dioscorea spp. With special empahasis on their starch content.

i. **Medicinal importance of steroids with special reference to diosgenin.**

Steroid plays an important role in modern civilization and human welfare. This is reflected by an estimate to have position second to antibiotics and in terms of drugs derived from higher plants , steroids are by far the most important (Farnsworth , 1977).

Steriods can be consider into several major groups on the basis of their application viz., corticosteroids, sex hormones and antifertility compounds. Corticosteroids are known to possess anti-inflammatory properties as they provide relief from arthritis , rheumatism and asthma. Sex hormones , both male and female , are generally used as substution therapy for deficiency in natual hormones. Testosterone , one of the most potent of male sex hormones, and its modifications are used to treat testicular insufficiency and as anabolic factor to build bodies effected by severe illness. Estradiol , estrone and progesterone , the female sex hormones , are useful in treatment of gynaecological disorders. Along with their identification , the female sex hormones serve as anti-fartility agents , the active ingradients of oral contraceptive.

ii. **Histrorical events in connection with the production of commercially important steroid drugs.**

Production of steroid drugs may be accomplished in principle , using any of three different approaches ; isolation of drugs itself from natural sources ; partial synthesis of drugs from suitable precursors of plant origin and total synthesis of artificial drugs. The first two approaches are commonley use now-a-days for the production of drugs on indrustaial scale and the third one involves cumbrous form the synthesis in laboratory condition and which may sometimes become un economical in nature (Vellus et al. 1965).

Earlier steroid production relied on animal sources such as horse urine , bull testes and cow ovaries (Butenandt et al. 1934). Several tons of those animal products were required and it was menace to obtain them easily. As the demand grew for hormones , partial synthesis from cholesterol came into practice since 1933 , and synthesis of hormones was put in the market Fernholz , 1933 ; Butenandt et al., 1934 ; Chakravarti et al. (1960). But due to multiple chemical steps of synthesis of hormones involving more then 1 , 00 , 000 per Kg. This encouraged the use of some what cheaper sterol such as stigmasterol (Fieser and Fieser , 1959) , but its steady supply was again limited.

The cost of progesterone obtained from plant sterol was observed to be around 80,000 pound per Kg. The increased amount of cost involvement and limited supply of starting material for the production of sex hormones encouraged further research. The

the production of sex hormones encouraged further research. The artificial synthesis of sex hormones and cortisones involves a lengthy process and at the same time is an expensive one. For these reasons naturally occurring steroids are , now-a-days , in great demand for their utilization during partial synthesis of cortisones and sex hormones (Applezweig , 1962 , 1969 ; Djerassi , 1966 , Tekada 1972). Contraceptive hormones and anti-inflammatory agents derived from corticosteriods are the two mejor groups of steroid presently manufactured on an industrial scale (weston, 1976.). The importance and widespread use of these steroid drugs has been discussed by Applezweig (1969 , 1974).

The dramatic increase in the scale of antifertility agent in recent years necessitates a greater supply of naturally occurring steroid precursor from which these drugs are prepared.

Initially an attempt was made to isolate cortisone directly from the organ of animal origin. But the output was extremely insignificant. Only 0.5 gms. of cortisone could be isolated from 450 Kg of incised beef adrenal cortex (Chakravarti et al., 1961). Gravity of this situation was realizes from the fact that Schering Laboratory , Berlin , needed 625 Kg. of ovaries from 50, 000 cows to obtain 20 mg. of pure crystalline progesterone (Butenandt and West Phal, 1934). Total systhesis of steroid involves cumbrous process and their commercial production requires huge expenses. So the conversion of cheap and easily available naturally occurring intermediates into desired steroid hormones appears to be the best way for their commercial

production. This led to an intensive phytochemical survey on vegetable sources during the last three decades to search staroid precursor of plant origin which would be cheaper and potentialy useful for the preparation of cortico-steroids , sex hormones and contractive steroids on industrial scale (Marker et al., 1977 ; Barua et al. 1953 , Correll et al., 1955 ; Chopra and Handa, 1963 ; Chakravarty et al. 1957 ; 1964).

During the last few decads , it has been noted that various steroid compounds such as stigmasterol (Fieser and Fieser , 1959) , cholesterol (Fernholz, 1933 ; Butenandt et al., 1934 ; Cakravarti et al., 1956). Bita-sitosterol (Chawla , 1977) , desoxycholic acid (Sarett , 1946 ;1948 , Correll et al., 1955) , sarmentogenin (Lardon and Reichstein , 1958), hecogenin (Applezweig , 1962 ; Rule, 1975) were attempted to be utilized as the basic steroid precursor for the partial synthesis of desired steroid drugs. For this purpose different plant materials such as Glycine max, Phytostigma venenosum, Strophanthus sarmentosus, Agave spp. Costus spp. and Dioscorea spp. were being used by various workers.

