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**REVIEW ARTICLES**

# Biological Activity and Pharmacological Prospects of Lupane Terpenoids: I. Natural Lupane Derivatives

**T. G. Tolstikova*, I. V. Sorokina*, G. A. Tolstikov*, A. G. Tolstikov**,[1] and O. B. Flekhter*****

** Vorozhtsov Institute of Organic Chemistry, Novosibirsk, Siberian Division, Russian Academy of Sciences, pr. akademika Lavrent'eva 9, Novosibirsk, 630090 Russia*

*** Institute of Technical Chemistry, Ural Division, Russian Academy of Sciences, ul. Lenina 13a, Perm, 614990 Russia*

**** Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, pr. Oktyabrya 71, Ufa, 450054 Russia*

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**Abstract**—The biological activity of natural and semisynthetic lupane triterpenoids is discussed in a two-part review. The first part is devoted to the pharmacological properties of natural lupane triterpenoids. Betulinic acid has proven to be the most effective antitumor agent among more than fifty natural lupanes.

*Key words: anti-HIV activity, betulin, betulinic acid, betulonic acid*

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## INTRODUCTION

Triterpenoids are widely spread in nature [1–3]. In the last few years, natural triterpenoids, especially those exhibiting antitumor activity, are of increasing interest of the specialists in the field of pharmacology and medicinal chemistry. More than 100 compounds promising as antineoplastic (cytostatic) agents have been revealed among natural triterpenoids of various structural types [4].

Semisynthetic analogues of natural triterpenoids are now gaining a growing acceptance as well. We should like to stress that triterpenoids characterized by both availability and significant biological activity become the preferential subjects of chemical transformations [5–8].

Lupane triterpenoids are of a particular interest. As to the availability of natural lupane derivatives, a number of them have unique natural sources. European white birch tree (*Betula pendula* Roth.) widely spread in Russia is one of such sources of betulin. The birch bark, which is a waste of wood processing and production of activated charcoal, contains up to 30% of betulin [9–11]. This source also permits isolation of up to 3 wt % of lupeol, the simplest lupane triterpenoid [12].

Biological activity of lupane triterpenoids had attracted attention as far back as in the 19th century. For example, betulin was suggested for the use as a component of antiseptic plasters [13]. Tar obtained by thermolysis of birch bark and containing lupane derivatives is known to be a component of Vishnevsky's ointment.

In the last decade, the real burst of interest in the pharmacological properties of lupane derivatives was observed after revealing promising antiviral (in particular, anti-HIV) and antineoplastic agents among these compounds. In particular, betulinic acid was recognized as a rather potent antitumor agent [14]. Active anti-HIV agents have been revealed among synthetic derivatives of betulinic acid [15, 16]. Biological tests and pharmacological studies of several dozens of natural lupane triterpenoids and several hundreds of semisynthetic compounds, betulinic acid derivatives being among them, have been carried out up to now.

This circumstance determined the structure of the present review. The pharmacological properties of natural lupane triterpenoids are discussed in the first part of this review, whereas the second part is devoted to the prospects of use of semisynthetic lupane derivatives.

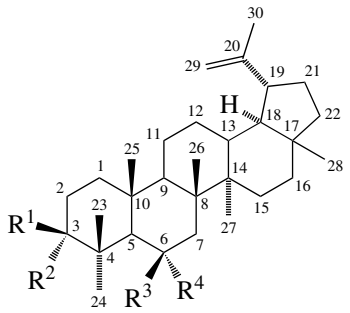
## 1. NATURAL LUPANE DERIVATIVES POSSESSING BIOLOGICAL ACTIVITY

Natural lupane derivatives whose biological activity has been reliably established can be conditionally divided into three structural types according to the oxidation degree of 28-methyl group: lupeol derivatives, betulin derivatives, and betulinic acid derivatives.

### *1.1. Lupeol and Its Derivatives*

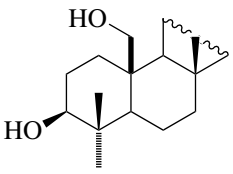
This group includes lupeol (**I**) itself; its esters (**VI**); compounds with functional groups in B, C, D, and E rings; and triterpenoids with a hydroxyl function and oxo groups at C-20, C-25, C-29, and C-30 (**II**)–(**XXII**).

[1] Corresponding author; fax: +7 (095) 954-2328; e-mail: tagtolst@presidium.ras.ru

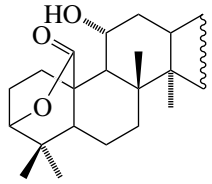


| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| (**I**) | OH | H | H | H |
| (**II**) | – | O – | H | H |
| (**III**) | OH | H | OH | H |
| (**IV**) | – | O – | – | O – |
| (**V**) | $COC_{17}H_{35}$ | H | H | OH |
| (**VI**) | $OCOCHCHC_6H_{13}(OH)_2$ | H | H | H |

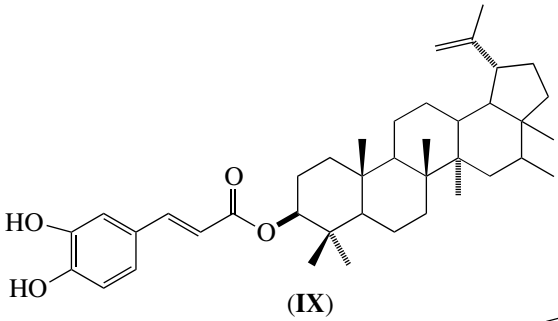
(**I**)–(**VI**)



