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Review on Betulin: A nano-sized Pentacyclic dihydroxy-Triterpenoid

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Abstract

Studies on plant secondary metabolites have gained renewed interest in recent years because these can serve as renewable chemicals for the development of a sustainable society. Betulin, a naturally occuring 6-6-6-6-5 nano-sized pentacyclic Triterpenoid is obatainable from the bark powdered of *White Birch (Betula papyrifera).* In this review, the self-assembly of betulin in various organic liquids into supramolecular architectures and its potential application in the adsorption of fluorophores and removal of toxic dyes has been discussed. The potential pharmacological activities such as cardiovascular, diabetes, cancer, liver and anti-inflamatory has also been discussed.

Graphical abstract

Keywords: *Natural, Triterpene, Betulin, fluorohores, pharmacology*

1. Introduction

Plants constitute an enormous reservoir of renewable chemicals, and contemporary organic chemistry increasingly emphasizes the use of plant-derived metabolites as sustainable alternatives to petroleum-based raw materials, thereby addressing present demands without jeopardizing future resources. Primary metabolites such as carbohydrates, proteins, and nucleic acids are indispensable for plant survival, whereas secondary metabolites are crucial for plant growth, defense against pathogens, pollination, and the production of color and aroma. To date, over 500,000 plant secondary metabolites have been isolated and structurally elucidated. Among these, terpenoids—built from repeating C_5 (isoprene) units and occurring as C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, and C_{40} compounds—represent one of the most significant classes. In particular, C_{30} triterpenoids occupy a unique position following Ruzicka's biogenetic isoprene rule, which proposed squalene as the universal precursor for all triterpenoids. Extensive research over the past six decades has strongly validated this concept and aided the structural elucidation of numerous new triterpenoids.

The spontaneous self-assembly of molecules in liquids to form supramolecular structures ranging from nanometer to micrometer scales has emerged as a rapidly growing field due to both fundamental interest and technological relevance. Self-assembly has been widely reported for several natural compounds, including sugars, amino acids, fatty acids, sophorolipids, and steroids. Despite the vast structural diversity and abundance of naturally occurring triterpenoids, their self-assembly behavior remained unexplored until relatively recently. During our investigations into terpenoids structures and dimensions, we discovered that natural triterpenoids are inherently nano-sized molecules. This observation suggested that their renewable nature, structural diversity, and multiple chiral centers could make them excellent bio-based supramolecular building blocks.

In this review, I summarize a naturally occuring 6-6-6-6-5 pentacyclic triterpenoid, betulin extractable from the bark of white birch undergoes self-assembly in organic liquids to form colloidal suspension and gel. Detailed morphological studies of the self-assemblies revealed the formation of flower like architectures of nano to micrometer diameters. The flowers were composed of petals of nanometric dimensions consisting of nanofibers, as evident by electron microscopic techniques and X-ray diffraction studies. The porous self-assemblies of 1 have been utilized for the adsorption of fluorophore such as rhodamine-B and the anticancer drug doxorubicin. Moreover, the removal of toxic dyes such as rhodamine 6G (rho 6G), crystal violet (CV), methylene blue (MB), and cresol red (CR) from their aqueous solution has also been demonstrated. The potential pharmacological activities such as anti-tumor, anti-inflamatory, and anti-viral has also been discussed.

2. Triterpenoid as Renewable functional nano entities

Triterpenoids are a 30-carbon subset of terpenoids, the largest class of plant secondary metabolites. These compounds occur in acyclic as well as mono- to pentacyclic forms, exhibiting more than 100 distinct skeletal diversities. Fused tetra- and pentacyclic triterpenoids, possessing several chiral centers, are more abundant in nature than acyclic, mono-, bi-, and tricyclic triterpenoids. In the 1950s, Ruzicka and his co-workers proposed that all triterpenoids were biosynthesized from the common precursor squalene (1). Numerous enzymological studies,[20] mechanistic analysis,[21,22] and computational investigation[23,24] have confirmed that squalene (1) or oxidosqualene (2) is activated by a cationic attack, followed by a cation–olefin cyclizations and subsequent deprotonation, leading to monocyclic and fused polycyclic structures. Due to free rotation about its single bonds, the acyclic precursor squalene is highly flexible. As cyclization progresses, molecular rigidity increases with the formation of ring systems, while flexibility is retained primarily in the side chain, resulting in a concomitant change in molecular dimensions. Detailed conformational analysis revealed that acyclic squalene can adopt more than 10,000 conformers. This number gradually decreases to monocyclic, bicyclic tricyclic, tetracyclic and pentacyclic triterpenoids. To investigate molecular length variations, examination upon 60 representative naturally occurring triterpenoids has been carried out, which revealed that all pentacyclic triterpenoids are narrowly confined between 1.2 and 1.3 nm.