In course of time diosgenin was noted to be more convenient in comparison to other precursors so far utilized for the preparation of sex hormones and contraceptive steroids and had been the choice of the world till the early seventies (Apple zweig, 1962 ; 1969 ; Djerassi ; 1966 ; Tekada ; 1972).

According to Hathi Committee's report (Chaturvedi and Sinha , 1980) annual requirement of diosgenin in India was estimated to be 60 tonnes and its annual production in our country was noted to be in the order of only 10-15 tones. The first systhesis of cortisone from dexoxycholic acid isolated from ox bile involved 32 successive steps , (Chakravorty and Roy Choudhury ,1974). Cortisone and its derivatives are noted to be oxysteroids in nature whereas the sex hormones , including the oral contracceptives , have no oxygen substitution in the molecule. Hecogenin therefore provides a partial starting material for the synthesis of the corticosroids , whereas diosgenin has been noted to be suitable for the manufacture of oral contraceptives and sex hormones. Diosgenin however can also be used for the corticosteroid synthesis by the introduction of oxygen into the 11 , L-position of pregnenenucleus during microbial transformation.

The matter of research on steroidal chemicals was proved to be fruitful when Fuji and Malsukawa , in 1936 , discovered diosgenin. Marker and his associate (1943) revealed the potential use of plant sapogenin for the synthesis of cortisones and other related drugs via 16-DPA. Since then diosgenin has been used as the most important and versatile precursor , being capable of transformation to all the types of steroidal drugs. The demand for steroidal compounds has been increased considerably and some 600 - 700 tones of diosgenin are being used now-a-days annually with the world-wide use of

hormones estimated to be 500 millions per annum (Panda , 1980). Strenuous efforts are being made to discover the high yielding strains of plants and to assure a regular supply of raw material by the cultivators of good quality plants and, in this respect , differt species of Dioscorea play a remarkable role.

Tuber of many Dioscoreas , commonly known as yams , have long been used for food as they are rich in starch. In addition to starch , some species contain steroidal saponins as well as other alkaloids. From suitable sources sapogenins are isolated by acid hydrolysis of the saponins. Preliminary fermentation of the materil often gives a better yield (Charkraborty et al., 1958). The water insoluble sapogenin is then extracted with a suitable organic solvent.

Untill 1970 diosgenin isolated from the Mexican yam was the sole source for the manufacture of steroidal contraceptives (Bammi and Randhawa, 1975). With the nationalization of the Mexicam industry , however prices increased to such an extent that manufacturers switched over to hecoginin as precursor for the synthesis of steroid compounds or two other sources of diosgenin e.g. Costus , Kallstromia , Balamites and Trigonella (Khanna, 1977) and even to the utilization of the steroidal alkaloids of Solanum species.

iii. Commercially important species of Dioscoreas and Costus in

connection with the production of diosgenin :

AS regards with Dioscoreas , D.composita , D.floribunda and to lesser extent D.spiculitora , D.mexicana are used in Mexico and Guatemala commersially for production of diosgenin , while in China , D.singierensis and D.prazeri are the commercial sources of diosgenin (ITC-1974) and are being used now-a-days in central and westren parts of India. D.prezeri and D.composita are commercialy used in eastern and north estern parts of the country. Out of these species again D.deltoidea is being preferred much as it is very easy to obtain pure diosgenin from this source (Chaturvedi and Choudhuri, 1980). It has been noted by Sarin et al. (1974) that the supply of Dioscorea tubers obtained from wild resources are likely to be exhausted in next 10-15 years due to large scale collection but poor natural regeneration. The situation is being further aggravated due to indication of raising these plants as commercial crops without commendable success. Hence attempt has been made to find out other sources of doisgenin ; and among the plants Costus speciosus (Koening) Sm. has been considered to be one of the most importent alternative sources of diosgenin (Sarin et al., 1977 , Shah et al., 1978) as it requires only one year to complete the cyle as compared to other species of Dioscorea (Selvaraj and Subhas Chandra, 1980) which normally require average of 3 to 5 years per cycle.

iv) Chemical examination of commercially important species of Dioscorea and costus available in North Bengal.

