CHAPTER - I

REVIEW OF LITERATURE

Medicinal and Other Uses of Different Species of Dioscorea

Yams of *Diascorea alata* form a cheap source of carbohydrate food and are extensively used by the people of Assam, Bihar, West Bengal, Madhya Pradesh, Orissa and Deccan in India. They are of immense value during the period of scarcity of food. For use as food yams are washed either as a whole or in slices and cooked or baked to eliminate water soluble part of them. Even the best one among the cultivated yams cause irritation in the throat or a feeling of discomfort when eaten raw. The acridity is due to crystals of calcium oxalate.

The cultivated species is comparable to potatoe in taste and quality. The yams of *D. alata* are used for alcohol production. *D. alata* yams contain on an average 21% starch. The starch grains are transparent oval, rounded, or trangular in shape and do not separate easily during extraction with water. The yams are considered anthelmintic and useful in leprosy, piles and gonorrhoea (Kirtikar and Basu 1933; Chopra et al, 1956). The yams of *D. bulbifera* is also used mostly as a famine food. It is used for the preparation of starch in Japan. Poisnous alkaloids, volatile acids and calcium oxalate present in the yams should be eliminated by suitable treatment to obtain an edible product. The yams of the plant are used in Kashmir for washing wool and as fish bait. Dried and pounded tubers are used as an application for ulcers; they are also used in piles, dysentery and syphilis. Bulbils of wild species are used as an application for sores.

The yams of *D. glabra* are eaten in Andaman islands and Khasi hills, but are not much liked as they become gluey when cooked. The yams of *D. oppositifolia* are used as an external application, after grinding and heating, to reduce swellings (Kirtikar and Basu, 1933). The nutritive value of edible yams of *D. pentaphylla* is nearly the same as that of *D. alata*. The flowers are often collected and used as vegetable. Leaves are also eaten at the time of scarcity. Yams are used to disperse swellings and as tonic. The yams of *D. prazeri* contain

saponin and are used as fish poison and for killing lice. The yams of *D. puber* are edible and are reported to be good though it may emit an offensive odour when cooked. (Chopra *et. al,* (1956). Lee *et al* (1999) has established antidiabetic activity of *D. batatas.*

Importance of *Discorce* sp. in Connection with 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 commonly used now-a-days for the production of drugs on industrial scale and the third one involves cumbrous form of synthesis in laboratory condition and which may sometimes become uneconomical in nature (Velluz 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 huge expenditure the use of some what cheaper sterol such as stigmasterol was encouraged (Fieser and Fieser, 19659), 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 artificial synthesis of sex hormones and cortisones involves a lengthy process and at the same time is an expensive one. For these reason 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 corticosteroides are the two major 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 (Chakrabarti et al., 1961). Gravity of this situation was realized 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 synthesis 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 utilization. This led to an intensive phytochemical survey on vegetable sources during the last three decades to search steroid precursor of plant origin which would be cheaper and potentially 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 decades, it has been noted that various steroid compounds such as stigmasterol (Fieser and Fieser, 1959), cholesterol (Fernholz, 1933; Butenandt et al., 1934; Chakravarti et al., 1956) sitosterol (Chawla, 1977), desozycholic acid (Sarett, 1946; 1948, Correl et al., 1955), sarmentogenin (Lardon and Reichstein, 1958), hecogenin (Aplezweig, 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 venesosum, Strophanthus sarmentosus, Agave spp., Costus spp.*, and *Dioscorrea spp.* were being used by various workers.

Fig.I shows the molecular formulae of several compounds. From national point of view, the two starting materials which are of maximum interest at present in India are diosgenin and solasodine, a steroidal alkaloid.

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; Dherassi; 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 synthesis 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 contraceptives have no oxygen substitution in the molecule. Hecogenin therefore provides a partial starting material for the synthesis of the corficosferoids 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 pregnene nucleus during microbial transformation.

The matter of research on steroidal chemicals was proved to be fruitful when Fuji and Malsukawa (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, different 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 material often gives a better yield (Chakraborty et al., 1958). The water insoluble sapogenin is then extracted with a suitable organic solvent.

Until 1970 diosgenin isolated from the Mexican yam was the sole sources for the manufacture of steroidal contraceptives (Bammi and Randhawa, 1975). With the nationalization of the Mexican industry, however prices increased to such an extent that manufacturers switched over to utilise different others precursor for the synthesis of steroid compounds.

Utilisation of Diosgenin for Production of Steroids in Phytochemical Industry

To the pharmacogonisist steroid chemistry has always been a fascinating subject. This is not only because of this complicated and interesting chemistry involved in steroid reactions but also because of the incipient recognition of numerous and diversified physiological functions and pharmacological effects, such as, influence on carbohydrate, protein fat and purine metabolism; on electrolyte and water balance, on the functional capacities of the cardiovascular system, the kidney, skeletal muscle, the nervous system and some organs and tissues.

Diosgenin is isolated from *Dioscorea* spp. by initial bydrolysis of the root with mineral acids followed by extraction of the liberated diosgenin in the hydrolized root cake by hydrocarbon solvents followed by isolation of the diosgenin from these solution. However, at least one company extracts the dioscin with polar solvents and isolate the diosgenin by hydrolysis of purified dioscin (Kunjithapadam, 1977). The outline of the scheme for the production of 16-DPA from diosgenin have been represented in Fig.2.

The 16-DPA is converted into the 16-17-epoxide with alkaline hydrogen peroxide. The epoxide is converted through the corresponding bromohydrin to

the 5-pregnene, 3-17 diol which is converted through a series of reactions of Reichstenin's Substances 5. Substances 5 is converted into hydrocortisone by a fungal hydroxylation and subsequently into prednisolone again by microbial enzymes. Recent work in this area includes use of immobilized enzymes of microbial cells for controlled transformation of this type. Fig. 3 shows the production of cortisone and Hydrocortisone from 16-DPA.

Progesterone is synthesized chemically but 11-hydroxylation of progesterone is caused by the fungus *Rhizopus arrizus*, later *R. nigricans* was found to hyxroxylate progesterone at 11-position in higher yields. 11-Hydroxy progesterone, available in high yield by microbiological oxidation of progesterone is also an attractive intermediate to cortisone (Dherassi, 1966). The outline of the synthesis of progesterone and 11-Hydroxy progesterone from 16-DPA has been represented in Fig. 4.

16-DPA is reacted with hydroxylamine hydrochloride and the resulting 20-Ketoxime is submitted to a Beckmann rearrangement to the amide which on hydrolysis with hydrogen chloride gives the 17-Keto compounds, DHA acetate (Fieser and Fieser, 1960).