3. Natural Sources of Betulin

Historically, betulin can be traced back to 1788, when it was first observed by Lowitz [8], but its name was first coined by Mason in 1831 [9]. Betulin is a lupane-type triterpenoid with two hydroxyl groups at the 3α and 28 positions (Figure 1). One of the hydroxyl groups is a primary OH group at carbon 28, and the other is a secondary OH group at carbon 3. An alkene moiety was also observed at carbon 20. The hydroxyl and alkene groups serve as binding sites for simple modifications, whereas the pentacyclic lupane skeleton contributes to the lipophilic nature of betulin, resulting in poor aqueous solubility. Betulin is isolated from the bark of white birch solvent extraction, and the content of betulin in the extract depends on the birch species and the part of the tree. It is found in various plant sources; however, its most abundant source is the Betula spp. Even so, betulin can be obtained from other non-plant sources, including mushrooms. Table 1 lists various sources of betulin, their extractants, and isolation methods.

4. Self-assembly of Betulin

Betulin 1 (Figure 1) is a pentacyclic dihydroxy triterpenoid (6–6–6–6–5 ring system) with a molecular length of approximately 1.29 nm. It is mainly isolated from the bark of white birch (*Betula papyrifera*), which is widely distributed in North America and Europe. Betulin is well known for its diverse biological activities, including anticancer, anti-inflammatory, and antiviral effects. Structurally, betulin is an amphiphilic molecule composed of a rigid, nanosized lipophilic backbone bearing a secondary hydroxyl group on the A ring and a hydroxymethyl group at the junction of the trans-fused D and E rings. Its structure closely resembles that of betulinic acid, except that the carboxylic acid group at the C-28 position in betulinic acid is replaced by a hydroxymethyl group in betulin.

To understand how this subtle functional group difference influences molecular organization, the self-assembly behavior of betulin was investigated in various neat organic solvents and aqueous solvent mixtures. Interestingly, betulin showed strong self-assembly tendencies in aromatic solvents and mixed aqueous systems, forming soft solid-like materials in several cases. For instance, an opaque gel formed instantly in a DMSO–water (1:1) mixture at 1% w/v, while opaque gels were also observed in neat o-, m-, and p-xylene at 5% w/v. In contrast, colloidal suspensions were obtained in solvents such as benzene, toluene, mesitylene, o-dichlorobenzene, and mixed systems including ethanol–water, DMF–water, and ethylene glycol–water (1:1).

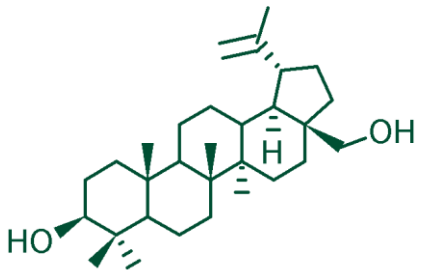

Figure 6. Betulin **1**, a 6–6-6-6-5 pentacyclic dihydroxy triterpenoid extractable from White birch (Betula papyrifera).

Morphological analysis using optical microscopy revealed the formation of flower-like structures with micrometer-scale dimensions. Similar petal-like architectures ranging from nano- to micrometer size were confirmed by transmission electron microscopy (TEM). Field-emission scanning electron microscopy (FESEM) of dried assemblies further showed well-defined flower-shaped objects, approximately 520 nm in size. Detailed examination indicated that these symmetric structures consisted of multiple two-dimensional petal-like sheets radiating from a common center. Each petal was formed from intertwined nanofibers with diameters of 27–48 nm, suggesting that many betulin molecules pack together within each fiber cross-section.

Wide-angle X-ray diffraction analysis of the dried self-assemblies revealed a lamellar arrangement with characteristic reflections at 1.2, 0.6, and 0.4 nm,

corresponding to a 1:1/2:1/3 spacing ratio. This pattern suggests a head-to-tail molecular packing stabilized by intermolecular hydrogen bonding. Similar diffraction profiles were observed for xerogels prepared from different solvents, indicating a consistent self-assembly mod**e** irrespective of the solvent system. FTIR studies further supported this conclusion by showing shifts in the O–H stretching frequencies compared to the neat powder, confirming the involvement of strong hydrogen bonding during assembly.

