J Phytol. Res 15 (2) : 243-250, 2002

BIOLOGICAL ACTIVITIES OF PHYTOSTEROLS : A REVIEW

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Phytosterols, the secondary plant products found in significant quantities in many plants are the precursors of pharmaceutically important steroidal products, including corticosteroids, sex hormones and oral contraceptives. This review elaborates the significance of the biological activities of phytosterols which are of vital importance for biotechnology based pharmaceutical industry.

Keywords : Biological activity; Corticosteroids; Hormones; Oral contraceptive; Phytosterols.

Introduction

The distribution of phytosterol has been reported in a number of plants. The distribution of sterol in bulk is in the microsomes (endoplasmic reticular membrane and mitochondria)[1]. Some of the commonly occuring phytosterols are brassicasterol, campesterol, stigmasterol, sitosterol, 24-methylene-cholesterol, spinasterol & fucosterol etc[1,2].

Presence of diosgenin in rhizome, root, stem and leaf of *Cortus marlorticanus* has been reported[3,4], Jain and Agarwal[5] while working with plant growth hormone (2,4-D, IAA, NA and GA_3) on *Trigonella foenumgraecum* found that the diosgenin and trigogenin increased considerably with spray of GA_3. It was also found that not only leaf, stem, root and rhizome contain phytosterol but the apple squeezes contain sitosterol, cholesterol, campesterol and 24-ethylidenephenol[6]. In grasses and legumes, Puff *et al*[7] found the contents of stigmasterol and campesterol. Decreasing content of carotene was found with advanced development of the phytosterols as investigated by them.

Phytosterols from leaves

The mature leaves had a significant higher sterol content than the immature leaves. Separation into free sterols, steryl esters, steryl glycosides and acylated steryl glycosides showed that the free sterols accounted for most of the sterol increase and stigmasterol was the principal sterol, responsible for this increase[8]. The presence of sitosterol, cholesterol, stigmasterol 24-ethylidenephenol were found in leaves of wheat, citrus species (lemon varieties), armand pine (*Pinus armandii*)[6,9]. Henry *et al*[10] found that sterols are biosynthesized mainly in the leaves. To support this, the report came from Grunwald[11], that sterol component were isolated non aqueously from chloroplast of *Phaseolus vulgaris* leaves, *Spinacia oleracea*, *Zea mays*.

Phytosterols from roots and tubers

Phytosterols were extracted from roots of *Verbesina rupestries*[12]. The dried roots of Ginseng contain phytosterols[13]. Not only this, sitosterol, stigmasterol, campesterol and β - D-glucosides of sitosterol and stigmasterol were detected in the roots of meadow clover, i.e. *Trifolium pratense*[14]. Two phytosterols were also isolated as fibrinolytic principles from the roots of *Spatholobolus suberetus* on the basis of spectral data and chemical conversion[15]. Roots of *Glycyrrhiza inflata* contain β - sitosterol[16].

The rhizomes of plants belonging to the genus *Dioscorea linn* are the main sources of diosgenin[17,18]. In China and India, rhizomes of *D. zingiverensis*, *D. deltoidea* and *D. panthaica*, *Costus speciosus* are sources of diosgenin[19].

Phytosterols from flower and seeds

Phytosterols content were reported in the essential oil of fresh flower of *Ligustrum japonicum*[20]. New potent sterols were also obtained from the flower of *Edgeworthia crysantha*[21]. Isolation and characterization of β - sitosterol from the flowers of *Acacia leucophloca* is described by Khan *et al*[22]. β - sitosterol and stigmasterol were isolated from the flower of *Lilium devidii*[23]. Some 18 sterols were also isolated from the aerial parts of *Kalanchoe pinnata* including four novel sterols[24].

