

Chemical and pharmaceutical technologies. Life safetyDOI: <https://www.doi.org/10.53002/168>**METHODS FOR BETULIN PURIFICATION: A REVIEW**¹**Bakibayev A.A., ¹Lyapunova M.V., ¹Razgulyaeva Yu.D., ¹Salina M.V., ¹Zaitsev A.V.*¹ *National Research Tomsk State University, Tomsk, Russian Federation*** Correspondent's E-mail: bakibaev@mail.ru***Author information:****Bakibayev A.A.** — Professor, Doctor of Chemical Sciences, National Research Tomsk State University, Tomsk, Russian Federation; e-mail: bakibaev@mail.ru.**Lyapunova M.V.** — Researcher, National Research Tomsk State University, Tomsk, Russian Federation; e-mail: lyapunova.mari@mail.ru.**Razgulyaeva Yu.D.** — PhD Student, National Research Tomsk State University, Tomsk, Russian Federation; e-mail: lyapunova.mari@mail.ru.**Salina M.V.** — Master's Student, National Research Tomsk State University, Tomsk, Russian Federation; e-mail: 89991237185@mail.ru.**Zaitsev A.V.** — Master's Student, National Research Tomsk State University, Tomsk, Russian Federation; e-mail: Deynerys02@mail.ru.

Abstract — Betulin, a pentacyclic triterpenoid abundant in the outer bark of Betula species, represents a valuable natural compound for pharmaceutical, biomedical, and cosmetic applications. Its further utilization, however, requires reliable technologies capable of providing material with a consistently high level of purity. This review examines current strategies for recovering and purifying betulin from birch bark, with emphasis on the relationship between physicochemical properties of the compound and the efficiency of individual separation processes. Solvent-based extraction, recrystallization, chromatographic procedures, and alternative purification techniques are discussed with consideration of solvent polarity, temperature-dependent solubility, hydrogen-bonding interactions, and crystalline solvate formation. Reported experimental procedures and patented technologies are evaluated with respect to product purity, solvent requirements, operational complexity, recovery efficiency, and prospects for technological scale-up. Methods employed for structural identification and purity control, particularly high-performance liquid chromatography and spectroscopic techniques, are also reviewed. Analysis of the published data indicates that recrystallization remains a practical route for obtaining highly purified betulin, although its effectiveness strongly depends on appropriate solvent selection and careful control of processing conditions. Differences in the composition of birch bark associated with species and geographical origin are additionally considered, with particular interest in less extensively investigated taxa such as Betula kirghisorum. The development of more selective, resource-efficient, and environmentally sustainable extraction and purification processes is expected to improve the production of high-purity betulin from renewable birch resources and expand opportunities for its subsequent chemical modification and practical application.

Keywords — betulin; Betula spp.; birch bark; pentacyclic triterpenoids; isolation; purification; solvent extraction; recrystallization.

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Абстракт — Бетулин — Betula туысына жататын қайың түрлерінің сыртқы қабығында едәуір мөлшерде жинақталатын табиғи пентациклді тритерпеноид. Оның биологиялық қасиеттерінің алуан түрлілігі және фармацевтика, биомедицина мен косметология салаларында пайдалану мүмкіндігі бұл қосылысқа деген ғылыми және практикалық қызығушылықты арттырады. Бетулин негізіндегі өнімдерді әзірлеудің маңызды алғышарттарының бірі — қажетті тазалық деңгейіндегі қосылысты тұрақты әрі тиімді алуға мүмкіндік беретін технологияларды таңдау. Осы шолу жұмысында қайың қабығынан бетулинді бөліп алу және тазарту үшін қолданылатын заманауи тәсілдер жүйеленіп, олардың ерекшеліктері салыстырмалы түрде қарастырылды. Негізгі назар еріткіштік экстракцияға, қайта кристалдандыруға, хроматографиялық бөлуге және физика-химиялық тазартудың басқа әдістеріне аударылды. Тазарту нәтижесіне әсер ететін еріткіштің полярлығы, температураға тәуелді ерігіштік, молекулааралық әрекеттесулер және кристалдық сольваттардың түзілуі сияқты факторлар талданды. Әдебиетте сипатталған тәжірибелік әдістер мен патенттелген технологиялар алынатын өнімнің тазалығы, еріткіш шығыны, технологиялық үдерістің күрделілігі және оны өндірістік деңгейге масштабтау мүмкіндігі бойынша салыстырылды. Бетулиннің құрылымын сәйкестендіру және тазалық дәрежесін анықтау үшін қолданылатын аналитикалық әдістерге, соның ішінде жоғары тиімді сұйықтық хроматографиясы мен спектроскопиялық талдау тәсілдеріне де назар аударылды. Жинақталған деректер қайта кристалдандырудың жоғары тазалықтағы бетулин алудың практикалық тұрғыдан тиімді әдістерінің бірі екенін көрсетеді. Алайда оның нәтижелілігі еріткішті дұрыс таңдауға және үдерістің технологиялық параметрлерін оңтайландыруға тікелей байланысты. Сонымен қатар қайыңның түрлік ерекшелігі мен географиялық өсу жағдайларының бетулин мөлшері мен құрамына ықпалы маңызды зерттеу бағыты болып табылады. Осы тұрғыдан Қазақстан аумағында таралған, салыстырмалы түрде аз зерттелген Betula kirghisorum түрі ерекше қызығушылық тудырады. Экологиялық қауіпсіз, ресурсты үнемдейтін және селективті экстракциялау мен тазарту технологияларын жетілдіру жаңартылатын өсімдік шикізатынан жоғары тазалықтағы бетулин өндірудің тиімді технологиялық негізін қалыптастыруға және оны әрі қарай химиялық модификациялау мен практикалық қолдану мүмкіндіктерін кеңейтуге ықпал етеді.