DHA acetate is reduced to the corresponding 17-alcohol with sodium borohydride in mixed methanol tetrahydrofuran solution and the 5-androstene-3-17-diol-3 acetate is converted into testosterone in a seris of reaction.

Again, for the synthesis of methyl testosterone DHA acetate is reacted with methyl magnesium bromide to form methyl androstene diol which is oxidized by Oppenauer procedure to methyl testosterone. (Fig.5).

Commercially Important Species of Dioscorea in India and Abroad

As regards Dioscoreas, *D. composita*, *D. floribunda* and to lesser extent *D. spiculitora* and *D. mexicana* are used in Mexico and Guatemala commercially for production of diosgenin while in China, *D. singierensis* is the commercial source of diosgenin. Various commercially important species of *Diascorea* used in different countries in the world has been represented in Table - 1. In India

D. prezeri and *D. composita* are commercially used in eastern and north eastern 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. Commercially important species of *Dioscorea* now-a-days utilised in pharmaceutical industry in India has been represented in Table - 2.

Table - 1: The Commercially important species of *Dioscorea* used in different countries in the world :

Sl. No.	Species	Name of the country
01.	*Dioscores bakanica*, Kosanin	Europe
02.	*D. belixensis* Lundell	Central America
03.	*D. composita* Hemal.	Mexico
04.	*D. deltoidea wall*	Nepal
05.	*D. floribunda* Mert & gall	Mexico, Central America
06.	*D. friendrichalli* Kounth	"
07.	*D. hundurenzis*, Knuth	"
08.	*D. medicana* Guill	"
09.	*D. spiculiflora*, Nemol.	"
10.	*D. sylvatica* Ecklen.	South Africa
11.	*D. villosa*, hundell	United States
12.	*D. singierensis* Kunth	China
13.	*D. prazeri* Prain & Burk	"

Table - 2 : Different species of *Dioscorea* yielding yams for commercial utilisation in India (Kunj thapada, 1982).

Sl. No.	Botanical Source	Sapogenin	% of Sapogenin	Habit and cycle
01.	*D.deltoidea* (Rhizone)	Diosgenin	2-5%	Growing wild in Himalayan region. Organised culture not very successful. Cycle : 5 years.
02.	*D.prazeri* (Rhizome)	Diosgenin	1-3%	North Eastern Region, can be cultivated in this region.Cycle : Not available.
03.	*D.floribunda* (Rhizome)	Diosgenin	2-5%	Central American sps grows well in peninsular and Northern India low altitude location. Cycle : 1-3 Yrs.
04.	*D.composita* (rhizome)	Diosgenin	2-4%	Central American sps. Cycle : Abour 3 Years

Cultivation of *Dioscorea sp.* in India

At a symposium held in Lucknow in the year 1952 Dr. R. N. Chakravarty of the School of Tropical Medicine Calcutta, was the first to suggest the possibility of exploitation of diosgenin containing Indian *Dioscorea sp.* He mentioned that *D. deltoidea* and *D. prazeri* growing wild in North Western and North Eastern Himalayan regions respectively contained appreciable amount of diosgenin. This

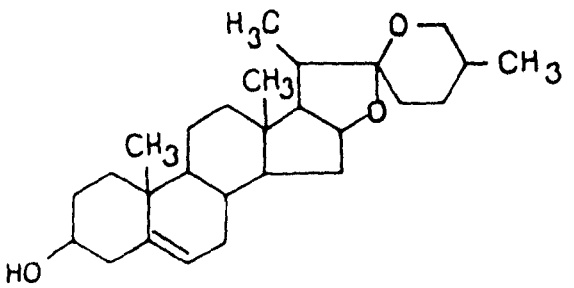
generated considerable interest in some pharmaceutical firms. An Indian company CIPLA entered the steroid field and began producing diosgenin in 1961. Subsequently CIBA-GEIGY, SEARLY and ORGANON also started manufacturing sex hormones. Glaxo (India) switched over to local diosgenin production and subsequently to its intermediate for producing beta-methasone. In 1966-67 CIMAP (CSIR) entered the commercial production of diosgenin in its drug factory, Jammu utilising the wild *D. deltoidea*. It was for the first time that in Eastern India the Directorate of Cinchona & other medicinal plants, Govt. of West Bengal started on a commercial scale for production of diosgenin and also entered to produce down stream products of diosgenin. At one time India depended almost entirely on the wild *D. deltoidea* for its diosgenin. the large scale collection of yams has resulted in dipletion of forest resources and in some areas complete eradication of the wild plant is well noticed. The natural regeneration of this species required more than seven years and it has been felt necessary to bring it under cultivation. Successful cultivation of *D. floribunda* and *D. composita* in Bangalore, Jammu, Goa and other parts of the country was reported from 1973 onwards.

Chemical Constituents isolated from different species of *Dioscorea* sp.

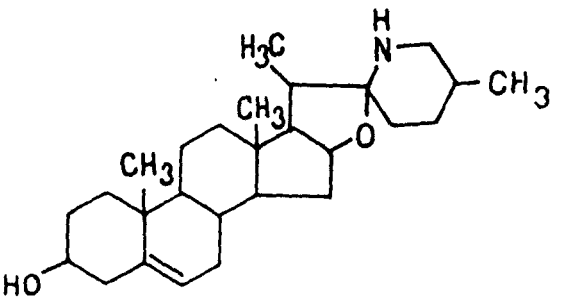
Different species of *Dioscorea* are the store house of various chemical constituents. Various chemical constituents which have been isolated so far from different species of *Dioscorea* are steroidal in nature and have been represented in the table 3.

Table- 3: Chemical constituents isolated from the yam of different species of *Dioscorea*

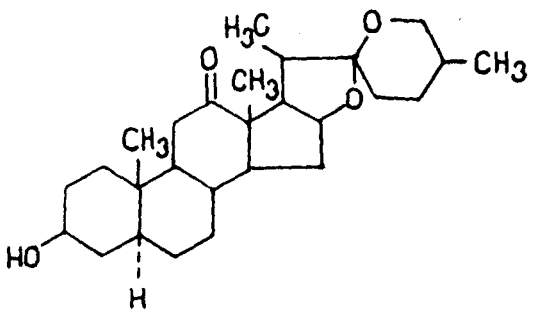
Species	Chemical Constituents	Reference
D. prazeri	*Diosgenin*	*Kunjitapadam, 1977*
	Prazerizenin A-glucopyranoside	Rajaraman and Rangaswami (1982).
	Prazerizenin A-ramnogluco pyranoside	Do
	Prazerizenin -D	Do
	Prazerol (9,10-dehydrophenathrene)	Biswas et al. (1988)
D. gracilima	Trisacharides of Diosgenin A and B	Rang & Vie Feng (1984)
D. panthiaca	Saponin	Li et at. (1986)
D. canposita	Diosgenin	Kunjithapadam (1977)
D. floribunda	Diosgenin	Do
D. deltoidea	Diosgenin	Do
D. hispida	Dioscorine	Lecte et al.(1988)
D. rotundata	Dihydrostilbene	Fagboun et at. (1987)
D. leullufera	p-hydroxyacetophenone	Gupta & Singh (1989)
D. septamoilea	Diosgenin B.	Lin and Yanyong (1985)
	Sitosterol	
	Palmitic acid	
	Diosgeninpalmitate	
	35-deoxytigogenin	
D. collettii var hypoglanca	Dioscin	Hu Dong et al (1996)
	Gracillin	Do
	Protoneodioscin	Do
	Protoneodioscin	Do
	Protogracillin	Do
	Methylprotodioscin	Do
	Methylproteodioscin	Do
	Methylprotogracillin	Do
D. delicata	Furostanol saponin	Haraguchi et al. (1999)