Cardamon consists of waxes and sterols (β - sitosterol and γ - sitosterol)[25]. In order to examine fragrant components, the essential oil of *Paulownia tomentosa*, was examined and among other compound, β - sitosterol, campesterol, stigmasterol were isolated from the steam distillation residue and methanolic extract[26]. The major sources of the sterol mainly the β - sitosterol are cotton seed oil, sugarcane and wax. It is a mixture of α - sitosterol, β - sitosterol and γ - sitosterol[27]. From the seed of Japanese yew (*Taxus cuspidata*) phytosterols were isolated. Several phytosterols were also identified as components of the seed oil[28]. Also a new phytosterol 4- desmethylsterols, 4-methylsterols and 14 α-methyl - 5 α- cholest - 9(II)-ene 3 β - ol which has never been observed in plant lipid have been isolated from egg palnt seed oil[29]. Extraction of corn oil food was found to contain β-sitosterol, campesterol and stigmasterol. The mixture is very cheap and regarded as an excellent substrate for direct fermentation of C-17 Ketosteroid intermediate of various steroid pharmaceuticals[30]. In soybean, sunflower, rape seed and corn oils, campesterol, β - sitosterols, brassicasterol and stigmasterol were analyzed[31].

Phytosterols from medicinal plants

Phytosterols are reported from a number of medicinally important plant. In many of the medicinal plants, the predominant sterol was found to be β - sitosterol[32]. Phytosterols were also reported from medicinally important plant like the *Paulownia tomentosa*[14] in leaves of *Pinus aronandii*[9] and *Nicotiana tobaccum*[8].

Henry *et al*[10] found that sterols are biosynthesized mainly in the leaves. And it was also observed by Sliwowski and Capsi[33] that only the 2-pro-hydrogen of MVA (mevalonic acid) was retained at the 15 α – position of sitosterol biosynthesized by excised petals of *Calendula officinalis* flowers. The biosynthesis of major phytosterol, campesterol, stigmasterol and sitosterol is inhibited by the application of fungicide. It also retarded the growth of the shoot, primary leaf and root of wheat and maize which may be due to the interaction of the fungicide with the sterol enzyme system[34].

Biosynthesis of sterols (phytosterols and cholesterols)

Acetyl - CoA is the source of all carbon atoms in sterols biosynthesis. The manner of synthesis of this complex molecule has been the subject of investigation by many workers[1,35]. Synthesis takes place in several stages. The first step is the synthesis of mevalonate, a 6-carbon compound, from acetyl - CoA. The next major stage is the formation of isoprenoid units from mevalonate by loss of CO_2. The isoprenoid units may be regarded as the building blocks of the steroid skeleton. Six of these units condense to form the intermediate, squalene, which in turn give rise to the parent steroid lanosterol. Cholesterol (sterol) is formed from lanosterol after several steps, with a loss of 3 methyl groups. In addition, cholesterol is converted to steroid hormones and bile acids and participates in the formation of membranes and of lipoproteins (Fig. 1 and 2).

Biological activities of phytosterols in plants

Sterols in plants may stabilize membranes in the same way as sterols function in animal cell membranes. It has been suggested that sterols might be involved in controlling the permeability of membrane[36]. Stigmast-5-en-3-β-ol(I) acts as inhibitor for insect grwoth (*Bombyx mori*) and development[37], C- 28-ecdysone, malcisterone A, is the predominant molting hormone in the embryonated egg of the milkweed bug. Dietary sterols are apparently utilized with little alteration during devlopment to the adult stage and egg production[38]. At the ripe stage of maturation of tomato, the level of phytosterols showed its maximum and the