Кілт сөздер — бетулин; Betula spp.; қайың қабығы; пентациклді тритерпеноидтар; бөліп алу; еріткіштік экстракция; тазарту; қайта кристалдандыру.

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Аннотация — Бетулин — природный пентациклический тритерпеноид, накапливающийся в значительных количествах в наружном слое коры растений рода Betula. Разнообразие биологических свойств и перспективы применения в фармацевтике, биомедицине и косметологии обуславливают устойчивый научный и практический

интерес к данному соединению. Одним из ключевых условий дальнейшего использования бетулина и создания продуктов на его основе является разработка технологически эффективных способов получения вещества с воспроизводимо высокой степенью чистоты. В настоящем обзоре систематизированы современные подходы к выделению и очистке бетулина из бересты и проведено их сравнительное рассмотрение. Основное внимание уделено экстракции органическими растворителями, перекристаллизации, хроматографическому разделению и другим физико-химическим способам очистки. Проанализировано влияние полярности растворителя, температуры, растворимости, межмолекулярных взаимодействий и образования кристаллических сольватов на эффективность разделения и качество конечного продукта. Представленные в научной литературе экспериментальные методы и запатентованные технологические решения сопоставлены по степени очистки бетулина, расходу растворителей, сложности технологического процесса и перспективам масштабирования. Рассмотрены методы идентификации и контроля чистоты получаемого соединения, в том числе высокоэффективная жидкостная хроматография и спектроскопические методы анализа. Обобщение опубликованных данных свидетельствует о том, что перекристаллизация остается практически значимым способом получения высокоочищенного бетулина, однако ее результативность в значительной степени определяется выбором растворителя и оптимизацией технологических параметров. Отдельное внимание уделено влиянию видовой принадлежности и географического происхождения растительного сырья на характеристики выделяемого бетулина. В этом аспекте особый интерес представляет сравнительно малоизученный вид *Betula kirghisorum*, распространенный на территории Казахстана. Совершенствование селективных, ресурсосберегающих и экологически приемлемых методов экстракции и очистки может создать технологическую основу для эффективного получения высокочистого бетулина из возобновляемого растительного сырья и расширить возможности его последующей химической модификации и практического применения.

Ключевые слова — бетулин; *Betula* spp.; береста; пентациклические тритерпеноиды; выделение; экстракция растворителями; очистка; перекристаллизация.

MAIN PROVISION

A comparative analysis of published and patented methods showed that recrystallization is one of the most accessible and effective approaches for obtaining high-purity betulin from birch bark extracts.

The efficiency of betulin purification is primarily determined by the physicochemical properties of the solvent, temperature conditions, betulin solubility, and the formation of crystalline solvates.

The combination of extraction and recrystallization with chromatographic and spectroscopic control provides a reliable approach to the isolation, identification, and purity assessment of betulin.

The selection of an appropriate solvent system and optimization of purification conditions can reduce the content of accompanying triterpenoids and other impurities while minimizing solvent consumption and process complexity.

The reviewed approaches provide a basis for the development of scalable and environmentally sustainable technologies for producing high-purity betulin from renewable birch bark resources.

