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Review

Recent advances in Phytosterol Oxidation Products

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ABSTRACT

Phytosterols and their oxidation products have become increasingly investigated in recent years with respect to their roles in diet and nutrition. We present a comprehensive review of recent literature on Phytosterol Oxidation Products (POP) identifying critical areas for future investigation. It is evident that POP are formed on food storage/preparation; are absorbed and found in human serum; do not directly affect cholesterol absorption; have evidence of atherogenicity and inflammation; have distinct levels of cytotoxicity; are implicated with high levels of oxidative stress, glutathione depletion, mitochondrial dysfunction and elevated caspase activity.

Contents

1. Introduction

Phytosterols are natural products which the past decade has seen a marked increase in their incorporation into nutraceutical formulations and diet. Phytosterols are commonly added to fortified foods as a phytosterol blend for economic reasons containing (but not limited to) β-sitosterol, stigmasterol, campesterol and dihydrobrassicasterol (Fig. 1). Although proven to exert health benefits via the lowering of low density lipoprotein cholesterol concentrations, the phytosterols are not without their problems due to their oxidative susceptibility.

Because of their inherent molecular structure, and close structural similarity to cholesterol, phytosterols are susceptible to

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oxidation to form hydroxy, epoxy, keto and triol derivatives which are collectively known as Phytosterol Oxidation Products (POP, Fig. 2). These derivatives have diverse biological functions of eminent interest to clinicians and this review will focus on the most recent developments in the POP field since the excellent review by Garcia-Llatas. [1]

2. Oxidation of phytosterols

Sterol autoxidation is oxidation of the steroid nucleus in an adventitious manner through storage, processing and preparation of foodstuffs whereas enzymatic oxidation is the biotransformation of phytosterols to their oxides via enzymatic pathways *in vivo*. POP may be generated *in vivo* through non-enzymatic oxidation of phytosterols as has been demonstrated for cholesterol [2] or by enzymatic oxidation [3]. Enzymatic activity of the intestinal microflora has been shown to result in the formation of cholesterol oxidation products (COP) [4] and could also result in POP formation *in vivo*. However, Grandgirard et al. [5] demonstrated that the plasma levels of sitostanetriol and campestanetriol were not increased following a 4 week feeding trial with a diet containing 1% phytosterol in rats. The authors concluded that the triol derivatives of phytosterols are not synthesised *in vivo* and it is highly probable that the presence of these compounds *in vivo* results from dietary origin. Similarly, there was no increase observed in the plasma levels of 7α-hydroxy or 7β-hydroxy derivatives in hamsters, following the consumption of stigmasterol or β-sitosterol-enriched diets [6].

3. Synthesis of POP

Significant steps have been taken towards the development of pure standards with a number of routes now published. A total synthesis approach was used in order to explore the chemistry of dihydrobrassicasterol and campesterol with concomitant synthesis of their oxides (Scheme 1) [7–9]. A similar route was chosen for the stigmasterol oxides from commercially available stigmasterol [10].

A different strategy for the synthesis of POP from a phytosterol mixture was recently described using semi-preparative HPLC as the purification method [11]. To this end, a mixture of phytosterols including avenasterol, brassicasterol, campesterol and β-sitosterol was converted to a complete mixture of POP which were assessed by chromatography and separated into their individual components. Four individual phytosterol oxides are reported as proof of this strategy: 7-ketocampesterol, 7-keto-β-sitosterol, 7-β-OH-campesterol and 7-β-OH-β-sitosterol.

4. Content of POP in foods

Phytosterol enriched foods are increasingly common in marketed products; analysis of the phytosterol content and stability over time is an essential measure to ensure product safety. A recent assessment of phytosterol enriched dark chocolate over a 5 month period of storage using sensory analysis and GCMS compared a formulation with palm oil, one with phytosterol esters and one with phytosterol esters and antioxidants [12]. Chemical analysis of the samples revealed the following: hydroperoxide concentrations peaked at 60 days for the samples held at 20 °C whereas in the samples held at 30 °C, the POP peaked at 30 days; antioxidants used (ascorbic acid and α-tocopherol) had little effect on storage outcome; the most commonly identified were 7-hydroxy, 7-keto, epoxides and triols with campesterol appearing the most susceptible.

Another recent study identified the degradation of phytosterols on storage over an 18 week period in an open environment. Rudzinska et al. [13] used GC and GCMS in order to quantify the phytosterol content of enriched margarines and identified that the phytosterol/phytostanol content dropped on storage at 4 and 20 °C from 7.9% to 6.3 and 5.5% respectively. In addition an increase in POP was seen for both temperatures with twice the original levels seen after storage at 20 °C. The most common oxidation product was seen to be the 7-hydroxy, followed by 7-keto and 5,6-epoxide. There is an obvious trend for loss of sterol/stanol content and formation of POP on storage at higher temperatures.

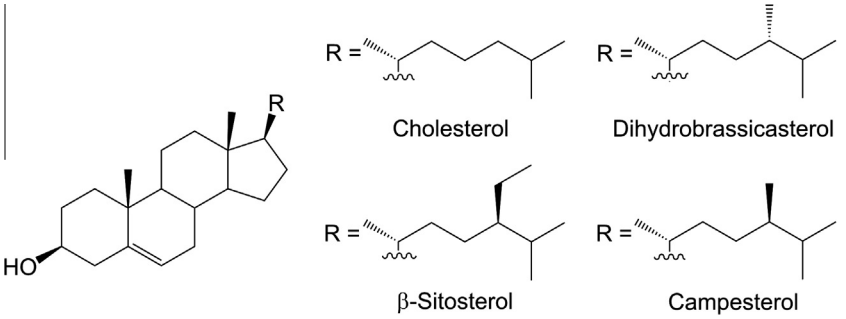

Fig. 1. Structures of cholesterol and common phytosterols.

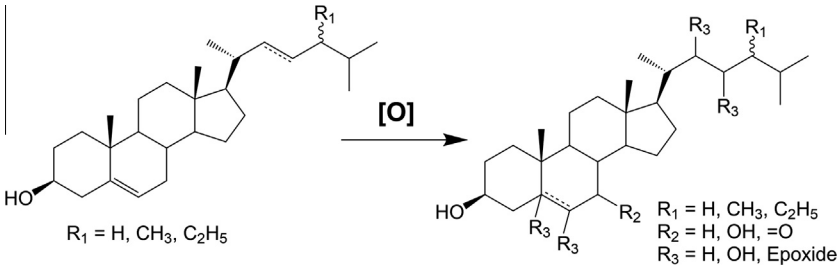

Fig. 2. Formation and general structure of the common oxides of cholesterol and phytosterols.

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