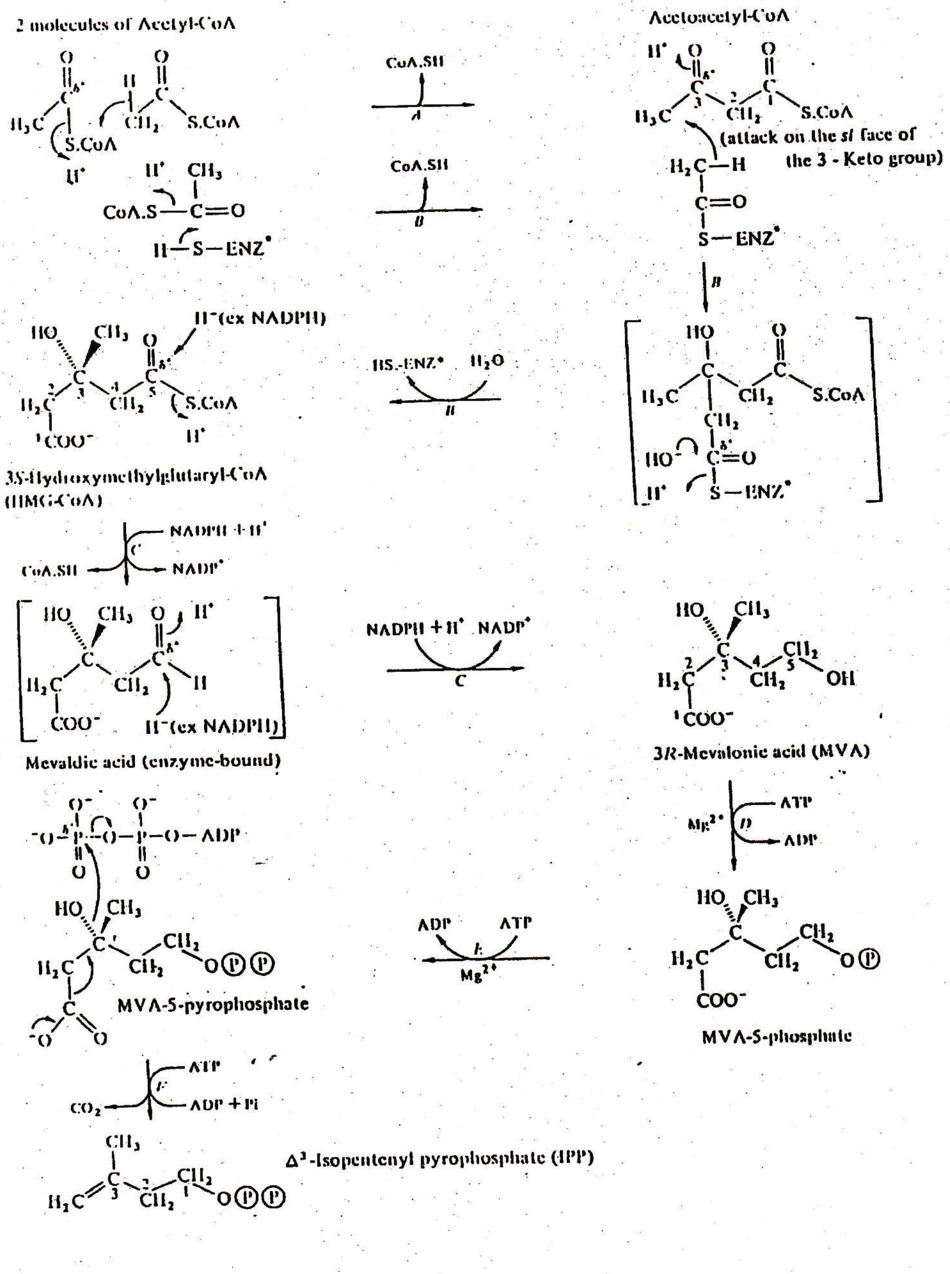

Fig. 1. The formation ofA^{3} - isopentenyl pyrophosphate (IPP) from acetyl - CoA. (A = Acetyl - CoA : acetyl - CoA C- transferase, EC 2.3.1.9; B and ENZ* - hydroxymethylglutaryl - CoA synthase, EC 4.1.3.5; C = hydroxymethylglutaryl - CoA reductase (NADPH), EC 1.1.1.34; D = mevalonate kinase, EC 2.7.1.36; E - phosphomevalonate kinase, EC 2.7.4.2; F = pyrophosphomevalonate decarboxylase, EC 4.1.1.33.)

