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Preparation and biological activities of castanospermine and 6-*O*-butanoyl castanospermine

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ABSTRACT

Castanospermine and 6-O-butanoyl castanospermine have attracted considerable interest because they have been found to have tremendous potential in some disorders such as diabetes, viral infection, immunosuppressive deficiencies and tumor metastasis etc. The preparative and biological aspects of castanospermine and 6-O-butanoyl castanospermine were covered in the review.

Key words: Castanospermine ; 6- *O*-butanoyl derivatives ; Preparation ; Biological activities.

INTRODUCTION

Castanospermine is an indolizine alkaloid, which is systematically named (1S,6S,7R,8R,8aR) - 1,6,7,8-tetrahydroxyindolizidine (Fig. 1). It was firstly isolated from the seeds of the monotypic Australian species *Castanospermum australe* [1]. Although there is castanospermine in all parts of the tree, the compound has the highest concentration in the seeds and seed pods, in which it occurs at levels of 0.3% fresh weight or higher [2] . It also has been identified in *Alexa leiopetala* and seven other species of the same genus [3]. Initially, castanospermine has attracted considerable interest by synthetic and medicinal chemists because it was a potent inhibitor of α- and β-glucosidase, which are involved in many important cellular processes in biological systems [4-7]. Later on, the other biological activities have been successively discovered [8-11]. As a result, castanospermine have their potential use in the treatment of a number of diseases such as s diabetes, viral infection, immunosuppressive deficiencies and tumor metastasis etc. In 1993, Hempel *et al.* determined high-resolution structures of castanospermine by X-ray diffraction and established the absolute configuration of castanospermine [12] . The castanospermine derivatives, 6-O-butyryl- castanospermine (Fig. 2), which is acylated at 6-hydroxy group of castanospermine, demonstrated broad antiviral activity in vitro, but it has higher antiviral efficacy and lower toxicity than castanospermine [13-20]. The compound is well absorbed in vitro and in vivo, and is readily converted to castanospermine by esterase [13]. 6-O-butyryl- castanospermine in combination with peginterferon alone (double combination therapy) or also with ribavirin (triple combination therapy) are under Phase II clinical trials for the treatment of patients with chronic HCV [13, 21, 22].

To date, a lot of studies on castanospermine and 6- *O*- butanoyl castanospermine have been carried out [1-20]. However, few reviews related to these studies were reported. The purpose of this paper is to review the literature concerning the preparative and biological aspects of castanospermine and 6- *O*-butanoyl castanospermine.

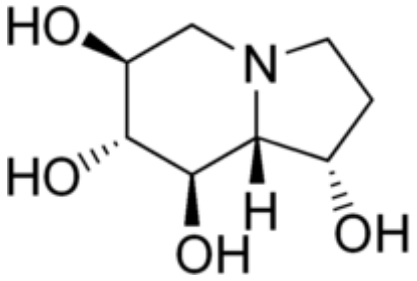

Fig. 1 The structure of (+)-castanospermine

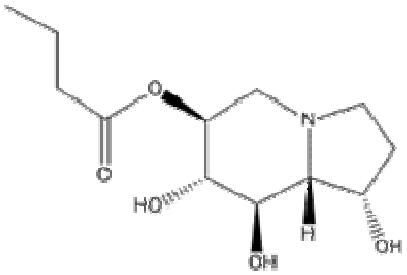

Fig. 2 The structure of 6-O-butyryl-castanospermine

PREPARATION OF CASTANOSPERMINE FROM NATURAL SOURCES

For the first time Hohenschutz *et al.* reported the purification of castanospermine from the toxic seeds of the Australian legume *Castanospermum australe* with 0.056% yield by extraction with ethanol, filtration, acidic cation exchange chromatography, crystallization [1]. However, the purification process included a tedious extraction and a subsequent elution with pyridine. Liu *et al.* invented a new procedure for the separation of castanospermine from *Castanospermum australe*, which prevent from the tedious extraction steps and the use of the obnoxious solvent pyridine while obtaining the desired product in 1.2% yield [23]. Alternatively, in order to produce castanospermine **,** Roja *et al.* established unorganized callus cultures from the leaves of the mature *Castanospermum australe* on a chemically defined nutrient medium of Murashige and Skoog supplemented with 2,4-dichlorophenoxyacetic acid and benzyladenine. Castanospermine was isolated from culture with the yield of 0.004% by gas liquid chromatography [24] . Furthermore, Nash also isolated the compound from the tree *Alexa leiopetale* [3] .