INTRODUCTION

Natural triterpenoids and their chemically modified analogues continue to be of considerable interest as promising sources of biologically active substances. Among this broad class of natural compounds, betulin occupies a particularly important position because of its availability, diverse biological properties, and potential for further structural modification. Highly purified

betulin has therefore become the subject of extensive research and is increasingly considered as a valuable

starting material for the development of pharmaceutical and cosmetic formulations [1–8].

Research on betulin has a long history extending over two centuries. Early studies describing its extraction from the outer bark of birch species and subsequent isolation in crystalline form appeared during the first half of the nineteenth century. Nevertheless, interest in this triterpenoid has remained substantial, largely because its practical utilization depends not only on its biological properties but also on the availability of reproducible methods for obtaining material of appropriate purity and quality. Existing extraction procedures allow betulin to be recovered from birch bark; however, further improvement of its isolation and purification remains important for both laboratory studies and potential large-scale applications. Consequently, optimization of extraction conditions, purification procedures, and reliable approaches to compound identification continues to represent an important area of research.

Particular attention may also be given to birch species growing under the specific ecological and geographical conditions of Kazakhstan. Investigation of natural triterpenoids isolated from endemic and locally distributed species, including the Kyrgyz birch (*Betula kirghisorum*), provides an opportunity to compare the qualitative and quantitative composition of bark-derived compounds among birch species

originating from different geographical regions. Such comparative studies may help clarify the relationship between geographical origin, environmental conditions, species-specific characteristics, and the accumulation of triterpenoid constituents in plant raw materials. These data may ultimately provide a scientific basis for evaluating *B. kirghisorum* as a potential local source of betulin and related biologically active compounds.

LITERATURE REVIEW

1. General Information on Betulin and Some of Its Derivatives

Betulin 1 is a naturally occurring pentacyclic triterpenoid belonging to the lupane-type triterpenes. Its systematic name is $3\beta,28$ -dihydroxylup-20(29)-ene, also referred to as lup-20(29)-ene- $3\beta,28$ -diol (Fig. 1).

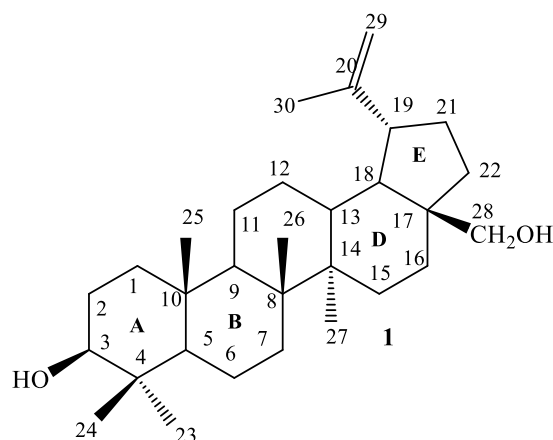


Figure 1. Betulin ($3\beta,28$ -dihydroxylup-20(29)-ene or lup-20(29)-ene- $3\beta,28$ -diol).

The structure of lupane-type triterpenoids is based on a complex polycyclic system containing a cyclopentanoperhydrophenanthrene (sterane) core composed of four fused cyclohexane rings and one saturated cyclopentane ring. According to the conventional numbering of the carbon skeleton, the rings are designated A, B, C, D, and E in order of increasing carbon atom numbers (Fig. 1). A characteristic structural feature of betulin 1, indicating its classification within this group, is the presence of a five-membered E ring and an α -isopropenyl group at the C-19 carbon atom [9].

Birch bark consists of two clearly distinguishable layers: the outer bark and the inner bark. The outer bark is particularly rich in extractive compounds. Betulin 1, which is responsible for the characteristic white color of birch bark, is the major component of nearly all birch

bark extracts. Lupeol 2 is a common accompanying compound during the isolation of betulin (Fig. 2). In addition to betulin 1, birch bark extracts contain several oxidized derivatives, including betulin aldehyde 3, betulonic aldehyde 4, betulinic acid 5, methyl betulinate 6, and 3-oxobetulinic (betulonic) acid 7 (Fig. 2).

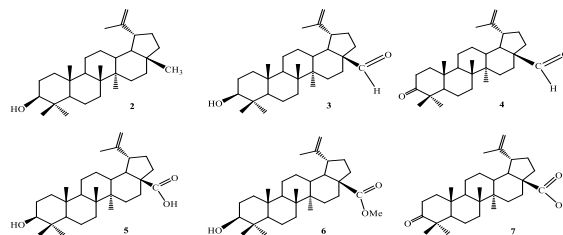


Figure 2. Terpenoids 2–7 accompanying betulin 1 during its isolation from birch bark.