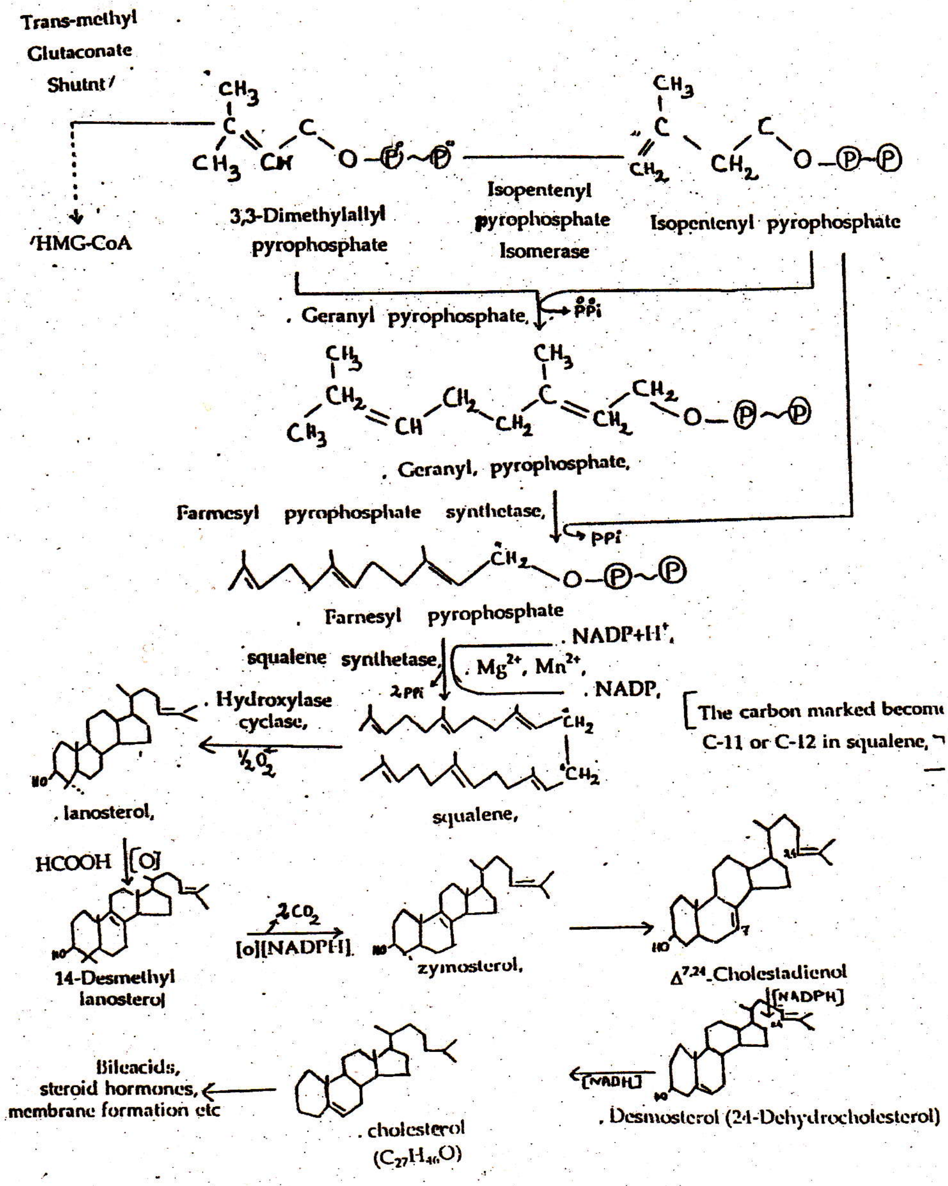

Fig. 2. Biosynthesis of cholesterol from Isopentenyl pyrophosphate.