PREPARATION OF CASTANOSPERMINE BY ORGANIC SYNTHESIS

Although castanospermine can be obtained from natural sources, the process would be limited by high expense and the availability of the plant sources. In another way, castanospermine can be obtained by organic synthesis, which was first described by Ganem and Bernotas in 1984[25]

.

Many efforts have been devoted to the total synthesis of castanospermine due to its biological activity and intrinsic complexity over the past 30 years (Table 1). Generally, the total synthesis of castanospermine was started from either a carbohydrate, tartaric malic acid, or an achiral substrate, a majority of which have started from sugars to date [26-28] . Julien Ceccon *et al.* accomplished a seventeen-step synthesis of castanospermine in 3.7% overall yield based on sequential enol ethermetathesis - hydroboration/ oxidation [29]. Thomas Jensen *et al.* described a nine-step route from methyl α-D- glucopyranoside in which a zinc-mediated fragmentation of benzyl-protected methyl 6-iodoglucopyranoside, ring-closing olefin metathesis, and strain-release transannular cyclization to afford the indolizidine skeleton serve as the key steps [30]. Scott E. Denmark *et al.* developed an eight-step syntheses of castanospermine in 18% overall yields from 2, 5-dihydrofuran. The asymmetric tandem [4 + 2]/ [3+ 2] cycloaddition between silaketal nitro olefin and chiral vinyl ether to create nitroso acetal was the key step of the total syntheses [31]. According to a strategy in which a carbohydrate- derived tricarbonyl precursor is converted to the indolizidine skeleton via a triple reductive amination reaction, Hang Zhao *et al.* were succeed in the synthesis of castanospermine in 22% overall yield[32]. In another strategy, Narayan S. *et al.* initially generated R-amino aldehydes via the 1,3-addition reaction of D-glucose-derived nitrone 2 with vinylmagnesium bromide, followed by N-O bond reductive cleavage, *N*-protection, and dihydroxylation followed by oxidative cleavage, and then R-amino aldehydes was applied for the synthesis of castanospermine, using the intramolecular aminomercuration methodology[33]. On the basis of a method where a highly diastereoselective vinylogous Mukaiyama- type reaction with either chiral or achiral aldehydes and a diastereodivergent reduction of tetramic acids allow the formation of three continuous stereogenic centers with high diastereoselectivities, the syntheses of castanospermine has been accomplished in nine steps and 13.9% overall yields [34]. Edward G. Bowen reported a synthesis of castanospermine in a fifteen-step sequence, where the C-1/8a stereodiad is obtained through the diastereoselective oxamidation of an unsaturated *O*-alkyl hydroxamate [35]. Linda Cronin *et al.* devised a synthesis route of castanospermine commenced from the

6-deoxyhex-5-enopyranoside, which incorporates the aldol reaction and a novel catalytic reductive amination cascade to establish the indolizidine ring [36]. Peter Somfai *et al.* developed an asymmetric 19-step synthesis of castanospermine starting from diene with 13% of yield [37]. Usually, the ideal procedure for the synthesis of castanospermine is short, high- yielding and highly selective. Among the aboved mentioned examples of castanospermine synthesis, the method developed by Scott E. Denmark *et al.* involve in the shortest steps with rather high yield [31] .