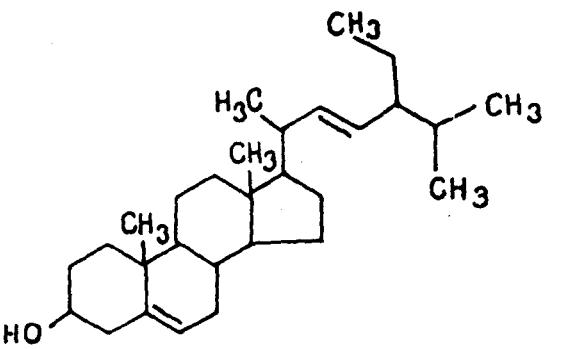
Diosgenin



Solasodine



Hecogenin



Stigmasterol

Fig: 1. Different types of Steroids and Steroidal alkaloid and their molecular structure utilised for the production of 16-DPA.

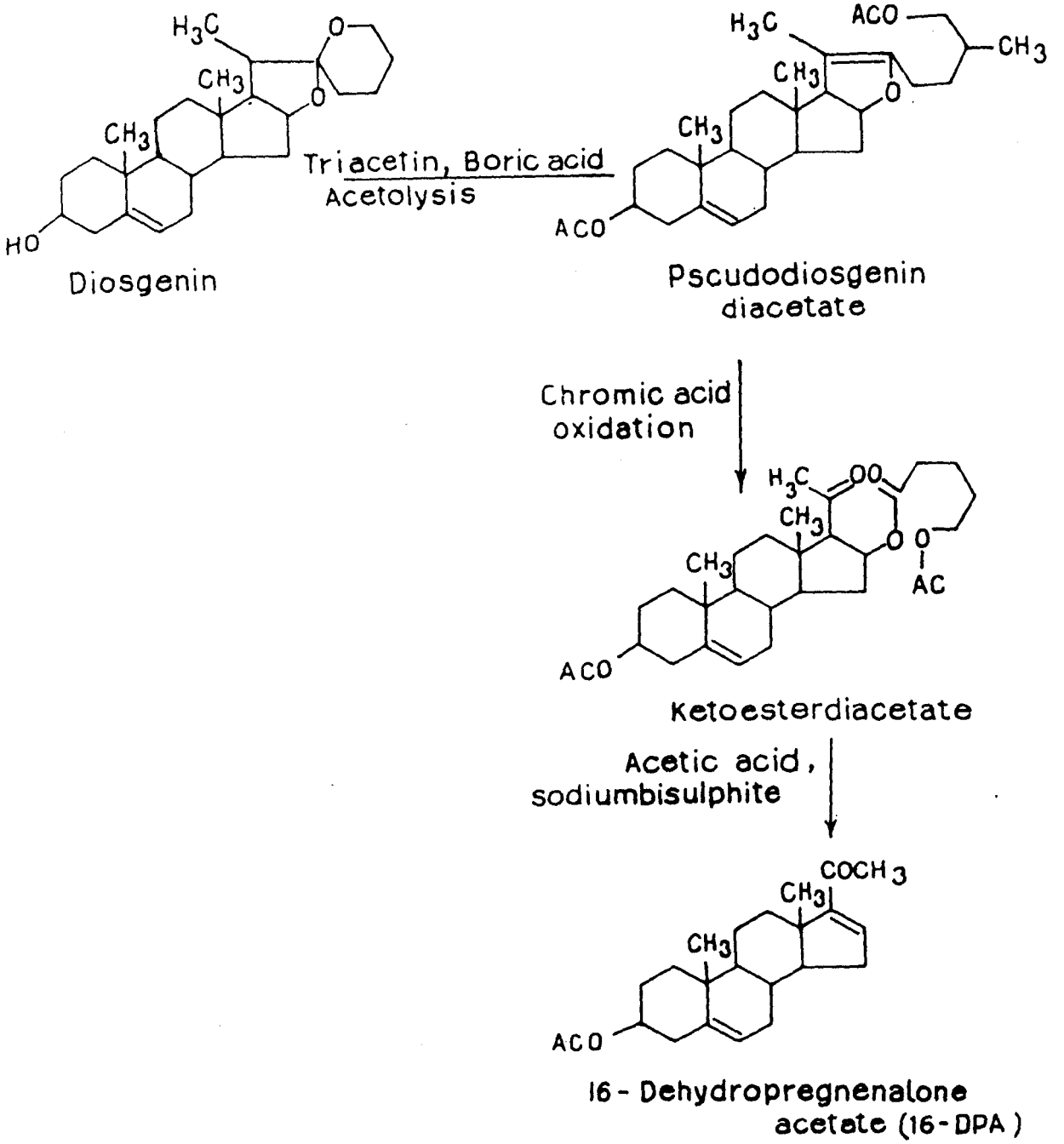


Fig. :2. The scheme representing production of 16-Dehydro pregnenalone acetate from Diosgenin .