Overall, these results demonstrate that even a minor functional group modification in triterpenoid molecules can significantly alter their self-assembly behavior. In particular, the hydroxymethyl group at the D/E ring junction plays a crucial role in directing supramolecular organization, as betulin forms flower-like architectures, whereas betulinic acid produces simple fibrillar structures under identical conditions.

Entry	Solvent	State	MGC	$T_{gel}{}^{0}$c
1	Benzene	G	2.50	>85
2	Toluene	G	1.43	70-72
3	o-xylene	G	1.06	75-78
4	m-xylene	G	2.50	>90
5	p-xylene	G	5.00	>90
6	Mesitylene	G	2.04	74-76
7	Brormo benzene	G	0.54	46-47
8	Chloro benzene	G	2.57	>85
9	o-dichloro bezene	G	0.41	44-46
10	Nitrobenzene	G	0.90	62-65
11	Methanol	CS	1.25	64-66
12	Ethanol	CS	3.4	-
13	n-Propanol	G	10.0	-
14	n-Butanol	G	10.0	76-77
15	n-Pentanol	G	10.0	>90
16	n-Hexanol	G	10.0	>90
17	n-Heptanol	G	10.0	78-79
18	n-Octanol	G	10.0	80-81

5. Application of self-assemblies in dye removal:

Development of biocompatible nano carriers has been an emerging area of research for efficient drug delivery.[87, 79,88] To find out whether the porous self-assemblies of betulin are capable of adsorbing various fluorophores including anticancer drugs, we have studied the adsorption of rhodamine-B and the anticancer drug doxorubicin as model compounds.[89,90, 91]Comparing the microscopic images under normal and fluorescence lights it was clear that both the fluorophores were adsorbed on the flower like self-assemblies of betulin (Figure 10a-d). The large surface area of porous self-assembled betulin facilitated the adsorption of rhodamine B and doxorubicin dyes. These results opens up the use of self-assembled betulin in the removal of toxic dyes[92] and targeted drug delivery vehicles. Removal of toxic dyes and other pollutants from contaminated water is of great concern for maintaining a clean and healthy environment.As dried self-assemblies of betulin derived from various organic liquids were highly porous in nature, we examined the utilization of these porous self-assemblies for the removal of organic dye molecules from their aqueous solutions.The removal studies carried out with three cationic dyes namely rhodamine 6G (Rho 6G), crystal violet (CV), methylene blue (MB) and one anionic dye cresol red (CR) revealed that the cationic dyes could be selectively removed very efficiently by the dried self-assemblies of betulin (Figure 11). When a weighed amount of the dried porous selfassembled betulin was kept in equilibrium with aqueous solutions of the dyes and the adsorptions of the dyes were monitored by UVvisible spectroscopy after a certain time interval, the reduction of the absorption intensities with time indicated the adsorption of dye molecules on the surface of the dried porous material.By comparing the initial absorption intensity of the dye solutions and the decreasing absorption intensities of the aqueous layer with time, it was observed that the dried porous self-assembled material could remove the cationic dyes rhodamine 6G (78.0 %), crystal violet (99.0 %), and methylene blue (62.0 %) from aqueous solution very efficiently. However, adsorptions of the anionic dye cresol red (CR) was negligible by the porous material. Photographs of the porous materials (Figure 11) after the adsorption of dyes clearly indicated the adsorption of dyes.

6. Pharmacological Activities of Betulin

6.1. Protective Effects of Betulin on Cardiovascular Diseases

Cardiovascular disease (CVD) includes several conditions that affect the heart and blood vessels, such as heart failure, cardiomyopathy, abnormal heart rhythm, reduced blood supply to the heart, and heart defects present from birth. Even though these diseases develop in different ways, they are the leading cause of death worldwide, accounting for about 17.9 million deaths (31% of total deaths). Cardiomyopathy is a disorder of the heart muscle in which the heart becomes unusually thick or enlarged and may be inherited or caused by other diseases.