Botanical Source	Chemical Constituents	Plant parts and yield	Referance
Dioscorea deltoidea	Diosgenin	Yams, 2%-5%	Kunjithapadam (1977)
	Diosgenin glucopyranoside	Yam	Rajaraman etal., (1976)
	Laevulinice acid	Yam	Chakraborty etal., (1960)
Dioscorea grezeri	Diosgenin	Yam 1%	Kunjithapadam (1977)
	Prezerizenin-A-gluco pyranoside	Yam	Rajaraman etal., (1976)
	Prezerizenin-A-ramnogluco-pyranoside	Yam	Do
Dioscorea composita	Diosgenin	Yam 2%-4%	Kujithapadam (1977)
Dioscorea floribunda	Doisgenin	Yam 2%-5%	Do
Costus speciosus	Diosgenin	Rhizome 1.5%-2.5% Seed - (traee)	Do Sing etal., (1980)
	Total sapogenin	Rhizome 3.86%	Das Gupta and Pandey (1970)
	B-cytosterol	Do	Do
	Tegogenin	Do	Do
	Costic acid	Root	Bawdekar and Kelker, (1965)
	Candinene	Rhizome	Do
	Cineole	Do	Do
	P-methoxy Benzophenol	Do	Sharma etal., (1980)
	Pinocarveol	Do	Do
	Carvacnol	Do	Do
	Saponin		Do

V. Production of diosgenin in Costus sp. and Dioscorea sp. under various treatements and conditions.

Post harvest incubation experiments proved very useful for maximising the amount of diosgenin that could be obtained from given quantity of Costus Rhizomes. (Chakravarty et al., 1976b). Although increase of diosgenin was noted in all the exogenously applied chemicals , 2,4D (100 ppm and sodium acetate (50 ppm) were found to be effective for maximum production of diosgenin in the tuber of Dioscorea deltoidea , in the seed of Trigonella foenumgraceoum (Hardmam and Brain, 1971) , in the rhizomes of Costus speciosus (Shah et al., 1978 and Dioseorea floribunda (Selvaraj and Subhash Chandra, 1980) during post harvest inclubation of rhizome.

The possible biochemical mechanism involved in such an increase of diosgenin due to incubation could be projected on two main routes: either release of pre-existent material or synthesis of diosgenin itself. (Hardman and Brain, 1971). It has been reported (Bluden and Hardman, 1963) that sapogenin exits in plants mainly as their glycosides , the saponins and the sugar part of the latter fraction is incorporated into the cell wall homopolymer (cellulose) by convalent bonds. Increased diosgenin yeild on incubation has been ascribed to be an enzymic process where the bound form of diosgenin could be released by hydrolytic activity of endogenous enzyme systems (Blunden and Hardman, 1963

). This statement finds support from experiments of Selvaraj and Subhas Chandra (1980) who traced an amount of diosgenin from the isolated cellulase of hydrolysis tuber samples of D. floribunda.

Dutta et al., (1977b) studied the processing of rhizome of C. speciosus for better yeild. They found deterioration in diosgenin content as a result of storage of undried rhizomes for different lenghts of time. They suggested that Costus speciosus rhizome must be first chopped off and dried immediately after harvest. Roy and Dutta (1978a)found that the rhizome of the plants having small diameter showed maximum diosgenin content. Which decreases with the increase in diameter of the same.

Culturel practiceses:

Literature on investigations partaining to caltural studies of C. speciosus is rather limited. In 1977 Sarin et al., recommended planting by rhizome piece with at least two available buds for which they used 1 m.x 0.75 m, spacing. An alternative method of propagation of the species were reported by Verma and Sharma (1978). According to them the steps were laid horizontaly in well covered bed after removal of the rhizome. Within a month rooting took place at each node of these stems. The rooted nodes were then used as planting materials.

The variation in diosgenin content in defferent portion of the rhizome as well as during the life cycle of the plant was also reported by Sarin et al., (1976). He found the nodes with sprouted stem contained maximum diosgenin in comparison to the internode and top portion of rhizomes. In addition to the above findings they have also reported a sharp decline in diosgenin content during the period of fruit formation. However , their subsequent studies on deflowering and defruting did not yeild any significant difference in diosgenin content (Sarin et al., 1977). The elaborate study by collecting the samples from various parts of the country revealed that there existed variation in diosgenin content from plant to plant and such variation were attributed to agroclimatic condition (Sarin et al., 1981) found a relationship between agroclimatic and physiographic conditions prevalent in a locality and the renge of diosgenin content in natural populations. Their extensives studies have revealed that an annual rainful below 1500 mm followed by marked dry period between winter monsoon season , with mean annual temperature below 24 degree centrigrade , is ideal condition for yielding good diogenin content in the plant. They identified commercially exploitable localities in India for the collection of raw materials form natural resources (Sexena el al., 1979) surveyed the plants from different ares of south eastern region of India and found fibrous rhizome with poor diosgenin content. Singh et al., (1979) estimated the potentiality of commertial exploitation of this crop and suggested the harvesting time for the plant. According to him the yield of dry rhizomes and

diosgenin production becames heighest when the plant was 21 months old. Sharma et al., (1980) in a field experiment observed the number of shoots , leaves and rhizomes as well as diosgenin increased significantly with the increase in weight of planting material. Increased rate of rhizome production was also reported (Balyain et al., 1980) due to application of poultry manure. Optimum level of 36.6 tones/hectre of manure has been suggested for economic cultivation of C. Speciosus plants.