The chemical composition of the bark of several birch species, including *Betula pendula* Roth. (*B. verrucosa* Ehrh.), *Betula pubescens* Ehrh. (*B. alba* L.), and *B. davurica* Pall., has been extensively studied [10]. The betulin content of the outer bark varies from 10 to 40%, depending on the birch species, geographical location and growing conditions, tree age, season, and other factors [11,12].

The diverse properties of betulin are closely associated with its biological role in plants. Betulin is synthesized and accumulated predominantly in the outer protective tissues, where it contributes to the plant's defense against adverse environmental factors. As a component of the protective bark layer, betulin contributes to resistance against radiation, bacteria, fungi, viruses, and insects. The remarkable durability of birch bark is illustrated by its ability to remain preserved in soil for more than 1,000 years without substantial decay, as demonstrated by the well-known birch-bark manuscripts discovered in Novgorod.

METHODS FOR BETULIN PURIFICATION

Betulin (1) is generally obtained as a white crystalline solid that may also display partially amorphous characteristics. Crystallization from commonly used organic solvents does not always produce well-developed large crystals. This behavior can be associated with the structural features of the molecule, particularly the predominance of weakly polar and lipophilic hydrocarbon fragments.

Structurally, betulin (1) contains an olefinic double bond together with two hydroxyl groups of different types: one primary and one secondary alcohol group. The presence of these functional groups substantially

affects its interactions with organic solvents and explains its comparatively greater affinity for media of intermediate polarity than for strongly nonpolar solvents. Betulin has the molecular formula $C_{30}H_{50}O_2$ and a molar mass of 442.72 g/mol. Literature data indicate some variation in its reported melting characteristics. A melting point of 261 °C and an optical rotation of $[\alpha] = +20^\circ$ in pyridine have been reported [14], while Hausmann reported a melting temperature of 258 °C [15]. The compound is odorless and demonstrates good stability upon exposure to atmospheric oxygen and light. It is also generally regarded as non-toxic. According to the literature, betulin (1) is not encountered naturally in an unbound free form [16].

From a physicochemical perspective, betulin is characterized by relatively low chemical reactivity and practically no solubility in water. After isolation, it commonly forms pale or nearly white prismatic crystals. Its solubility increases considerably in several organic media, particularly at elevated temperatures. Betulin dissolves comparatively well in boiling alcohols and is also soluble in ether, chloroform, benzene, pyridine, tetrahydrofuran, and dimethyl sulfoxide. Among alcohols, the reported solubility trend is 1-butanol > 1-propanol > ethanol > 1-pentanol > 1-hexanol > methanol. For ester-based solvents, the corresponding sequence is ethyl acetate > methyl acetate > ethyl formate = methyl formate [17].

In contrast, betulin exhibits limited solubility in nonpolar organic media [18,19]. Its solubility, however, is strongly dependent on temperature as well as solvent properties. This effect can be illustrated by comparing acetone and cyclohexane. At 15.2 °C, approximately 5.2 g/L of betulin can dissolve in acetone, whereas its solubility in cyclohexane is only about 0.1 g/L. Increasing the temperature to 35.2 °C raises these values to 13.7 and 0.67 g/L, respectively. These data demonstrate the pronounced influence of both solvent polarity and temperature on the dissolution behavior of betulin.

Despite its lower solubility in hydrocarbons, the use of nonpolar media can provide important advantages during purification [20]. In particular, recrystallization from such solvents minimizes the formation of solvent-containing crystalline phases. Polar solvents, by contrast, are capable of participating in hydrogen-bonding interactions with the hydroxyl groups of betulin. Hydrogen bonds may therefore be established both between betulin molecules themselves and between betulin and solvent molecules, facilitating solvate formation [20].

Most conventional procedures used for the purification of crude betulin rely on recrystallization from polar protic or aprotic media [21]. Typical solvents include lower aliphatic alcohols, acetone, chloroform, ethyl acetate, and dichloromethane. Although these solvents are suitable for dissolving and recrystallizing betulin, their application has several limitations. In particular, betulin may form crystalline complexes or solvates with the solvent. Furthermore, the selectivity of such systems toward structurally related accompanying components is often insufficient. Crude birch-bark extracts may contain lupeol, polyphenolic compounds, and oxidation products of betulin, including betulinic and betulonic acids as well as related aldehydes. Their efficient separation during conventional recrystallization can therefore be difficult. Another important disadvantage is the loss of the target compound, which may reach approximately 40% during purification.