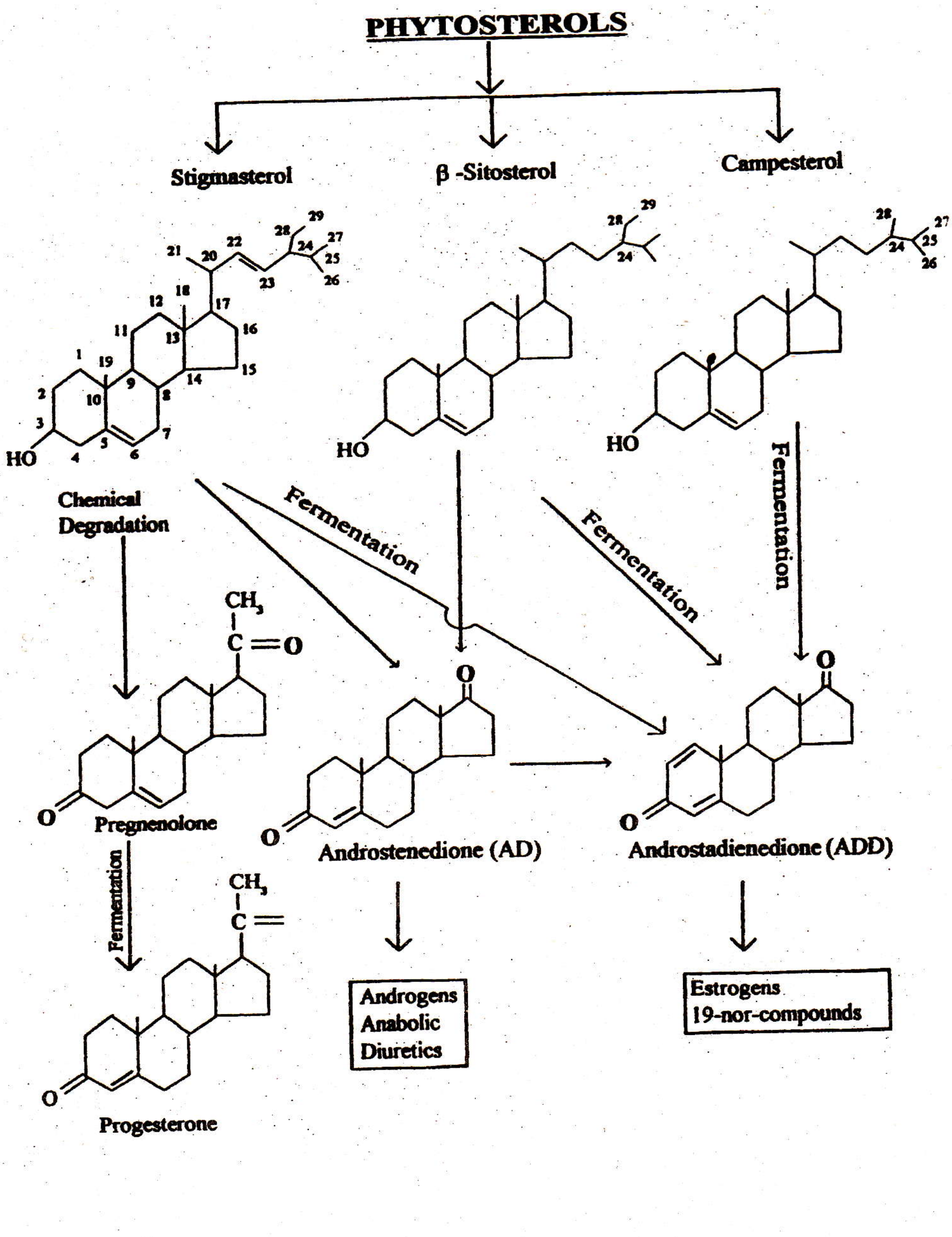

Fig. 3. Summary flow - chart for partial synthesis of steroidal drugs from Phytosterols (Asolkar and Chadha, 1979).

content declined as tomatoes became senescent. As the fruit matured, stigmasterol became the dominant phytosterol with β - sitosterols closely behind[39].

