Taraxasterol and β-sitosterol: new naturally compounds with chemoprotective/chemopreventive effects*

Minireview

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Substantial attention has been given to primary cancer prevention in daily life. Dietary factors are through to contribute to as much as one-third of the factors influencing the development of cancer. Ones of the components of a plant-based diet are β-sitosterol and taraxasterol, compounds attracting our specific attention. This review summarizes the biological activities of presented phytosterols (anti-inflammatory, cholesterol-lowering, anti-microbial, anti-bacterial, anti-fungal effects). Our interest has been focussed especially on their anti-tumor and chemopreventive activity. They have been shown experimentally to inhibit colon and breast cancer development. They act at various stages of tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of cell differentiation. They effectively inhibit invasion of tumor cells and metastasis. With regard to toxicity, no obvious side effects of phytosterols have been observed in studies to date, with the exception of individuals with phytosterolemia. The exact mechanism by which dietary phytosterols act is not fully understood. However, some mechanisms have been offered. Therefore, they have a bright future in clinical application. Further investigation to explore their potential in tumor treatment may prove to be worthwhile.

Key words: β-sitosterol, taraxasterol, anti-tumor activity, chemoprevention, dietary supplementation

Sterols are a group of naturally occurring substances derived from hydroxylated polycyclic isopentenoids having a 1,2-cyclopentanophenanthrene structure. These compounds contain a total of 27–30 carbon atoms (the number of carbon atoms in the biosynthetic precursor squalene oxide [61]) in which a side chain with carbon atoms ≥7 is attached at the carbon 17 position (C17). Their structures are closely related and varied depending on the extent of modifications of the ring system and side chain variations. In general, the sterols can be categorized into three subclasses: (I) 4,4-desmethylsterols; (II) 4α-methylsterols; and (III) 4,4-dimethylsterols (Fig. 1) [2].

Sterols are known to have a wide range of biological activities. Plant sterols (phytosterols), in particular, are important agricultural products for health and nutrition industries [2]. They are common components of plant foods, especially plant oils, seeds and nuts, cereals and legumes [40]. Reported phytosterol data for some plant foods and vegetable oils have shown that nuts and oils contain higher levels (≥1%) of sterols than fruits and vegetables (<0.05%) (Tab. 1) [2]. It is obvious that vegetarian diets contain higher amount of phytosterols as compared to conventional western diet [34].

The most common phytosterols are campesterol, β-sitosterol (SS) and stigmasterol. Structurally, these compounds are similar to cholesterol, except for an additional hydrocarbon chain at the C-24 position [40]. Although they are structurally similar to cholesterol, they have been shown to exert significant unique biochemical effects in both animals and humans [15].

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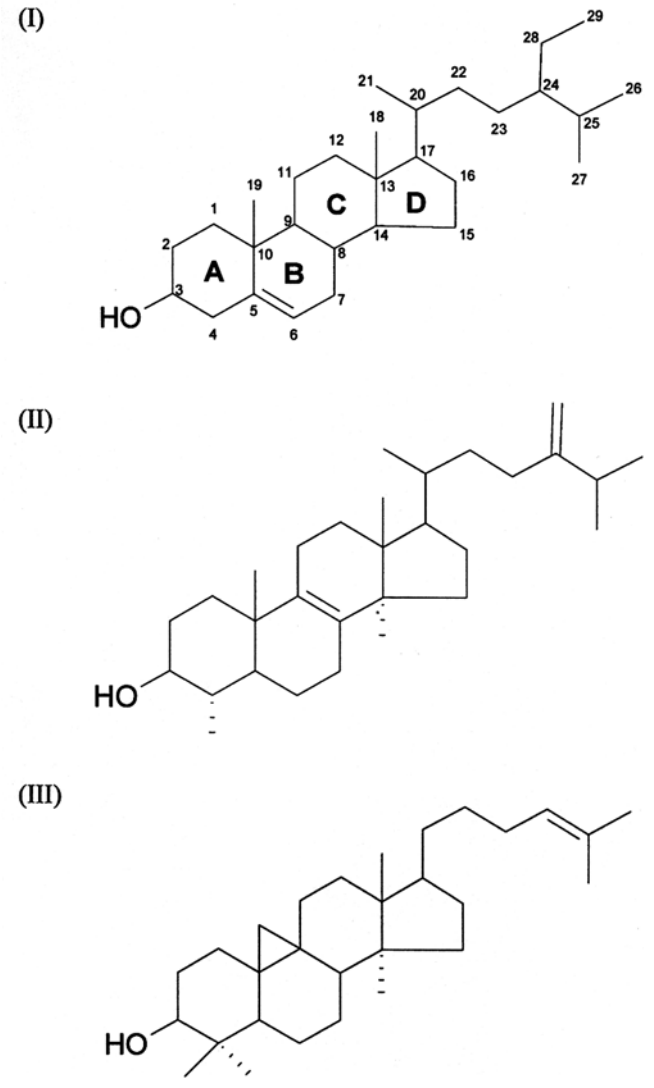