VI. Isolation, purification and quantitative estimation of diosgenin in Costus and Dioscorea sp.

The chemical method recomended by Marker et al., (1942) for the isolation of sapogenin have been noted not to be feasible from commercial point of view. According to their procedure the steroidal saponin was extracted by ethyl-alcohol from ground plant material , which was acid hydrolyzed to liberate the sapogenin from the glycoside. The crude sapogenin was obtained after ether extraction of the hydrolyzate and was purified by charcoal and recrystallized several times. Often recrystallize in the acetate form was required before pure sapogenin was obtained.

A procedure for isolating diosgenin , somewhat similar to the one described by Rothrock et al., (1957) , has been presented in a recent patent by Sarin et al., (1976). The process for the isolation of diosgenin from tuber consists of

three major operations ; preparation of the tubers , hydrolysis of the saponin and extraction of the diosgenin. Rothrock et al., (1957) found that hydrolysis of fresh pulverised tubers with 2N HCl at boiling temperature for two hours was sufficient for hydrolysis of saponin. Chakraborty et al., (1958) standardized a procedure in which formation of 25-spirosata 3, 5 diene during acid hydrolysis was avoided by aqueous hydrolysis and simultaneous extraction of sapogenin. Subsequently , Chakrabarty et al., (1970) used a modified method in which they used 2N Hcl in ratio of 1:10 for hydrolysis of dry powder sample for 5 hours in a boiling water bath. Crude sapogenin obtained on extraction with pet. ether (40 deg.C - 60 deg.C) was washed with NaOH solution and evaporated to give a product which was chromatographed over neutral alumina. Relatively pure sapogenin was then acetylated and diosgenin was estimated in the acetate form. Preston et al., (1961) used (1.5)N HCl to dydrolyze the sample for 5 hours. Selvaraj and Subhash Chandra (1980) found the hydrolysis of dry powder of D. floribuna with 2.5 (N) HCl for 2 hours would give a product which , on cromatography over alumina would offer diosgenin having the yield of 86.8 - 87 . 6% of total sapogenin. Chakravarti et al., (1961) proposed a procedure in which the oven dried material was powdered and transfered to a soxhlet extracter and extracted with light pet. ethar (40 - 60 degree centregrate) for 8 hours. The extract was concentrated to about 50 ml. , when crystal of diosgenin began to apper. At this stage the flux containing the crystal was refluxed for 1 hour. After cooling crystals were filtered throguh a tared

sintered crucible and washing was performed with a fresh quantity of 50 ml. of cooled pet. ether to make the crystal free form any colouring matter. Futher crystals , if any from the mother liquor were similarly recovered and added to the bulk. The crucible was dried in an oven at 100 degree centrigrate for 2 hours , dessicated and weighed.

Later Gandotra et al., (1977) suggested a suitable method which was modified later by Panda and Chatterjee (1980). According to the procedure fresh rhizome was cleaned with running water ; after clanning the roots the excess of adhering water was remove by wipping with a clean cloth. The rhizome was dried ground and hydrolyzed in an autocleve at the pressure of 15 lbs. For 15 minutes in the prsence of 4% HCl. The slury was filtered under reduced pressure. The residue was washed with distilled water to free it from acid. The acid free residue was dried in an oven and extracted with Hexene in a soxhlet apparatus for 8 hours. The solvent extract was concentrated , chilled in ice to obtained diosgenin. The diosgenin was weighed after drying in an oven for 2 hours at 80 deg.C.

It has been noted from the literature that during the preparation of tubers for processing , either fresh or dry tubers were pulverized through a micro pulverizer (Bantam) eqipped with 0.013 inch (Slotwidth) herringbone screen (Rothrock et al., 1957). Moriss et al (1958) used sliced fresh tuber. These were macerated in presence of water with a high speed blender. Accoring to Chakraborty et al., (1958) the size of the particle

of tubers is of considerable inportance from the stand point of efficient extrction. Sixty mesh powder has been found to be suitable for extraction.

It has also been noted that during hydrolysis of saponin different workers expressed different views. Earlier workers , Marker et al. (1942) ; Fuji and Mathsukuwa (1936) ; hydrolyzed under contitions varying from 2 N HCl. refluxed for 2 hours to alcoholic H2 SO4 treated for 20 hours. In none of these cases optimum hydrolysis codition was shown. Rothrock et al., (1957) found that diosgenin was completely hydrolyzed by 4 N HCl solution refluxed for 4 hours.