Haseeb *et al*[40] found that the concentration of phytosterol was higher in healthy plant. Several phytosterols, stigmasterol, campesterol and β - sitosterols, were shown to have potent anti-complimentary activities. Dietary phytosterol modulates desaturase activities. Phytosterols make the membrane more rigid but do not induce changes in the relative phospholipid composition. Disappearance of free fatty acid and increase in the saturation degree of phospholipids and sterol amount were observed in root cells[41].

Sterol composition appears to be a useful marker in the determination of taxonomic affinities in the order Caryophyllales[42]. Sitosterol and stigmasterol acts as the virus inhibitor agent occurring in *Artemesia annua*[43]. Phytotoxicity test of the sterols revealed biological activity against root seedling growth of radish[44]. It was found that sterols are strongly protonated in strongly acidic media and form carbocations[45]. Steroids are precursors of many pharmaceutically active steroids including corticosteroids, sex hormones and oral contraceptives[46]. Yvonne[47] found that progesterone inhibits intracellular sterol transport. Progesterone also inhibits the entry of plasma-membrane cholesterol into the cell.

Medicinal application of phytosterols and steroidal drugs

Phytosterols produce a wide spectrum of therapeutic effects in animals including antitumour properties. Phytosterols have been shown experimentally to inhibit colon cancer development. With regard to toxicity, no obvious side effects of phytosterols have been observed in studies to date, except in individual with phytosterolemia, an inherited lipid disorder. Further characterization of the influence of various phytosterol subcomponents on lipoprotein profiles in humans is required to maximize the usefulnes of this non-pharmacological approach to reduction of atherosclerosis in the population[48]. Plant sterols have been shown to reduce dietary cholesterol absorption and hence, total and low density lipoprotein (LDL) & cholesterol concentrations in humans[49]. Berqes *et al*[50] reported the effectiveness of β- sitosterols in the treatment of benign prostatic hyperplasia. Withaferin A(WA), a steroidal lactone inhibited tumour growth and increased survival[51].

Conclusion

Even though the role of steroidal drugs in plants still remains uncertain, it takes a major significant role in animals to possess anti-inflammatory, immuno-suppressive and anti-allergic activities by the corticosteroids and also serve as an oral contraceptive, antifertility and even employed in various gynaecological disorders like habitual abortion, menopausal syndrome and infertility by the sex hormonal steroidal drugs. Thus, the increasing demand of steroidal drugs especially the corticosteroids and oral contraceptives in the market for large quantities has resulted in the depletion of many natural resources.

Approximately three-fourth of the raw materials for chemical synthesis of the steroidal hormonal drugs produced has depended on diosgenin obtained from the plant *Dioscorea species*[17,18]. From the middle 1950's to the early 1960's, over 50% of all steroid manufacturers in the world are originated from Mexican diosgenin[19]. However, this diosgenin is now used mainly for the production of corticosteroids but not for the hormones, oral-contraceptive of spironolactone (diuretics)[19]. Hence, the extensive search for suitable raw materials other than diosgenin as a starting material for the synthesis of oral contraceptive is imperative.

In this review, the naturally occuring phytosterols such as campesterol, stigmasterol, sitosterol, solasodine, hecogenin and spinasterol etc. are currently the most promising precursors. Stigmasterol alone contributes 15% of the total steroid drug produced in the world[52]. These phytosterols can be used as an alternative and plant source for the synthesis of pharmaceutically active steroidal drugs[53] by the conversion of phytosterols to C-19 or 17-ketosteroids such as androst-4-ene-3, 17-dione (AD) and androsta-1, 4-diene-3, 17-dione (ADD) and can serve as starting materials for the preparation of androgens, estrogens, anabolic steroids, 19 norsteroids (Fig. 3).

Hence, the review of biological activities of phytosterols will be the utmost important for the further researchers of the pharmaceutical manufactures in the near future to meet the sudden drastic increase demand of the steroidal drugs in the market at low cost.

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