Figure 1. Chemical structures of: (I) 4,4-desmethylsterol, β-sitosterol; (II) 4α-methylsterols, obtusifoliol; (III) 4,4-dimethylsterols, cycloartenol.

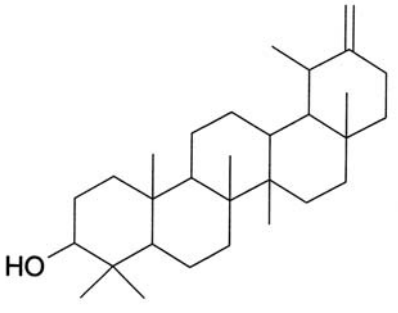

Figure 2. Chemical structure of taraxasterol.

Taraxasterol (TS) (Fig. 2) is a monohydroxy triterpene found in arnica, burdock, chicory and dandelion. The distribution in plants of TS is not extensive (Tab. 2), but the biological activity of this compound is very interesting.

Table 1. Some reported sterol concentrations in selected foods and vegetable oils (mg/100 g) [58]

Food	Phytosterol
Potato	5
Tomato	7
Pear	8
Lettuce	10
Carrot	12
Apple	12
Onion	15
Banana	16
Fig	31
Garbanzo bean	35
Kidney bean	127
Soybean	161
Pecan	108
Almond	143
Cashew nut	158
Peanut	220
Sesame seed	714
Peanut oil	207
Olive oil	221
Soybean oil	250
Cottonseed oil	324
Safflower oil	444
Sesame oil	865
Corn oil	968
Rice bran oil	1190

Chemoprevention/diet

Phytosterols have anti-carcinogenic properties. Some studies have suggested that populations with low breast cancer incidence often consume diets high in phytosterols [40]. It has been shown that dietary consumption of phytosterols is lower in developed countries (80 mg/day) as compared to Asia countries (400 mg/day), where the incidence and/or death rate from these cancers are at minimum [7].

Populations at low breast cancer risk consume more dietary phytosterols than those at high risk. For example, the Japanese consume a plant-based food and low animal fat diet that is rich in phytosterols. Their diet concentrations range from 8 to 12 μmol/l. The Japanese also have a low incidence of breast cancer. In contrast, high breast cancer incidence populations in Western countries, such as United States, have low vegetable and high fat intakes which are associated with low dietary phytosterol of 2–6 μmol/l. Thus, phytosterol intake may explain in part the protective effect of a vegetable-rich diet on breast cancer incidence that has been observed in some epidemiological studies [40].

Despite the experimental and ecological evidence for a role of phytosterols in protection against breast cancer, there is insufficient information describing diet influence on serum phytosterol levels and how, in turn, phytosterols might influence breast cancer risk. The human body does not synthesize phytosterols endogenously. Circulating phy-