.During extraction and purification of diosgenin from Dioscorea sp.various hydrocarbon solvents have been used and out of which pet. ether , Skellysolve B , Skellysolve c , and Esso heptene proved most useful (Rothrock, 1957). The yield of diosgenin for assay and development work was based on a write crystalline product melting at 200 deg.C. or higher. This material of about 95 to 100% purity was found satisfactory in the usual test for the preparetion of 7-dehydro-diosgenin acetate. According to Rothrock (1957) , in order to obtain diosgenin of still higher purity it can be prepared by recrystallization from methyl-ethyl-ketone, ethyl alcohol-acetic acid 1 : 1 or chloroform-methanol 1 : 1. Chaudhuri et al., 1979 purified diosgenin after column chromatography over neutral alumina using chloroform acetone (3 : 1) as eluent. The extract was

crystallized from methanol to give fine needle shaped crystal melting at 202 deg.C. to 204 deg.C. The purity of chromatographed produced was cheeked by TLC over silicagel G using benzene-chloroform (1 : 2) solvent system. The spot was detected under UV light after spraying with 50% phosphoric acid. During estimation of diosgenin in Costus speciosus Sarin et al., (1981) followed the method of Gandotra (1977).

VII. Bio synthesis of diosgenin and related saponins in plants:

It has been noted earlier that steroidal saponin arises via the mevalonic acid pathway to produce " squalene ". The subsequent cyclization of squalene to give chlolesterol is well established (Croey et al., 1966). Cholosterol , has recently been shown to be incorporated into a number of C-27 sapogenins with side chain cleavage (Haftman, 1967). Consequently it appears that cholestrol is rapidly formed and metabolised. The ability of cholesterol to serve as precusor for other 27 carbon , sterols was shown by its conversion to tigogenin , gitogenin and diosgenin (Bennett and Heftman, 1965). Joly et al., (1969) showed that open chain saponins (5 Furostene-3p , 22 , 26-triol 3 b chacoside 26 b D-glucopyrexoside) are formed from cholesterol. He showed that in Dioscorca floribunda homogenates cholesterol has been converted directly to diocin i.e. diosgenin glocosides.

In plants the sapogenins are combined with sugar to

form the saponins. Generally , the sugars are in a branched chain and are attached to the C-3 position of the steroid moiety , saponins with the open side chains also exists (Joly et al., 1969 a , 1969 b) by forming a glycoside (Heftmann , 1967).

Cholesterol (Bennett and Haftman 1965 (a) ; Joly et al., 1969 c and Sitosterol) are the precursors in the formation of saponis as cholesterols is directly converted into diosgenin. With Sitosterol , however , removal of a two carbon unit from C-24 may proceed oxygenation. The sequence in which oxygen is introduce at position 16 , 22 and 26 is unanswered , but indirect evidence strongly suggests that oxygenation at C-26 is the first step (Bennett et al., 1970) ; and is not dependent upon a 24 bond (Joly et al., 1969 c) , cholesterol , however does not appear to be an obligatory step in the bio-synthesis , since desmosterol is converted to saponins without going through cholesterol (Tschesche et al., , 1974).

viii. **Effect of physical factors on growth and development of plant with special emphasis on the production of diosgenin in Costus speciosus and Dioscorea spp.**

Quantity of light :

After the discovery of photoperiodism in connection with the flowering of the plant Garner and Allard (1920) and various other authors have utilized the principle for commercial

development of economically important plant in India and abroad (Hendricks et al., 1956 ; Wareing , 1956 ; Nitsch , 1957 ; Vence Pure 1975 ; Nandi and Chatterjee 1978 b ; Singh and Nanda , 1981). Akahori et al., (1970) reported that in Dioscorea tokoro this application of light increased the amount of yomegenin and tokarogenin in long way conditions. Akahori etal (1970) reported that the amount of sapogenin in aerial parts of D. tokoro could be increased by long day photoperiodic condition. Similar observation was also noticed by Karnick (1972) in D. deltoidea. Nandi and chatterjee (1978) in D. pentaphylla , and D. composita.

Light influences a number of biochemical, physiological and morphological characteristic of plants including steroid metabolism. Generally dark grown plants contain mere steroodal constituents on a dry weight basis than light grown plants (Duperon 1968 ; Bush et al., 1971). The bio synthesis of sterol from mevalonic acid has been observed to become higher (Bush and Grunwald , 1973). The light effect on metabolism gives rise to a change in the component of sterol very similar to that of senesence (Grunwald 1978). It is well known that light is one environmental factor that not only acts on photosynthesis but also on the process of senesence . Wool house (1967) , with ageing cell organelles , showed a loss in structure , when eventually only the plasmalemma and some empty residues remained and opined that reduced light intensities chanced this process. The increased accumulation of some sterols in plants during this

phase of plant growth has been linked to the dis-organisation of intra cellular organelles (Duperon , 1971).