Table 1: The procedures for the total synthesis of castanospermine

Starting material	Methods	Yield	Steps	Reference
dichloro enol ether	Sequential enol ether metathesis - hydroboration/ oxidation tandem	3.7%	17	[29]
methyl α-D- glucopyranoside	zinc-mediated fragmentation, ring-closing olefin metathesis, strain-release transannular cyclization	22%	9	[30]
2,5-dihydrofuran	Asymmetric tandem cycloaddition between silaketal nitro olefin and chiral vinyl ether	18%	8	[31]
methyl α-D- glucopyranoside	Triple reductive amination	22%	9	[32]
D-glucose-derived nitrone	Intramolecular 5-*endo*-Trig Aminomercuration of β-Hydroxy-γ-alkenylamines	15.8%	9	[33]
tetramic acid derivative	vinylogous Mukaiyama-type reaction a diastereodivergent reduction of tetramic acids	13.9%	9	[34]
tribenzyl D-glucono-δ-lactone	the diastereoselective, oxamidation of an unsaturated *O*-alkyl hydroxamate	n.a.	15	[35]
6-deoxyhex-5-enopyranoside	aldol reaction, reductive amination cascade	1.2%	12	[36]
diene	asymmetric hydroxylation, epoxidation, intramolecular cyclization	13%	19	[37]

n.a. means not available

SYNTHESIS OF 6-*O*-BUTANOYL CASTANOSPERMINE BY CHEMICAL AND ENZYMATIC METHODS

It is found that the 6- *O*-butanoyl analog of castanospermine showed an improved antiviral activity as compared with the parent compound [13]. Therefore, 6-*O*-butanoyl castanospermine synthesis in a short and efficient way constitutes an important and urgent target. However, castanospermine bears four secondary hydroxyl groups of similar reactivity, hence regioselective acylation at the 6-position represents a challenging goal for synthetic and medicinal chemists [38]. Liu *et al.* reported the selective synthesis of 6-*O*-butanoyl castanospermine in a total of five steps starting from castanospermine by chemical methods, which required the protection and deprotection of neighboring hydroxyl groups [39]. Anderson *et al.* described a one-pot route for the preparation of 6-*O*-butanoyl castanospermine, in which castanospermine was reacted with dibutyltin oxide in methanol and subsequently the product was *in situ* treated with an acid chloride and triethylamine to provide the desired 6-*O*-butanoyl castanospermine in yields ranging from 18% to 44% after flash chromatography [40]. In order to increase the yield of 6-*O*-butanoyl castanospermine, Tyler *et al.* improved the one-pot procedure reported by Anderson *et al.* In their methodology, castanospermine was treated with bis(tributyltin) oxide in toluene. A key step was removal of water through heating reflux of reaction, which resulted in an improved yield of 83% [41]. Enzymatic methods, with their unparalleled specificity and mild reaction condition, are an attractive way for acylation of hydroxyl group instead of the chemical method [42]. Deborah synthesized several esters of castanospermine via subtilisin-catalyzed regioselective acylation in pyridine [43] . However, lipase have not successfully used for the synthesis of 6-*O*-butanoyl castanospermine until now.

BIOLOGICAL ACTIVITIES OF CASTANOSPERMINE AND 6-*O*-BUTANOYL CASTANOSPERMINE

Castanospermine displays an impressive range of biological activities in that it is a potent and competitive inhibitor of endoplasmic reticulum resident α-glucosidase I as well as glucosidase II, and intestinal maltase and sucrase [44]. Therefore, castanospermine shows potential therapeutic value in the treatment of diseases as varied as cancer, diabetes, the infections of human viral pathogens including parainfluenza, dengue virus, HSV-2 and HIV-1 [45-51] (Table 2). Inhibition glycoprotein processing by castanospermine treatment resulted in a significant inhibition metastasis of B16-F10 murine melanoma cells [45] and tumor cell growth in nude mice [46] . Liu *et al.* established that castanospermine was effective in inhibitory action against increase of blood glucose in sucrose and starch-administrated mice, hence castanospermine would be useful for anti-diabetic purposes [47] . Castanospermine also has been found to have particularly noteworthy pharmacological effects on suppression of the infectivity of a number of viruses. Tanaka Y *et al.* investigated the antiviral effects of castanospermine on human parainfluenza virus type 3 (HPIV3). They postulated that castanospermine can prevent the first steps of HPIV3 envelope glycoprotein processing [48]. The extensively studied inhibition object of castanospermine is the human immunodeficiency virus responsible for AIDS, which suppressed syncytium formation and interfered with infectivity [49, 50] . More recently, castanospermine has been found to restrain all four serotypes of dengue fever virus in vitro and to prevent mortality of viral infection mice at a daily dose of 10 mg/kg body weight [16, 51]. Castanospermine has also been shown to be a novel immunosuppressive agent, which prolongs cardiac allograft survival [52] , acts synergistically with tacrolimus[53] , and alleviates experimental autoimmune encephalomyelitis