Studies suggest that betulin helps protect the heart. In heart cells, betulin reduced cellular stress, improved heart tissue structure, decreased excess fat accumulation, and enhanced cholesterol removal by increasing ABCA1 levels. In diabetic mice, betulin reduced heart damage, improved heart function, and lowered inflammation and cell death. Long-term betulin treatment reduced plaque formation and increased plaque stability in blood vessels. In high-fat diet mouse models,

betulin slowed atherosclerosis by improving cholesterol efflux through increased ABCA1 and ABCG1 levels, mainly by suppressing SREBP signaling and lipid-producing genes.

6.2 Protective Effects of Betulin on Diabetes

Diabetes mellitus is a chronic metabolic disorder affecting hundreds of millions of people worldwide and is associated with severe micro- and macrovascular complications. Betulin, a lupane-type triterpenoid, has shown notable antidiabetic potential in multiple experimental models. Studies demonstrate that betulin improves glucose tolerance, enhances insulin sensitivity, lowers blood glucose and lipid levels, and suppresses inflammatory responses through modulation of pathways such as SREBP, PPAR-γ, and NF-κB. It also inhibits key carbohydrate-digesting enzymes, including α-amylase and α-glucosidase, thereby reducing postprandial hyperglycemia. Additionally, betulin supports diabetic wound healing by regulating pro-inflammatory cytokines. Collectively, these findings highlight betulin as a promising therapeutic candidate for diabetes and related metabolic disorders.

6.3 Ptotective effect of betulin on cancer:

Current cancer therapies are often limited by severe side effects, driving interest in safer, natural alternatives. Betulin, a plant-derived lupane-type triterpenoid, has shown broad anticancer potential in numerous studies. It suppresses tumor growth by inducing apoptosis, autophagy, cell-cycle arrest, and mitochondrial dysfunction, while also inhibiting angiogenesis, inflammation, metastasis, and cancer cell migration. Betulin has demonstrated effectiveness against a wide range of cancer types in both in vitro and in vivo models and has enhanced the efficacy of conventional chemotherapeutic agents when used in combination. These findings suggest that betulin may serve as a promising low-toxicity anticancer and chemo preventive agent.

6.4 Protective Effects of Betulin on Liver Diseases

Liver diseases arise from multiple causes and often lead to severe functional impairment. Betulin has demonstrated significant hepatoprotective effects in various experimental models, particularly against alcohol- and drug-induced liver injury. It alleviates steatosis, inflammation, fibrosis, and oxidative stress while improving liver function markers and lipid profiles. These protective effects are mediated through modulation of key pathways, including SREBP-1, Sirt1/AMPK, NF-κB, inflammasome signaling, and antioxidant defenses. Overall, betulin emerges as a promising natural agent for the prevention and treatment of hepatic damage.

6.5 Protective Effects of Betulin on Inflammation

Inflammation is a protective biological response that helps the body defend against tissue injury and infection, but its prolonged activation can lead to chronic disease. While acute inflammation is beneficial and characterized by neutrophil involvement, persistent inflammation dominated by macrophages and lymphocytes contributes to pathological conditions. This process is regulated by complex signaling networks involving inducers, sensors, mediators, and effectors, with cytokines, chemokines, and lipid mediators playing key roles. Since chronic inflammation underlies many diseases, natural compounds capable of modulating multiple inflammatory pathways are increasingly preferred over single-target drugs due to their improved safety and therapeutic potential.

7. Conclusions

Plants serve as invaluable sources of a wide array of bioactive compounds that are essential for human nutrition, health promotion, and therapeutic applications. Triterpenes constitute a significant group of plant secondary metabolites, structurally defined by five fused rings and biosynthesized from the linear precursor squalene. In this review, I have discussed Betulin, *6-6-6-6-5*, pentacyclic naturally occurring dihydroxy triterpenoid extractable from the bark of White birch self-assembled hierarchically in organic and organo-aqueous liquids yielding 3D flower-like architectures of nano- to micrometer diameters via the formation of fibrillar networks. The porous self-assemblies have potential application in the adsorption of various fluorohores including anti cancer drug doxorubicin and removal of toxic dyes from its aqueous solution. The molecule has also exhibit diverse biological and pharmacological activities. Betulin exhibited strong protective effect against severe diseases such as cardiovascular, diabetics, cancer, liver and inflammation etc. Overall, these studies show that betulin has strong potential for treating diseases. However, more preclinical research is needed to better understand its effects. Further studies on its safety, behavior in the body, and ways to improve its solubility and absorption are also required.

8. Acknowledgements

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9. Notes and References

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10. About the author(s)

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