Light treatment

The discovery of Circa (1935) , the effect of different quality of light responsible for growth and development of plant , opened a new avenue towards advancement in plant physiology. After that verious information are available in this respect , Down (1956) , Siginva (1962) , Moore 1980 ; Walton et al., (1982) , Rao et al., (1982). The effect of the quality of light has been considered to be due to the interference of phytochrome and in this respect also information are available in connection with the structure and the function of the pigment (Parker et al., (1946) ; Borthwick et al., (1952) ; Butler et al., (1959) ; Siegelman and Butler (1965) ; Hillmann (1964 ; 1967). Every one at least agrees that phytochrome is a tetra pyrole and that intermediate forms between Pr and Pfr do exeist (Linschitz et al., (1966) These intermediate , may represent different forms of the protein component of the phytochrome system although Pr. and Pfr. almost certainly represent two distinctive conformational states of the protein. This protein has in fact credited with enzyme activity (Tezuka and yamamoto, 1969) which raises other intriguing prospect for light regulation of metabolic processes. Very recently Woitzik and Mohr (1988) observed the control of gravitropism in plant by phytochrome and noted strong effect of red and far red light treatment on blue light mediated

phototropism. Hunt et al., (1989) observed spectral quality of light influencing many aspects of plant growth and development. According to Karlsson (1988) , phytochrome is not involved in the red light (R) enhancement of the stomatal blue light response in plant. The stomatal response to blue light (BL) in plant was enhanced by back ground red light.

Lopez-Figueroa and Niell (1988) observed that the amount of Chlorophyll was accumulated in greater quantity in presence of blue light and pointed out the involvement of specific blue light photo recepter cryptochrome. Basu et al., (1988) observed that uptake of L-leucine could be enhanced by 50% over control by red light irradiation which was reversed by far red light. Reduction in loosening of cell wall has been observed due to the effect of blue light (Cosgrove, 1988).

Work in Soviet Union Voskresenskaya, (1950) gave an early indication of light quality effects on photosynthetic products. Leaves were noted to be added more dry weight in red light then in blue light Mc Cree (1971) , but , in red light , 68% of the added dry matter was due to carbohydrates compared with 42% in blue light , Das and Raju (1965) has revealed that blue light stimulates accumulation of protein and other non-carbohydrate substances. Though diosgenin systhesis is very much related to carbohydrate metabolism (Bennett and Haftman, 1965) ; Jolly et al., (1969) , no report has so far been made in connection with the effect of quality of light on diosgenin

synthesis.

ix) **Effects of chimicals on growth and development of plant with spceial emphasis on production of diosgenin and other bio chemical parameters in Costus spceiosus and Dioscerea spp.**

Growth regulators and growth hormones

From literature it appears that IAA retards the growth of plant and delays the onset of senesence of attached and detached leaves ; (Leopold, 1960) IAA is known to promote rooting in the stem cuttings of many plants (Thiamann and Behnke-Rogers, 1950). It has been supported by other workers (Audus, 1960 ; Leopold, 1960 and Hess, 1962).

Althrough auxins have been shown to have variety of actions (Bonner and Varner, 1965) their effects on secondary products have been studied only sporadically.

It has been noted that auxins and phenoxy acids have significant effect on the production of steroids or other related compounds in both plants and inex plants during culture ; (Genus 1975 , Hardman and Stevens, 1978) , Joans, (1969). In their experiments IAA and 2 , 4-D have been noted to increase diosgenin content in several plants such as Trigonella , Balanites

(Khanna , 1977) and Dioscorea , (Marshall and Staba, 1976).

Chaturvedi and Choudhuri (1980) reported that in tuber of Dioscorea deltoidea, IAA and 2 , 4-D combination yielded maximum diosgenin content.

Eversince the discovery of Kinetin as a stimulator of cell division in tobacco pith tissue (Miller et al., 1955) it has evoked considerable interest and is well known for its role in all phases of plant growth and development (Miller., 1961 , Kuraishi, 1959 ; Steward et al., 1961).

Van Overbeck et al., (1961) noted that kinetin treated leaf remained green as compared to other chemical. This was supported by Richmond and Lang (1957). Debata and Murty (1981) also showed that kinetin was effective on photosynthesis and specially on chlorophyll content. Wiss (1960) showed kinetin to delay seed germination though Miller (1958) and khan (1966) showed kinetin to be a germination promoter. Gupta (1970) in his study showed the inhibition of germination in presence of kinetin.

Bhattacharya and Varsha (1981) in their experiments with several plants showed that MH in higher concentration inhibited rooting though lower concetration of MH enhanced rooting. Gopal Rao (1968) observed that MH , in combination with low temperature treatment , increased growth of the plant

over control to 24.3%. He also showed that seed hormonization of MH inhibited the growth of seed ling more in comparison to other hormones. MH has been noted to show antimitotic effect (Greulach and Atchison, 1950). It also acted as a respiratory inhibitor (Naylor and Davis , 1950). Moore (1980) noted early flowering in plant when MH was applied during cold. Inhibition of flowering by MH was first reported by Nylor, (1950). Klein and Leopold (1953) suggested that inhibitory effects of MH on flowering may be as a result of growth inhibition rather than a direct effect upon the flowering stimulus.