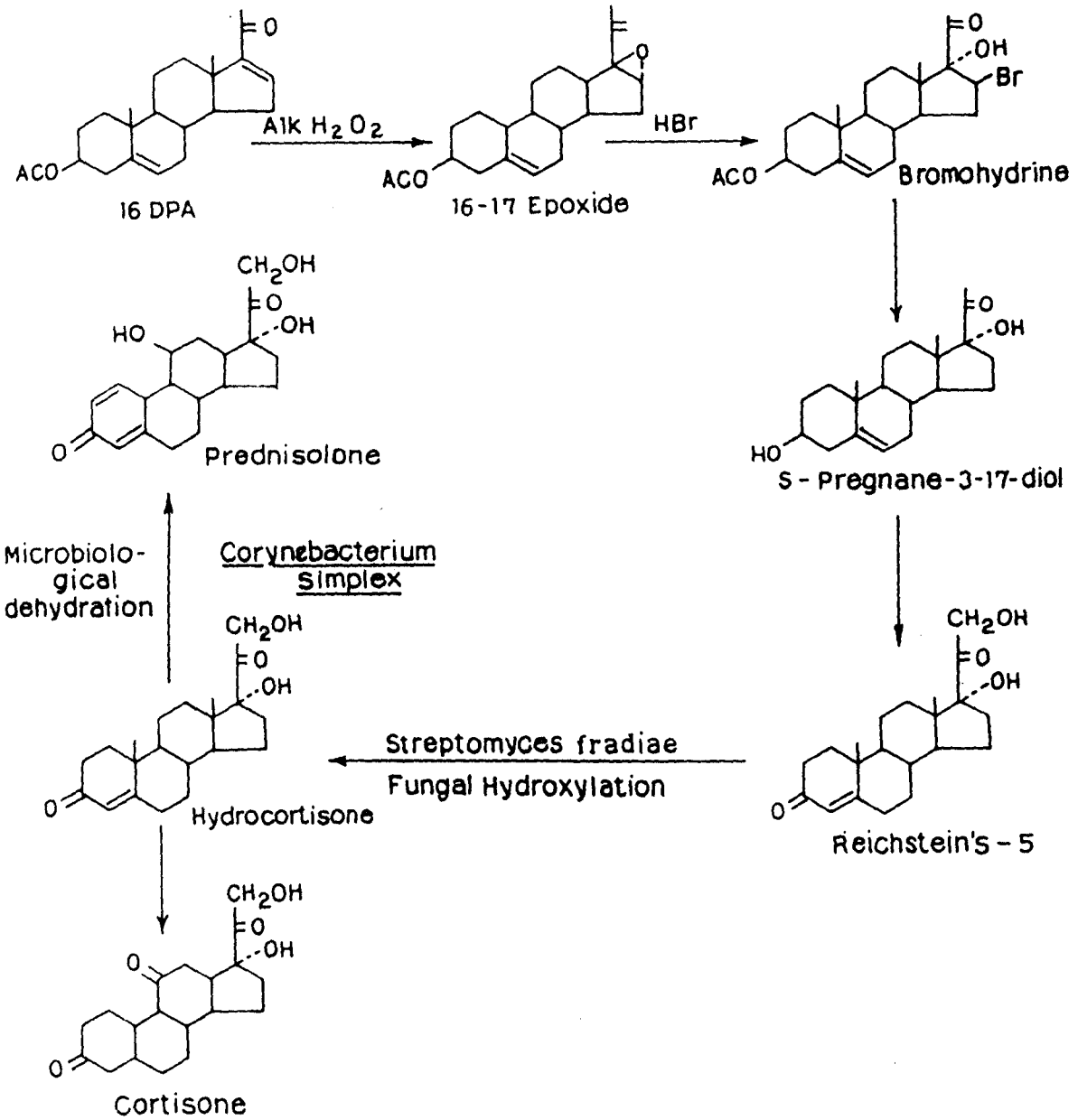


Fig: 3 Shows synthesis of corticosteroids from 16-DPA.

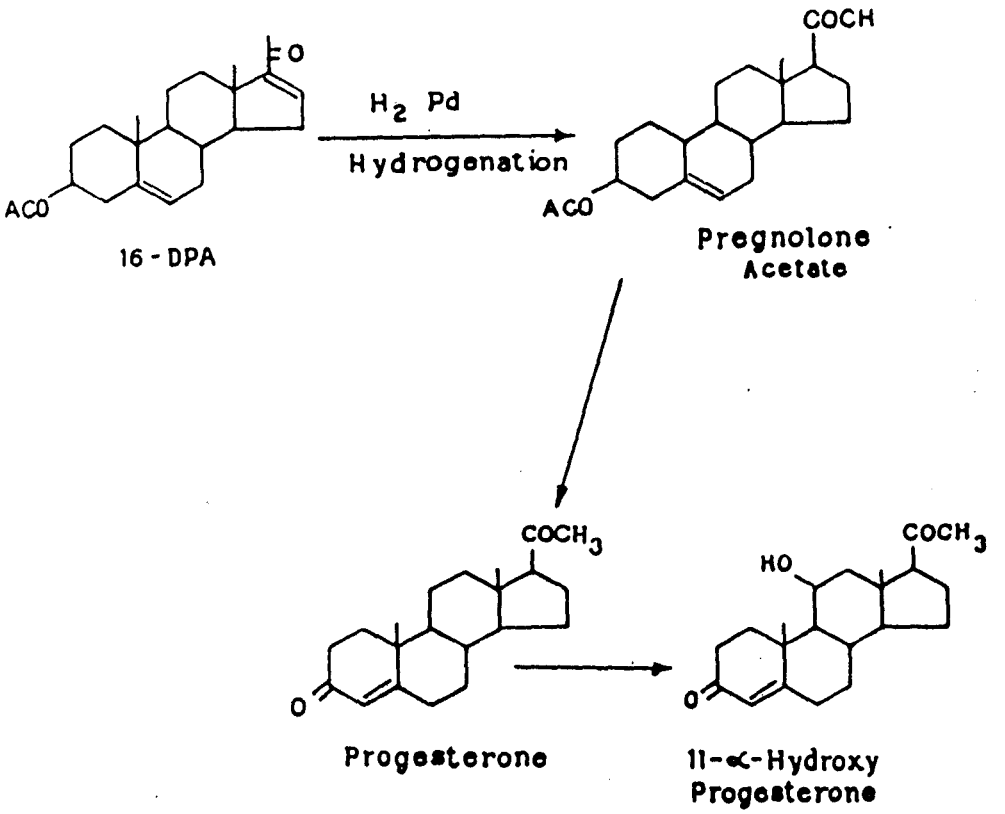


Fig. 4. Shows synthesis of progesterone and 11 - Hydroxyprogesterone from 16 - DPA.