(EAE) [54] and adjuvant induced polyarthritis [55]. In addition it demonstrates plant growth regulation activity, preventing root length elongation of dicot roots by 50% at 300 ppb, while the effective concentration against monocot roots is 200 ppm [8]. Furthermore, castanospermine has inhibited the lysosomal a-glucosidase and produced a lysosomal block leading to the abnormal storage of glycogen [6].

Table 2: Biological roles of castanospermine and 6- *O*-butanoyl castanospermine

material	**Biological role**	Reference
castanospermine	A tumor growth inhibitor	[45,46]
castanospermine	An antidiabetic agent	[47]
castanospermine	An antiviral agent (parainfluenza, HIV-1 and dengue virus)	[48-51]
castanospermine	An immunosuppressive agent	[52-55]
castanospermine	A plant growth regulator	[8]
6-*O*-butanoyl castanospermine	An antiviral agent (HCV, HIV-1, dengue virus ,HSV-1, HSV-2)	[13-15,17,56,57]

The prodrug, 6- *O*-butanoyl castanospermine, which also inhibits a broad range of viruses in vitro, has been used in human clinical trials to improve pharmacokinetics and overcome the water- solubility and gastrointestinal upset. 6-*O*-butanoyl castanospermine is developed by MIGENIX Inc for the treatment of HCV infection. It disturbs the correct folding and assembly of E1-E2 pre- budding complexes, thereby giving rise to an inhibition of the production and secretion of neo- virion from infected cells and the decreased infectivity of released virions [13]. It also has antiviral activity for HIV which is manifested by a decrease in syncytia as well as the production of virus with altered gp120 and a reduced infectivity [14] . Abhay P.S. Rathore *et al.* demonstrated that 6-O-butanoyl castanospermine strongly inhibited all four DENV serotypes partly due to the misfolding and accumulation of DENV non-structural protein 1 (NS1) in the endoplasmic reticulum [17]. 6-0-butanoyl castanospermine has also been tested orally in mice, cutaneously infected with herpes simplex virus type 1 and found to produce a significant delay in lesion development and reduced the amount of virus recovered from the brain [56]. Similarly, it blocked the growth of herpes simplex virus type-2 [57].

CONCLUSION

Currently, castanospermine and 6- *O*-butanoyl castanospermine arouse considerable interests among researches because they have been found to have tremendous potential in some disorders such as diabetes, viral infection, immunosuppressive deficiencies and tumor metastasis etc. The main preparation methods of castanospermine were involved in organic synthesis and extraction from plant cell and tissue. However, little attention was paid to the production of castanospermine from microorganism. It was reported that another glucosidase inhibitor, 1-deoxynojirimycin, was found in *Morus alba L.* and *B. subtilis* [58, 59]. Therefore, it is possible to obtain castanospermine with microbial origin. For the synthesis of 6-*O*-butanoyl castanospermine, although there are some successful examples, the optimization of synthesis parameter by enzyme such as lipase is worth investigating in depth.

In eukaryotes, most proteins synthesized in the ER are glycoproteins. ERα-glucosidases I and II inhibitors , results in terminal glucose retention on N-linked glycans ,which disturb the folding, sorting, secretion and function of the glycoproteins [60]. In summary, it affected a number of molecular events in cells related to all sorts of glycoproteins.

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