Bhatia(1978) observed that beneficial effect was due to MH in connection with yield of certain secondary metabolites in plants. Bhattacharya and Varsha (1980) showed that MH inhibited rootings at higher concentration. According to Gupta (1970) MH delayed initiation of bolting, flowering and fruiting. He also noted complete inhibition of flowering at higher concentration of MH. Although MH may inhibit cell division (Greuaea and Atchison, 1950) it may promote cell enlargement also. MH did not inhibit internode elongation in plants but can produce early flowering in plant when applied during cold treatment (Moore, 1980).

Tatum and Curme (1951) suggested that the accumulation of sucrose in the leaves after treatment with MH might alter to the C/N ratio to the extent of causing collapse of pollen grains in plant. MH has been noted to affect diosgenin synthesis particularly when it was applied at lower

concentration (Mandal and Chatterjee, 1984).

As regards the effect of the chemical on carbohydrate metabolism it was noted that hydrolytic activities were high under the treatment of NAA. NAA did not appear to be related with root formation. NAA was noted to adversely affect synthesis of strarch. This might be of the reason of failure of NAA to promote root formation over control (Basu et al., 1966).

NAA was observed to show male sterility in plants, (Moore, 1980). Production of seedless fruits, due to the treatment of NAA was notied by Gustafson (1936) and Hagemann (1937). NAA was found to be less deleterious to IAA and IBA (Boxy and Chatterjee 1967). Acharya Chowdhury (1968) showed that there was a rise of nucleic acid level as a result of NAA application. He showed significant decrease of total nitrogen as well as the non reduciong sugar. This is perhaps due to the fact that the conversion of sugar to nucleic acid might be facilitated by the exogenous application of NAA. IBA stimulated root growth in Dioscorea floribunda and D. composita cutting (Martin and Delphin, 1969).

Martin and Delphin (1969) noted that IBA stimulated root growth and treatment with ethylene chlorohydrine in a sealed container. At the rate of 0.25 mg/gm air space was noted to stimulate root growth in D. floribunda and D. composita cutting.Production of seedless fruits by the application of IBA

was noted by Gustafson (1936) ; and Hagemann (1937). In comparison to IAA , IBA was found to be more drastic in its action on growth. Swelling of hypocotyl was well marked due to IBA treatment (Boxy and Chatterjee , 1967).

Kano et al., (1982) showed that chlorocholine chloride (CCC) had the highest retarding effects on anthesis and at the same time it reduced the fruit-size and the content of solid sugars and vitamin C. Pappiah and Mathuswamy (1978) showed that CCC induced early flowering and yeild.

Shortening of internodes , thickness of the stem and some changes in the colour of the leaves , are affected by the treatment (CCC). Shah et al., (1978) reported the maximum yeild of plant due to CCC when applied at a very low concentration (25 ppm) Koinov et al., (1981) observed that the leaf ageing was retarded by CCC because of the improvement of photosynthesis activity.

It has been observed that the size of fruit in plant was reduced by the treatment of ethrel in comparison to the control shown by Pressman et al., (1983). Ethylene has been recognised as a potent accelerator of abscission (Zimmerman et al., 1931 , Gawadi and Avery , 1950). This was also supported by several workers (Hall ; 1952 , Acharya Choudhuri, 1963).To evevaluate the action of ethylene Biggs (1957) reported that

ethylene reduced auxin level and accelerate abcission. Similar observation was also reporeted by Addicott (1955).

Phenoxy acid like PAA effect was more or less identical with that of control. The effect was comparatively marked over untreated seedlings during later stage of root development. Hypocotyl growth was also inhibited in 2,4D and 2,45T treatment and those effefts were comparatively marked during initial growth phase. Gupta (1970) showed the increase of dry weight of root hypococyl due to the effect of phenoxy acids. Mchintosh et al., (1983) reported that 2,3,5 trichlorophenoxy acetic acid decreased club root weight by 40% without affecting weight of tops and clubs. All other phenoxy acetic acid starting from mono-to-penta and all monomythyl , dimythyl , phenoxy acid and 3,5 dimethoxy phenoxy acetic acid had no effects on clubs or tops , 3,4D treatment inhibited root growth and healthy as well as infected plants.

Gupta (1970) also further reported that amount of total nitrogen in root was much less in PAA and 2-C1 treated seedlings on 4th days which was later on followed by increased in TN upto 12 days. In 2, 4D and 2, 4,5T treated seedilings an increase in total nitrogen could be seen uniformly in both the roots and hypocotyl throughout the period of observations.