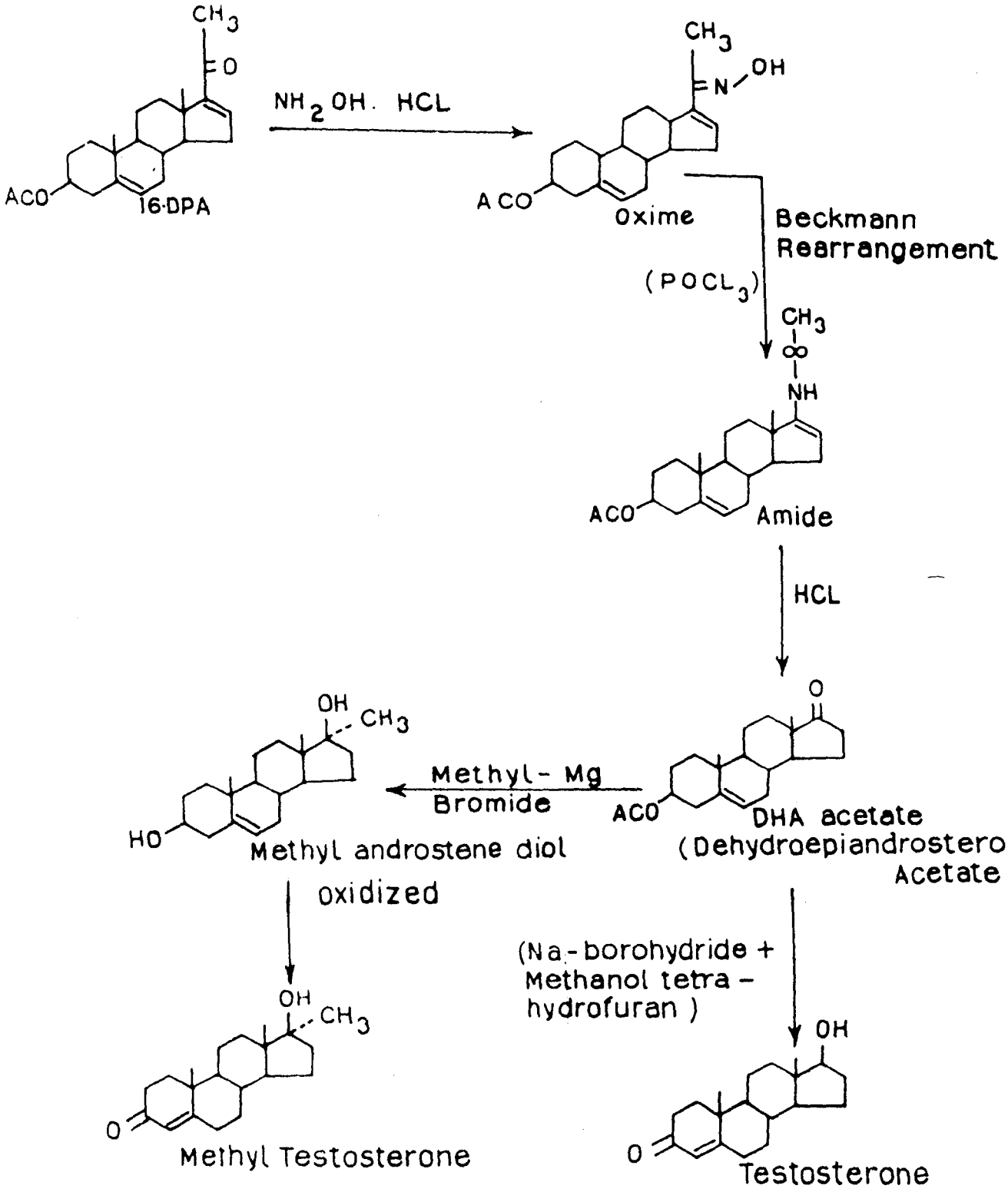


Fig. : 5. Shows synthesis of Testosterone and Methyl Testosterone from 16 - DPA .

Isolation and Purification of Diosgenin in *Dioscorea* sp.

The chemical method recommended by Marker et al, (1943) for the isolation of sapogenin have been noted not to be feasible from commercial point of view. According to their procedure the steroidal saponin extracted by ethyl-alcohol from ground plant material, was acid hydrolized 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 recrystallization 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 operation 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 pharmaceutically in unimportant diene form of diosgenin (Fig. 6) Acid hydrolysis was avoided by aqueous hydrolysis and simultaneous extraction of sapogenin. Subsequently, Chakraborty et al. (1970) used a modified method in which they used 2N HCl in the 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°-60°C) was washed with NaOH solution and evaporated to give a product which was chromatographed over neutral alumina. Ralatively pure sapogenin was then acetylated and diosgenin was estimated in the acetate form. Preston et al., (1961) used (1.5)N HCl to hydrolyze the sample for 5 hours. Selvaraj and Subhash Chandra (1980) observed that the hydrolysis of dry powder of *D. floribuna* with 2.5 (N) HCl for 2 hours would give a product which, on chromatography 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. ehter (40°-60°C) for 8 hours. The extract was concentrated to about 50 ml., when crystal of diosgenin began to appear. At this

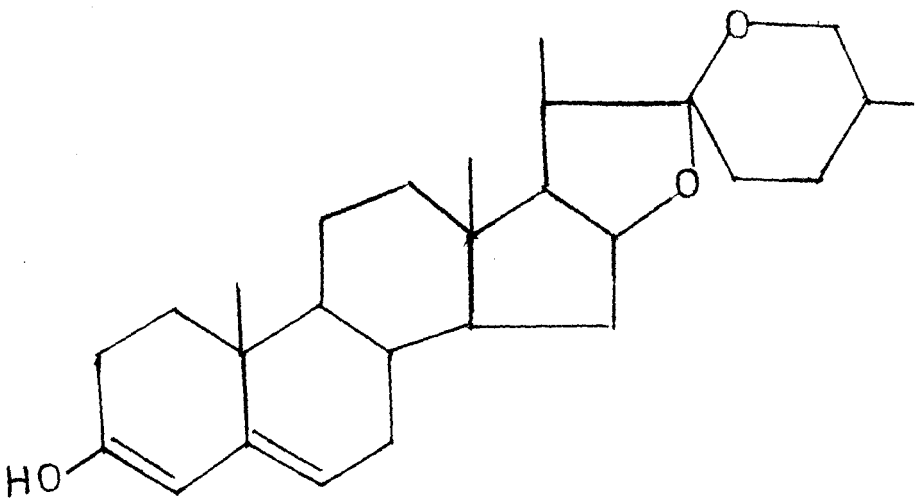


Fig.6 Diene form of diosgenin

stage the flux containing the crystal was refluxed for 1 hours. After cooling crystals were filtered through a sintered crucible and washing was performed with a fresh quantity of 50 ml. of cooled pet. ether to make the crystal free from any colouring matter. Further 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 centrigrade 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 warer. After cleaning the roots the excess of adhering water was removed 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 presence 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 hexane 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°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) equipped 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. According to Chakraborty et al. (1958) the size of the particle of tubers is of considerable importance from the stand point of efficient extraction. Sixty mesh powder 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 conditions varying from 2 N HCl refluxed for 2 hours to alcoholic H_2SO_4 treated for 20 hours. In none of these cases optimum hydrolysis condition 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 crystalline product having m.p.200°C. This material of about 95 to 100% purity was found satisfactory in the usual test for the preparation of 7-dehydrodiosgenin in 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 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 (m.p.202-204°C). The purity of the product was checked 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.