Table 2. Occurrence of β-sitosterol and taraxasterol in folk medicine

Latin name	Extracted from	Compound	Ref.
Aglaia rubiginosa	leaves	SS	[49]
Amphipterygium adstringens		SS	[6]
Astilbe chinensis		SS	[54]
Cabernet Sauvignon	grape skin	SS	[64]
Calendula officinalis	flowers	TS	[18]
Carthamus lanatus L.		SS,TS	[37]
Casearia membranacea		SS	[12]
Chrysanthemum morifolium		TS	[62]
Cirsium japonicum DC	rhizomes	SS	[66]
Citrus changshan-huyou		SS	[65]
Coeloglossum viride var. bracteatum	rhizomes	SS	[29]
Coccoloba acrostichoides		SS	[16]
Cyanotis arachnoidea		SS	[57]
Cynara scolymus		TS	[62]
Dalbergia hainanensis	leaves	SS	[63]
Dorstenia barteri var. subtriangularis	twigs	SS	[41]
Dorstenia elliptica	twigs	SS	[1]
Euphorbia marschalliana		SS	[30]
Euphorbia segetalis	whole plant	SS	[35]
Fragaria ananassa	fruit	SS	[50]
Gerbera piloselloides	roots, rhizomes	SS	[60]
Hedysarum polybotrys Hand.-Mazz	root	SS	[26]
Hemistepta lyrata Bunge	whole plant	SS,TS	[48]
Holodiscus discolor (Pursh) Maxim	leaves	SS,TS	[27]
Hypericum laricifolium		SS	[20]
Jatropha curcas seeds		SS	[5]
Juniperus communis L.		SS	[51]
Lamium macalatum L. var Kansuense		SS	[19]
Ligustrum delavayanum	leaves	SS	[44]
Miconia rubiginosa		SS	[53]
Moringa oleifera Lam.		SS	[24]
Parkia biglobbossa	seeds	SS	[5]
Pfaffia paniculata	roots	SS	[43]
Psidium guajava	leaves	SS	[9]
Salix oritrepha		SS	[22]
Schisandra sphaerandra	stems	SS	[25]
Smilax perfoliate		SS	[14]
Trichodesma amplexicaule Roth.		SS	[52]

tosterols are derived exclusively through intestinal absorption [15, 34, 40]. Plasma phytosterol levels in mammalian tissue are normally very low due primarily to poor absorption from the intestine and faster excretion from liver compared to cholesterol. Only 0.3–1.7 mg/100ml of phytosterols are found in human serum under normal conditions compared with daily dietary intakes of 160–360 mg/day, but plasma levels have been showed to increase up to two-fold by dietary supplementation. Phytosterols are able to be metabolized in the liver into C_{21} bile acids via liver other than normal C_{24} bile acids in mammals [34].

MUTI et al [40] suggested the hypothesis that a plant-based diet rich in cereal fibers, soy and flaxseed can increase circulating levels of SS. Their study included data from 99 hyperandrogenic postmenopausal women. The hyperandrogenic women were selected for their study because they are at an increased risk for breast cancer. These women were randomized into either a dietary intervention group (52 women) or a control group (47 women). Women randomized to the dietary intervention group were instructed to follow the specific diet for the term of 18 weeks. The control group received only a general recommendation to increase vegetable and fruit consumption. The results of their study have shown that circulating levels of phytosterols can be affected by dietary modifications. Their findings indicate that phytosterols, in particular SS, can be used as biomarkers of exposure in observational studies or as compliance indicators in dietary intervention studies of cancer prevention.

JU et al [32] hypothesized that SS could modulate the growth of estrogen-dependent human breast cancer cells *in vitro* and *in vivo*. Their study evaluated the estrogenic and anti-estrogenic effects of SS on the proliferation of Michigan Cancer Foundation 7 (MCF-7) cells *in vitro*. SS (>1 μmol/l) increased MCF-7 cell proliferation. Treatment with 150 μmol/l of SS increased cell growth by 2.4 times compared to negative control group. The effect of dietary SS on the growth of MCF-7 cells implanted in ovariectomized athymic mice was also evaluated. Dietary SS did not stimulate MCF-7 tumor growth. However, dietary SS protected against 17β-estradiol-stimulated MCF-7 tumor growth and lowered circulating 17β-estradiol levels.

Metastasis plays a major role in morbidity and mortality from breast cancer. AWAD et al [8] investigated the effect of phytosterols on some steps of the metastatic process: tumor cell invasion, adhesion, and migration. In addition, cell growth and cell cycle progression were evaluated. Human breast cancer cells MDA-MB-231 were supplemented with SS, cholesterol and campesterol. SS inhibited tumor cell invasion through Matrigel and adhesion of cells to plates coated with collagen I, collagen IV, fibronectin, and laminin compared with cholesterol treatments and controls. SS inhibited cell growth by 70% compared with controls and induced cell cycle arrest at the G_2/M phase. They concluded that, among phytosterols, SS may offer protection from breast cancer metastasis by inhibiting cell invasion of the basement membrane, which may be mediated by its ability to limit the adhesive interaction of the tumor cell and basement membrane.