An alternative strategy aimed at improving the purity of betulin involves recrystallization from high-boiling hydrocarbons. Nonane, which has a boiling point of approximately 151 °C, has been investigated as a representative solvent for this purpose [22,23]. In this procedure, crude betulin is heated under reflux with nonane until complete dissolution of the target compound is achieved.

Recrystallization using nonane has been reported to produce betulin with a purity of up to 98%. The principal advantage of this approach is that a high-boiling hydrocarbon medium reduces the likelihood of forming stable betulin–solvent complexes. At the same time, differences in the physicochemical behavior of the individual components of crude alcoholic birch-bark extracts make their selective separation possible.

The nonane-based procedure may therefore provide not only highly purified betulin but also additional valuable fractions containing polyphenolic compounds and phytosterols. Betulin obtained at approximately 98% purity can be employed in subsequent chemical transformations without an additional purification step. Another technological advantage is the possibility of recovering nonane after the purification process and returning it to the production cycle. Consequently, solvent consumption and overall processing costs can potentially be reduced.

High-purity betulin can also be prepared indirectly through the hydrolysis of its ester derivatives. In particular, betulin diacetate can be hydrolyzed by heating in ethanol in the presence of excess potassium hydroxide. This transformation has been described as an effective route for producing betulin with a purity of approximately 98%.

Chromatographic techniques provide another approach to the purification of lupane- and oleanane-type triterpenoids. Studies [24,25] examining betulin, betulin diacetate, and allobetulin demonstrated favorable results when column chromatography was performed using aluminum oxide as the stationary phase and acetone as the mobile phase. GC–MS analysis indicated that chromatographic treatment increased the proportion of the target triterpenoids by approximately 4–7%, with the final purity reaching about 95–98%. The procedure effectively removes accompanying substances and yields materials suitable for further use in organic synthesis and in electrochemical, microbiological, spectroscopic, and related investigations. Its reported practical benefits include relatively straightforward operation, short processing times, and the ability to obtain highly purified products.

Other purification strategies have also been described. For example, benzene and chloroform have been employed to separate polar and nonpolar contaminants from betulin-containing materials [26]. Another procedure combines alkaline treatment with subsequent removal of accompanying substances using xylene as an aromatic hydrocarbon solvent [27]. These approaches illustrate the variety of solvent systems that have been investigated to exploit differences between the physicochemical properties of betulin and those of associated extractives.

Crystallization conditions have a pronounced effect on the morphology and dimensions of the resulting betulin crystals. In study [26], crystallization of an ethanolic betulin extract produced small, flat, transparent, colorless crystals with an average size of approximately 0.2 mm. In contrast, gradual cooling of a hot saturated ethanol solution to room temperature resulted in substantially larger crystals, with lengths reaching approximately 12 mm. Structural studies have shown that crystallization from ethanol produces an orthorhombic betulin phase containing ethanol in the crystal lattice. The resulting solvate contains betulin and ethanol in a 1:1 ratio, with the two components

associated through hydrogen-bonding interactions [20,28,29].

Melting behavior remains one of the traditional physicochemical parameters used for evaluating the identity and purity of isolated betulin. Published melting temperatures cover a relatively broad interval of approximately 251–261 °C [27], although values near 255 °C are reported most frequently. Thermal observation of ethanol-derived betulin crystals reveals several successive transformations. At approximately 130–140 °C, darkening of the crystals accompanies the release of ethanol. Sublimation becomes noticeable at around 160–170 °C, while heating to 180–200 °C leads to the appearance of new transparent microcrystalline formations on the surface of the initial crystals. Complete melting occurs at approximately 255–256 °C. Subsequent cooling does not restore the original crystalline structure; instead, the material solidifies as an amorphous, glass-like phase. Atmospheric-pressure sublimation has likewise been reported to produce bright white powders consisting of amorphous spherical microparticles [26].

Overall, the available data demonstrate that the efficiency of betulin purification is governed by several interrelated factors, including solvent polarity, temperature, hydrogen-bonding ability, solvate formation, and differences in the solubility of betulin and accompanying birch-bark constituents. Conventional recrystallization, hydrocarbon-based purification, hydrolysis of betulin derivatives, and chromatographic separation each provide different advantages and limitations in terms of purity, product recovery, solvent consumption, and technological complexity. Further optimization of these processes is therefore important for obtaining betulin of reproducible quality and for improving the utilization of birch bark as a renewable source of valuable triterpenoids. Efficient isolation and purification may, in turn, broaden opportunities for the subsequent chemical modification and practical application of betulin and its derivatives.

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