Quantative Estimation of Diosgenin in *Dioscorea* sp.

In connection with various methods so far utilized for the quantitative determination of diosgenin, Selvaraj (1971) extracted diosgenin and estimated it gravimetrically. He took yams of *Dioscorea* which were washed, dried and powdered. 20 gms of the powder was taken, hydrolysed with HCl (2.5 N) for 3 hrs. and filtered. The residue was repeatedly washed to make it acid free. It was then dried for 6 hrs. at a temperature of 100°C. It was later on extracted with petroleum ether (40°C-60°C) in soxhlet for 3 hrs. The extract was concentrated and cooled and then filtered. The diosgenin was calculated on dry weight basis.

Glyzine et al (1981) utilized GLC for quantitative determination of diosgenin in *Dioscorea.* The raw material was ground and hydrolysed with 2(N) HCl. The product obtained was dried and extracted with a mixture of chloroform and ethyl alcohol (1:9 v/v) to obtain diosgenin. After isolation GLC was used for estimation of diosgenin content. Later the GLC technique was developed by Azorkova *et al.* (1978). They determined the diosgenin content in *Dioscorea* to 0.11-2.71% using GLC. Tang et al (1979) also utilised the GLC for quantitative analysis and identification of steroidal sapogenins of *Dioscorea.*

Pasehnichenko et al (1978) estimated the colorimetric method for the determination of glycoside bound diosgenin inthe suspension culture of *D. deltoidea* using concentrated H_2SO_4 and 1% formaldehyde.

Recently Raman et al (1995) studied diosgenin involving ^{1}H and ^{13}C spectral assignment of the compound by using two dimensional NMR technique and resulted in unambignous spectral assignments specially in the convoluted region of the spectra.

Bio synthesis of Diosgenin and related steroids in *Dioscorea* sp.

It has been noted that steroidal saponin arises via the mevalonic acid pathway to produce "squalane". The subsequent cyclization of squalane to give cholesterol is well established (Croey et., 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 cholesterol is rapidly formed and metabolised. The ability of cholesterol to serve as precursor 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 3, 22, 26-tropl 3.chaco side 26, D-glucopyrenoside) are formed from cholesterol. He showed that in *Dioscorea floribunda* homogenates choleserol was converted directly to dioscin i.e. diosgenin glucosides.

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 (Joly et al. 1969 a,b).

According to some authors (Bennettal Haftman) 1965 ; Joly et al. 1969) cholesterol and sitosterol are the precursors to form of saponin and cholesterol is directly converted into diosgenin, Sitosterol, however, require a two carbon unit from C-24 and may proceed oxygenation. The sequence in which oxygen is introduced 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). Cholesterol, however does not

appear to be an obligatory step in the bio-synthesis, since desmosterol is converted to saponin without going through cholesterol (Tschesche et al. 1974).

Factors Affecting Growth and Development *Dioscorea* sp. and Production of Diosgenin

Karnic (1975) reported that *D. prazeri* and *D. deltoideal* from different locations in India yielded varying percentage of sapogenin content at various stages of growth. Sapogenin concentration increased with the age of the tuber. The optimum content of sapogenin was found when the plants were just shedding i.e. in dormant stage, which appear to be the best for commercial exploitation.

Gangadhara (1974) pointed out distinct effect of external and internal factors on productivity patterns of active principles in some *Dioscorea* sp. and such an increase in productivity was found to be functions of topographical, ecological, enviornmental as well as of some biochemical factors. Enyi (1970) found that there was a positive correlation between rainfall and vine growth, vine weight and tuber yield of *D. cayanensis* which required long growing season for maximum production; whereas in *D. alata, D. rotundifolia* and *D. esculenta,* much shorter growing season was required. According to Gooding (1970), rainfall below 100 cm during the eight months growing season appeared the limiting factor in *D. alata.* Wilson and Mapother (1970) concluded that in *D. deltoidea* plants raised from seedlings and set out in May, tuber yields were meximum after seventeen to nineteen months. Diosgenin percentage augmented during the first year after which there was a little fluctuation in its content.

Shelvaraj et at. (1971) investigated the distribution of diosgenin in one year old tubers of three sapogenin bearing species i.e. *D. composita, D. floribunda* and *D. deltoidea.* The intact tuber was separated into three different portions used generally for propagation i.e. crown, median and tip. The sapogenin content was found to be more in the dorsal portion in the tuber than the ventral portion in *D. composita* and *D. deltoidea* on fresh weight basis. However, in *D. floribunda* the ventral portion had the maximum content. Tip portion of the tuber in *D. composita,* the median portion of the tuber in *D. floribunda* and

D. deltoidea had the maximum sapogenin content. They also reported that the diosgenin content of *D. floribunda* tuber had closer correlation with the dry matter production of two years old tuber. It was claimed that *D. floribunda,* planted in April in Bangalore region, was the best as it gave quicker sprouts and higher tuber yields. Bammi and Randhawa (1975) did not find any correlation between girth of tuber and diosgenin content but a positive correlation was obtained between available phosphorus in soil and diosgenin content in tuber, whereas the level of available potassium in the soil was negatively correlated with diosgenin content.

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Bammi and Randhawa (1975) suggested that 40 to 60 gms. pieces of *D. floribunda* tuber as a planting materials were more economical. They also suggested that as the crown portion of the tuber contained less sapogenin compared to median and tip, the crown portions could be used as planting materials having the maximum survival percentage to get the maximum return. Nutritional conditions affect the general growth of the plants as well as actual

formation of active principles. Cruzado et al. (1965) claimed that complete fertilization in *D. composita* increased diosgenin content. The fertilization effect had also been studied by Ferguson and Hynes (1970) in *D. esculenta* and *D. alata*. Nandi and Chatterjee (1978) showed the phosphorus fertilization to enhance extension growth of *Dioscorea*. The enhancement of diosgenin content by phosphate and its decrease by nitrogen fertilizers in *D. bulbifera* var *pulchella, D. pentaphylla* and in *D. composita* have also been observed. Singh *et al.* (1981) observed significant interaction between N and P, N and K and N with P and K on diosgenin yield. They also noted that the tuber yield/plant and tuber production per unit area of *D. floribunda* to be increasing significantly with increase in N and P dose. Khan and Shakeel (1990) showed that application of N. P. fertilizer at 135 Kg N and 65 Kg. Phosphorus perhactre as basal does gave higher yield as compared to control.