Epidemiological and experimental studies in general have implicated dietary cholesterol as a factor in colon carcinogenesis, primarily through the production of its end products (coprostanol and other neutral sterol and bile acids) by colonic microflora. Secondary bile acids probably play a major role in the development of colonic tumors. It has been suggested that a high ratio of secondary to primary bile acids may increase the risk of colon cancer. Additionally, it has been proposed that cholesterol and its metabolites may be involved in the etiology of colon cancer. Some studies have demonstrated that SS decreases serum concentrations of total and LDL cholesterol [17, 33, 36]. The me-

chanisms of hypocholesterolemic action include inhibition of cholesterol absorption and decreased excretion from the liver. It has been suggested that supplementation of phytosterols have anti-colonic tumor activities in addition to other beneficial actions in animals. High amounts of phytosterol may possess negative influence on reproductive system and occasionally may also cause diarrhea in animals. However, presently it can be assumed that at moderate levels of plant sterols offer advantages as safe and inexpensive primary cholesterol lowering agents for use in humans [34].

Recently phytosterol esters have been enriched in functional foods (margarines, natural remedies) to make use of the cholesterol-lowering effect of phytosterol, maximal at an intake of 2 to 3 g/day [20, 31, 33, 34]. FAHY et al [21] studied the cytotoxicity and uptake of ingested SS in human intestinal cells in culture. Another aim was to determine, if SS would interfere with α-tocopherol or β-carotene uptake by these cells. Human adenocarcinoma Caco-2 cells were supplemented for 24 h with increasing concentrations (0–12.5 μmol/l) of SS. Cytotoxicity was assessed by neutral red uptake, lactate dehydrogenase release (LDH) and fluorescein diacetate/ethidium bromide (FDA/EtBr) assays. SS had no significant effect on Caco-2 cell viability assessed using LDH and FDA/EtBr assays. The highest concentration of SS was taken up by Caco-2 cells in culture. The results demonstrated a reduction in the uptake of β-carotene when Caco-2 cells were supplemented with 20 μmol/l SS. SS did not interfere with α-tocopherol uptake by the cells.

The Korean fermented vegetable food, kimchi, has been demonstrated to have anticancer functional properties. PARK et al [45] examined the effect of kimchi samples, the dichloromethane fraction (DCM fr.) from cabbage kimchi, and the active compound, which has been identified as largely SS, on the Ras-dependent signaling pathway. When the DCM fr. and SS were used to treat Rat1 fibroblasts overexpressing human insulin receptors (HIRc-B) and microinjected with oncogenic H-Ras^{v12}, the DNA synthesis of injected cells was decreased, suggesting that kimchi might block the signaling pathway of oncogenic Ras^{v12}, thus preventing the proliferation of transformed cells. This study provides additional evidence that kimchi and its active components, including SS, have potential in both the prevention and treatment of cancer, and presents convincing evidence that the anticancer effects may be a result of an inhibition of Ras oncogene signaling.

Antioxidant activity

Minor components of virgin olive oil may explain the healthy effects of the Mediterranean diet on the cardiovascular system and cancer development. The uncontrolled production of reactive oxygen species (ROS) and arachidonic acid (AA) metabolites contributes to the pathogenesis of cardiovascular disease and cancer, and inflammatory cells infiltrated in the atheroma plaque or tumor are the major source of ROS and eicosanoids. MORENO [39] determined the effect of SS on superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and nitric oxide ($^{\cdot}NO$) levels. SS decreased the $O_2^{\cdot-}$ and H_2O_2 production induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). These effects were correlated with the impairment of [^{3}H]AA release, cyclooxygenase-2 (COX-2) expression, and prostaglandin E_2/leukotriene B_4 synthesis in murine macrophages RAW 264.7 stimulated by TPA. SS exerted its effects after 3–6 h of preincubation. SS also reduced the $^{\cdot}NO$ release inducted by TPA, which was correlated with the impairment of inducible nitric oxide synthase levels. This may be correlated with the modulation of nuclear factor-kappaB activation.