Chaturvedi and Choudhuri (1980) recently marked that tuber callus of D. deltoidea showed prolific growth on a modified

Sehenk and Hildbrandt's agar medium , a combination of 2,4D and IAA when the callus synthesized diosgenin (1.6%) during 60 days of incubation.

Suthar et al., (1980) showed that 2,4D; 2,45T in combination with GA3 and Kinetin enhanced diosgenin contains in callus tissues of D. assyptica considerably. High concentration of growth hormones restricted callus growth.

Liang et al., (1981) stated that respiration rate of potato tuber slices increased by the application of 2,4 Dinitrophenol (DNP).

According to Dall Olio et al., (1969) the chemical 2,4 5 TIBA in concentration of 50 and 100 ppm. delayed flowering and induced morphological changes in plants.

Inhibition of flowering was also recorded by Gupta (1970) by the application of TIBA at the early stage of flowering. Similar observation was also reported by Giuseppe and Seriti (1969).

Acharya Choudhuri (1968) reported that TIBA had a remarkable effect in hastening flowering. Besides promotion of flowering various abnormalities in reproductive parts were shown

in tomato plants. Similar observation was also reported by Zimmermann and Hitcochock (1949) and Giuseppa and Seriti (1969). Production of tillering was reported by Yamad et al., (1963) in presence of TIBA. Dall olio et al. (1969) reportrd that TIBA seemed to interfare with the transport of alkaloid from root to the leaves of the plant. Chatterjee (1963) observed acceleration of abscission due to TIBA.

Inorganic nutrients

NPK fertilizer has been found to be most effective in increassing the diosgenin content in C.speciosus (Singh et al., 1980 a).

Bacterial fertilizer

The role of micro organism in solubilizing the non available phosphate to make it available to plants is well known (Panova, 1956 ; Sundara Rao, 1965 ; Bardiya and Gaur, 1974 ; Gaur and Ostwal, 1972 ; Kabesh et al., 1975). It has been noted that several micro-organisms can solubilize phosphorous from rock phosphates and other insoluble phosphatic material (Koning, 1963 ; Sundara Rao and Sinha, 1963 ; Agnihotri, 1970 ; Arora and Gaur, 1979 ; Kundu and Gaur, 1981). The availability of phosphate for terrestrial forms of life depends on the continued solubilization of insoluble phosphate deposites, a process in which micro-organisms play an important role. Their metabolic products

solubilize the phosphate of calcium phosphate and hydrogen sulphid produced dissolves Ferric phosphate (Stanier,Adelberg and Ingraham, 1976).

The rate of soil biological responses to the addition of the organic compounds as an indicator of the micro-flora ability to regulate the conditions of soil habitat, Aristovskaya et al., (1988). According to them the ability of the micro-organisms to regulate the conditions of a soil environment is indicative of their ecological adaptability and resistance. In order to assess the regulatory mechanism which limits the range of variations in the chemical properties of soil, the authors porpose to use the rate and intensity of its biological responses to the addition of available organic compounds. They considered that the possibility of applying this procedure to the determination of an inhibiting or stimulating effect exerted by anthropogenic factors on the functional characteristic microflora might have occurred. For instance, they have noted that the biological response of a soil to the addition of glucose and starch changes when the soil is contaminated with heavy metals and when different cultivation methods are used.

Observation on pharmacognosy in costus speciosus and Dioscorea spp. with special emphasis on their starch content

Chakravarti et al., (1958) was the first to study rhizome of different species of Dioscorea with special reference

to starch granules. The pharmacognostic characters of the rhizomes of C.speciosus were studied by Tewari et al., (1947), while morphological and anatomical characters of the leaves collected from different geographical locations of the country were investigated by Sing and Srivastava (1980), Panda and Chatterjee (1980) analysed the microscopic characters of the rhizomes and found distinct relationship between the size and orientation of starch grains and diosgenin content.

In anatomical studies (Panda and Chatterjee 1980) show a distinct relationship between the microscopic characters of the starch grains and corresponding diosgenin content in the rhizome of Costus speciosus could be established. In higher altitude rhizomes, where a consistent high diosgenin content was prevalent, the starch grains were large in size their number and frequency of distribution in ground tissue as well as vascular bundle were lesser. In rhizomes of lower altitude the grain were abundant with relatively higher frequency, more in number/ cell and smaller in size.

Chakravarti et al., (1960) also stated that distribution of different sp. of Dioscorea could be made by starch grain characteristics.

The pharmacognostic characters of the rhizomes of C.speciosus were studied by Kapahi et al., (1977), while

morphological and anatomical characters of the leaves and stem collected from different geographical locations of the country were investigated by Sing and Srivastava (1980).