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 , 1996 ; Nitsch, 1957 ; Vence pure 1975 ; Nandi and Chatterjee 1978 ; Singh and Nanda, 1981). Akahori et al., (1970) reported that in *Dioscorea tokoro* this application of light increased the amount of yamogenin and tokarogenin in long day conditions. Akahori et al (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* and Wright et al (1989) in *D. Zingiberensis*

Light influences a number of biochemical, physiological and morphological characteristic of plants including steroid metabolism. Generally dark grown plants contain more steroidal constituents on 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 changed this process. The increased accumulation of some sterols in plants during this phase of plant growth has been linked to the disorganisation of intra cellular organelles (Duperon, 1971).

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 various informations are available in this respect, (Down, 1956; 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 information is 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). Phytochrome is a tetra pyrole structure and intermediate forms between red and far red of phytochrome exist.(Linschitz et al.,1966) These intermediates, represent different forms of the protein component of the phytochrome system although they represent two distinctive conformational states of the protein. This protein has in fact credited with enzyme activity (Tezuka and yamamoto, 1969) which raises the 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 Karisson (1988). Phytochrome is not involved in the red light enhancement of the stomatal blue light response in plant. The stomatal response to blue light 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. 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 than in blue light (Mc Cree, 1971), But in red light, 68% of the added dry matter was due to carbohydrates as compared to 42% in blue light Das and Raju (1965) has revealed that blue light stimulates accumulation of protein and other non - carbohydrate substances. Though diosgenin synthesis is very much related to carbohydrate metabolism (Benntt and Haftman, 1965 ; Jolly et al., 1969) but no report has so far been made in connection with the effect of quality of light on diosgenin But the effect at climatic factors on diosgenin production in *D. zingiberensis* has been worked out by Wright et al (1989).

Khan and Shakeel (1990) worked out the opitimum period of growth of D.deltoidea. They observed that monsoon planting provided more number of sprouts and higher yield of rhizomes.

From literature it appears that IAA retards the growth of plant and delays the onset of senescence 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; (Leopold, 1960 and Hess, 1962).

Although 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 in explant during culture; (Genus 1975, Hardman and Stevens, 1978; Joans). In their experiments IAA and 2, 4 - D have been noted to increase diosgenin content in several plants including *Dioscorea,* (Marshall and Staba, 1076).

Chaturvedi and Choudhuri (1980) reported that in tuber of *Dioscorea deltoidea*, IAA and 2, 4-D combination yieled maximum diosgein 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). Seasonal changes in abscisic acid in *D. florilemda* has been worked out by Farooqui et al (1989) and seasonal variation in diosgenin content in this leaves of *D. composita* has been studied by Hensl et al (1987).

Bhatia (1978) observed the beneficial effect was of MH in connection with yield of certain secondary metabolites in plants. Bhattacharya and Varsha (1981) 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 could 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 has been 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 noted by Gustafson (1936) and Hagemann (1937). NAA was found to be less deleterious to IAA and IBA (Boxy and Chatterjee 1967). Archarya Chowdhury (1968) showed that there was a rise of nucleie acid level as a result of NAA application.

He showed significant decrease of total nitrogen as well as the non reducing sugar. This is perhaps due to the fact that the conversion of sugar to nucleie acid might be facilitated by the exogenous application of NAA. IBA stimulated root growth in *D. floribuda* 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. floribuda* and *D. composita* cutting. Dormancy in seed germination has been observed in *D. composita* (Viana and Felippe,1990)

Application of biotechnology in *Dioscorea*

Mass multiplication of Dioscorea through tisse culture was first demonstrated by Chaturvedi (1975).In a confirmatory report by Lakshmi Sita et al (1976), the role of tisse culture has also been emphasized in propagation of *D. floribunda*. Since the first publication (Chaturvedi, 1975), nuch advancements had been made by way of increasing this rate of multiplication, derivation of minimal rooting medium, standardization of techniques and testing.the method on a large scale. Clonal multiplication of *D. deltoidea* by in vitro culture of single node leaf cutting, and shoot apex culture was also reported for the first time by Chaturvedi et al (1977). Induction of rooting in stem cuttings was quite difficult as compared to similar explants of *D. florilenda*. In an optimum treatment containing 1.5 mg/l NAA + 1.0 mg/l indole butyric acid (IBA) or vice versa, only 50% cutting could be rooted. Later on it was found that a combination of 4 auxins i.e. NAA, IBA, 2-4-D and chlorogenic acid considerally improved the rooting percentage. A large number of invitro raised plants of *D. deltoidea* were observed growing normally in soil under glass house conditions.

Shoot apices of *D. deltoidea* measuring 1 mm in length and comprising meristam and 2 youngest leaf primardia were successfully regenerated in to complete plants withen a period or 90 days, which grew normally in potted soil and developed tubers (Chaturvedi *et al.* 1977) Besides clonal propagatan successful short apex culture is especially useful for the elimination of viruses,

several of which are reported to infeet Dioscorea (Water Worth *et al.* 1974).

There have been two reports on regeneration of plants from somatic callus cultures of *D. deltoidea* (Grewal *et al.* 1976; Mascarenhas *et al.* 1976), but the exact mode of differentation of plant lets was not brought out. Chaturvedi (1979) showed that the differentiating cells of callus cultures of *D. floribunda* and *D. deltoidea* followed the embryo genetic planted formation. By closely investigating the histology or anatomy of embryogenesis even the "single cell state" in embryoid formation was traced, in the proembryogenic tissue for both the *Dioscorea* spp. Embryogenesis was induced in the treatments containing 0.25 to 0.5 mg/l BAP plus 1 mg/l indoleacetic acid in both the calli of *D. floribunda* Embryogeneisis was augmented in tuber callus of *D. deltoidea* when the conc of IAA was increased to 3 mg/l in the above pair, where as in its leaf callus it could be induced, through to a lesser extent, by substituting Zeatin for BAP.

Chaturvedi (1979) isolated embryoids from the callus culture of *D. floribunda* Mature embryoids presented a large assay of different sizes and shapes from bell and funnel shaped to disc and star shaped, however the most interesting were the "dicotyledonous" embryoids.