Anti-intitiation and anti-promotion activity

TAKASAKI et al [55] investigated the inhibitory effects of extract of the roots of Taraxacum japonicum (Compositae) on two-stage carcinogenesis of mouse skin papillomas induced by combinations of two different types of initiators, 7,12-dimethylbenz[a]anthracene (DMBA) and ($\pm$)-(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide (NOR-1), and two different types of promoters, 12-O-tetradecanoylphorbol-13-acetate (TPA) and a mycotoxin fumurosin B1. This extract exhibited strong anti-tumor-promoting activities on the two-stage carcinogenesis of mouse skin tumor induced by DMBA as an initiator and TPA as a promoter, as well as on that induced by DMBA and fumurosin B1. Further, the extract exhibited anti-tumor-initiating activity on the two-stage carcinogenesis of mouse skin tumor induced by NOR-1 as an initiator and TPA as a promoter. Their results suggested that an extract of the roots of the Taraxacum plant could be a valuable chemopreventive agent against chemical carcinogenesis. Later, TAKASAKI et al [56] isolated eleven triterpenoids from the roots of Taraxacum japonicum (Compositae). They were examined for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) induced by tumor promoter TPA, in Raji cells as a primary screening test for anti-tumor-promoters. Of these compounds, TS and taraxerol exhibited significant inhibitory effects on EBV-EA induction. Furthermore, TS exhibited potent anti-tumor-promoting activity in the two-stage carcinogenesis tests of mouse skin using DMBA as an initiator and TPA as a promoter, and TS showed a remarkable inhibitory effect on mouse spontaneous mammary tumors using C3H/OuJ mouse. These results strongly suggest that TS could be a valuable chemopreventive agent.

TS isolated from the flowers of Compositae plants Cynara scolymus (artichoke) and Chrysanthemum morifolium

(chrysanthemum), respectively, showed strong inhibitory activity against TPA-induced inflammation in mice. At 2 μmol/mouse, this compound inhibited markedly the tumor-promoting effect of TPA (1 μg/mouse) on skin tumor formation following initiation with DMBA (50 μg/mouse) [62].

SS isolated from Chaenomeles sinensis Koehne in relatively high yields was tested for anti-tumor-promoting activity using soft agar colony assays with mouse epidermal cells JB6. Anchorage-dependent preneoplastic cells (JB6 cells) are transformed into anchorage independent neoplastic cells by treatment with a promoter TPA and are then able to grow in soft agar. SS had an inhibitory effect on soft agar colony induction by TPA with an IC_{50} of 39.6 μmol/l [23].

Possible molecular mechanisms

In an attempt to investigate the mechanism by which phytosterols offer protection from some types of cancer, AWAD et al [7] investigated the effect of SS on the mevalonate and MAP Kinase (MAPK) pathways in human breast cancer cells MDA-MB-231. SS supplemented cells had reduced cholesterol synthesis, when using ^{3}H-mevalonolactone as substrate, which suggests that the inhibition in this pathway is downstream of mevalonate, where processes such as isoprenylation of proteins may take place. Mevalonate supplementation to cells treated with SS did not completely correct the observed growth inhibition by SS. There was no effect of SS on the concentrations low (21–26 kDa) or high (44–74 kDa) molecular weight isoprenylated proteins in these cells. On the other hand, both the quantity and activity of MAPK was elevated in the cells supplemented with SS. These data suggest that the down regulation of cholesterol synthesis from mevalonate and stimulation of the MAPK pathway may play roles in the inhibition of MDA-MB-231 cell growth by SS.

CHOI et al [15] examined the effect of SS on the growth of HCT116 human colon cancer cells. Treatment with SS resulted in a dose-dependent growth inhibition coupled with the characteristic morphological features of apoptosis and with the increase of a sub-G_1 cell population. Apoptosis-inducing concentrations of SS induced caspase-3 and caspase-9 activation accompanied by proteolytic cleavage of poly(ADP-ribose)-polymerase. In addition, SS-induced apoptosis in HCT116 cells was associated with a decreased expression of the anti-apoptotic Bcl-2 protein and mRNA and a concomitant increase of the pro-apoptotic Bax protein and mRNA, and with release of cytochrome c from the mitochondria into the cytosol. SS treatment also inhibited the expression of cIAP-1, a member of the inhibitors of apoptosis proteins, without significant changes in the level of cIAP-2. Taken together, these findings provide important new insights into the possible molecular mechanisms of the anti-cancer activity of SS.