In cultures showing optimum differentiation, even more than fifty plant lets were formed per culture. Further several "crops" of plantlets were obtained from a single differentiating culture by repeactedly excising the shoot and sub culturing the remaining portion. The callus regenerated plants showed variation in vigour, leaf, shape and size and in their ploidy level; from diploid to poly ploids and aneu ploids. Rarely, albino mutants to those having variegated leaves were also isolated from differentiating leaf callus of *D. floribunda* (Chaturvedi, 1979:)

An easy method for obtaining a large number of tetraploids of *D. floribunda* in a short time by the application of colchicine in aseptic culture was developed such tetraplord shoots were rapidly mass multiplied through in vitro culture of single node leaf cuttings and a large number of tetrapoid plants of *D. floribunda* were obtained growing in soild. This process has great significance for obtaining

tetraploid *D. floribunda* which may be expected to produce bigger tubers with higher diosgenin content. A competent embryogenic suspension culture of *D. opposita* has been obtained. Embryogenic callus was induced from stem segments cultured on an agar solidified M.S medium containing 2, 4 D for one month following placement as the embryogenic callus in a liquid medium containing 24 - D. The embryogenic tissue began to proliferate rapidly established suspension cultures consisted almost entirely of early stage proembryo with very little contamination from embryonic tissues. (Nagasawa et al 1989) The growth of *D. deltoidea* at high sugar concentrations was studied by Ball *at al* (1989). The ratio of fresh weight to dry weight of cells was observed to be dependent on the initial sugar concentration. However, it remained farely constant as long as the sugar was presant in the growth medium. Mitchell *et al* (1995) studied the effect of explant source, culture medium strength and growth regulators on the in vitro propagation of three Jamaican yams, i.e. *D. cayenensis,* D. trifida and D. rotundata. The results suggest that modal segments excised from young fast growing lines of these species are the best explant source for the purpose of commercial micropropagation.

Ravishankar and Grewal (1940) considered that AT Pase activity was an index of growth capability of cultured cells of *Dioscorea* and the character could be used as a marker for screening cell lines for improvement of growth in *Dioscorea* Browing of explants and callus tissue interrupts the growth of callus and its redifferentiation in tissue culture of chinese yam, *D. opposita.* Culture methods and conditions were examined by Kuginuki and Nishimura (1989) to prevent the browning of cultureed tissue of the plant. Browning of callus tissue was observed to be suppressed with vigorous growth in liquid medium in vertically rolated culture. The callus induced from axillary bud or young male influorescence showed little browing compared with that of others. When plant growth regulators was added to the M.S. medium callus growth was opitimum and the incidence of browing was the lowest at 2,4 -D and B.A. Addition of cytokinin in the medium enhanced the browning of the callus. Twyford et al (1990) worked out characterisation of the yam of *D. alata, D. cayenensis, D. rotundata, D. esculenta* and *D. bulbifera,* during tissue culture using isoelectric focusing of peroxidase and acid phasphatase isoenzymes.

While the diosgenin is synthesised particularly in the growing shoots of *Dioscora*, the tuber is only the storage organ for diosgenin and does not synthesize it (Bennelt *et al* 1963; Barker *et al,* 1966). However dispelling this belief, Chaturvedi, and Srivastava (1976) for the first time demonstrated with *D. deltoidea* that even tuber tissue could biosynthesize diosgenin. Initially, the percentage of diosgenin was 0.7, which was stepped up to more than two fold i.e. 1.6 by making certain changes in the composition of medium but with out adding any precursors.This is perhaps the highets percentage of diosgenin reported from *Dioscorea* callus cultures without feeding precursors. The two fold increase in fresh weight of callus in 2 months obtained in this study is also the highest record so far (Chaturvedi, 1970). It may be mentioned that earlier, diosgenin production from cultures, obtained by callusing the entire seedlings of several species of *Dioscorea* has been reported. (Kaul and Slaba, 1968; Mehta and Staba 1970). High content of diosgenin and fast rate of growth of callus as obtained by Chaturvedi (1979) hold promise for its large scale cultivations in fermentors and continous production of good amount of diosgenin. Besides the tuber callus, the callus tissue of leaf, stem and root of *D. deltoridea* were also grown satisfactorily. Vanek *et al.* (1999) studied biotrans formation of terpenoid. censtituent other than diosgenin in *D. deltoidea.* The leaf callus of *D. floribunda* showed so far the highest rate of proliferation ic 60 told increase, in fresh weight in 2 months, whereas the tuber callus of *D. prazeri* was grown to a limited extent Chaturvedi and Choudhuri (1980) marked that tuber callus of *D. deltoidea* showed prolific growth on a modified Schenk and Hild brandt's agar medium, a combination of 2.4. D and IAA when the callus synthenized diosgenin (1.6%) during 60 days of incubation. Suthar et al (1980) showed that 2, 4 D ; 2, 4,5 T in combination with GA3 and kinetin enhanced diosgenin contents in callus tissues of *D. assyptica* considerably. High concentration of growth hormones restricted callus growth. Ishida (1988) improved diosgenin production in *D. deltoidea* cell culture by immobilization in polyurethane foam cubes. Inmobilization increased the total diosgenin production by 40%. Increased efficiency in diosgenin production was greatest in 3% sucrose, high concentration inhibited diosgenin production. Lipsky *et al.* (1989) identifical diosgenin during in vitro tissue cultures of *D. caucasica.* Paukev. (1988) analysed diosgenin in cell culture

of *D. deltoridea*. Sengupta *et al* (1987) studued the behaviour of chromosomes and production of diosgenin and sterol in different hormonal regimes during different phases of callus growth of *D. floribunda* An increase in the amount of phytosterol was noted in culture during morphological differentiation. Sengupta (1989) also observed steroid formation during morphogenesis in callus cultures of *D. floribunda*. Roberts *et al.* (1989) studied diosgenin formation by freely suspended and entrapped plant cell cultures of *D. deltoidea*. A low rate of O_2 supply completely inhibited formation of diosgenin. A high O_2 supply rate led to the greatest formation of diosgenin in 30g/l sucrose. Entrapped plant cell culture with polyurethane foam led to delayed development of a suspension culture and to the formation of significantly higher concentration of diosgenin. *D. deltoide*a callus exposed to blue, red or white light was observed to be superior to dark incubated culture interms of growth of tissues and diosgenin and sterol production (Ravi Shankar. and Grewal, 1990). Renard *et al.*(1991) observed carbohydrate and principal ion consumption and production of steroidal sapogenins by *D. villosa* cell suspension. Development of media for growth of *D. deltoidea* cells and in vitro diosgenin production has been established by Ravi Shankar and Grewal (1991) According to them nutrient stress i.e. dipletion of nitrogen increased the production of diosgenin.

The *Dioscorea* is a slow propagating plant and cannot cope with the demand for its diosgenin value. Thus, tissue culture, specially the rapid propagation could be practiced with the aim to produce high yielding variety of *Dioscorea.*

There are 2 lines of approach for development of tissue culture method for rapid propagation of plants. i.e. (i) Regeneration of plants through organ culture without the intervening callus formation i.e. clonal propagation. (ii) their differentiation from callus culture. The latter affords faster rates, but rates are variable because callus culture is genetieally unstable (D'amata-1977). *Dioscorea* is reported to be affected by virus disease (Ruppel *et al.* 1966). It would, therefore, be worth while to find out cultural condition for growth of its shoot spices.