SS isolated from the grape skin of Cabernet Sauvignon was assayed for COX-1 and COX-2 enzyme inhibitory activity. At 100 μg/ml SS inhibited the COX-2 enzyme by 11%, but did not show activity on the COX-1 enzyme [64].

Anti-inflammatory activity

TS isolated from the flowers of Catharamus tinctorius, Chrysanthemum morifolium and Helianthus annuus, exhibited considerable activity against TPA-induced inflammatory ear edema in mouse and tumor promotion in mouse skin. Its 50% inhibitory dose was 0.3 mg/ear [4].

Others

Aloe vera gel has a beneficial effect on wound healing. Because angiogenesis is an essential process in wound healing, MOON et al [38] hypothesized that Aloe vera gel might contain potent angiogenic compounds. SS, purified from the final fraction of Aloe vera gel, showed a potent angiogenic activity in the chick embryo chorioallantoic membrane assay. In the presence of heparin, SS stimulated neovascularization in the mouse Matrigel plug assay and the motility of human umbilical vein endothelial cells in an *in vitro* wound migration assay. Thus SS could be a novel plant-derived angiogenic factor which may have potential pharmaceutical applications for the management of chronic wounds.

HENDRIKS et al [28] evaluated both efficacy and safety in humans of long-term consumption of spreads containing plant sterol esters. Hundred and eighty-five healthy volunteers consumed daily 20 g spread enriched with plant sterols for one year. SS concentration in serum was increased from 1.86 to 2.47 (μmol/mmol total cholesterol) ($p<0.0001$). It was shown that the consumption of plant sterol esters-enriched spread is an effective way to consistently lower blood cholesterol concentrations and is safe to use over a long period of time.

TS obtained through a systematic chemotaxonomical study with Mexican plants of the Asteraceae showed antimicrobial activity against Staphylococcus aureus [59].

Fractions prepared from the methanol extract of Buchholzia coriacea stem bark exhibited a high concentration-dependent anti-bacterial and anti-fungal activity compared to the standard antibiotics, ampicillin and tioconazole. In the brine shrimp lethality assay, the methanol extract was found to be non-toxic with LC_{50} of 1031 μg/ml. The two main compounds present in the most active fraction were isolated and identified as lupeol and SS [3].

Toxicity and side effects

No obvious side effects have been observed after long-term feeding of phytosterols in animals and humans. Phytosterol treatments were reported to cause certain side effects at very high doses [34]. These compounds exhibit relatively little potential for toxicity because they are poorly absorbed [17].

Sitosterolemia, a rare inherited lipid storage disease, is characterized chemically by the increased plant sterols and 5α-saturated stanols in plasma and tissue with premature atherosclerosis. The absorption rate of phytosterols is very high in the patients. The large quantities of SS and cholestanol in sitosterolemic liver competitively inhibited cholesterol 7α-hydroxylase for bile acid synthesis, which may eventually lead to a decrease of bile acid production and deficient pool size [13, 34, 47]. Mutations in the human genes encoding ABCG5 and ABCG8 have been shown to cause sitosterolemia with a reduced biliary secretion as well as a strongly enhanced intestinal absorption of plant sterols (SS, campesterol) [10, 11, 46, 47].

Furthermore, SS has been shown to affect reproductive tissue in that SS may act as an abortifacient in animals. High doses (0.5–5 mg/kg per day subcutaneously) of SS in rats reduced sperm concentrations as well as weights of testis and accessory sex tissues in a time-dependent manner [19].

Conclusion

Dietary factors are through to contribute to as much as one-third of the factors influencing the development of cancer. There is now considerable evidence from *in vitro* and *in vivo* studies to suggest a protective role of plant based diets on tumorigenesis [39]. Phytosterols or plant sterols are the counterparts of cholesterol in animals, which exist in many forms. The most abundant one is SS and therefore there is a growing interest in the elucidation of the biological roles of this phytosterol. The distribution of TS in plants is not extensive, but the biological activity of this compound is very interesting. Both compounds have been shown to act at various stages of the tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of tumor cell differentiation. Anti-proliferative effects on cancer cells were associated with activation of the sphingomyelin cycle, cell cycle arrest and stimulation of apoptotic cell death. However, the underlying mechanisms of its action are not fully understood. With regard to toxicity, no obvious side effects of phytosterols have been observed in studies to date, with the exception of individuals with phytosterolemia.

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