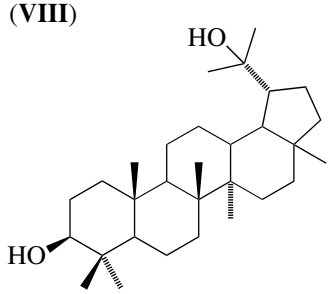
(**VII**)



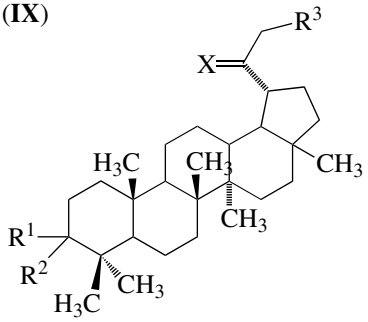
(**VIII**)



(**IX**)



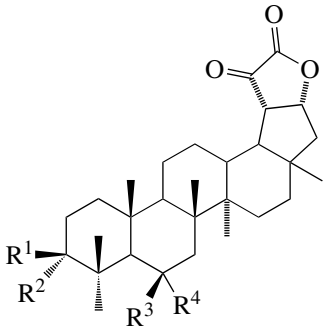
(**X**)



(**XI**)–(**XIII**)

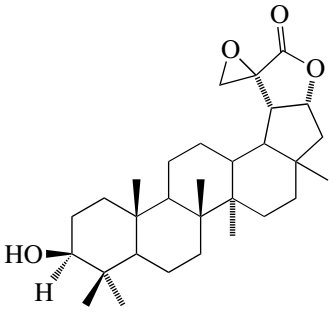
| | $R^1$ | $R^2$ | X | $R^3$ |
|---|---|---|---|---|
| (**XI**) | OH | H | O | H |
| (**XII**) | OH | H | $CH_2$ | OH |
| (**XIII**) | – O – | | $CH_2$ | OH |

Lupeol (**I**), which is produced by many plants and, hence, is rather available, was the subject of comprehensive studies. It was found to be an effective cytostatic. Its high inhibiting effect toward human leukocytic elastase (HLE) was reported in [17]. Lupeol was also shown to suppress the growth of HL-60 human leukemia cells by inducing their apoptosis [18]. Its ability to induce differentiation of B162F2 murine melanoma cells via activation of the inhibitor of p38 proapoptotic mitogen-activated protein kinase (MAPK) was also established [19]. A high activity of this triterpenoid against NSGLG-N6 human large cell bronchopulmonary carcinoma was also found [20]. It was particularl shown that the suppression of malignization of murine melanoma is achieved not only by the inhibition of cell proliferation, but also by a direct cytotoxic effect of

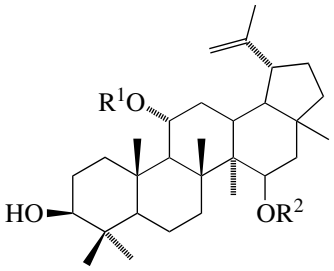


(**XIV**)–(**XVI**)

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| (**XIV**) | OH | H | H | H |
| (**XV**) | – O | – | H | H |
| (**XVI**) | – O | – | – O | – |



(**XVII**)



(**XVIII**)–(**XXII**)

| | $R^1$ | $R^2$ |
|---|---|---|
| (**XVIII**) | a) | H |
| (**XIX**) | b) | H |
| (**XX**) | a) | b) |
| (**XXI**) | a) | a) |
| (**XXII**) | a) | $COC_6H_5$ |

(a) coffeic acid residue
(b) *p*-coumaric acid residue

lupeol at the stage of cell differentiation [21]. Lupeol inhibits growth of epithelial cells of umbilical melanoma HUVEC, is slightly active against tumor culture cells A2780, and has no effect on the growth of SK-MEL-2, A549, and P16-F10 (melanoma) murine tumors [22]. A selective catalytic inhibition of human topoisomerase II (Topo II) by lupeol (**I**) was also reported [23].

A topical administration of lupeol at a dose of 1–2 mg/mouse prevented the formation and growth of model skin cancer induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [24].

Lupeol was shown to recover the damage of carbohydrate fragments of DNA caused by free radicals. Lupeol was capable of decreasing the level of microsomal lipid peroxidation induced by iron ascorbate *in vitro* [25].

Lupeol administration for 10 days at a dose of 40–80 mg/kg of body mass to animals resulted in a decrease in urea and creatinine content in blood and lipid peroxidation. At the same time, an increase in glu-

tathione content in blood and catalase activity under the conditions of cisplatin-induced nephrotoxicity was observed [26]. These results enable to regard lupeol as an effective antioxidant exhibiting chemopreventing effect.

An ability of lupeol to prevent other pathological states in the experiment is also of interest. For example, an ability of lupeol to decrease the risk of renal calculus formation and also to affect the process of nephrolith solution have been shown. These data permitted to attribute lupeol to nephrolytic agents [27].

A protective activity of lupeol *in vitro* upon lipid peroxidation induced by copper salts has been established [28]. Lupeol and its acetate are known to possess irritative effect in the experiments on the open ear in mice [29].

The inhibiting effect of lupeol against plasmodium of the 3D7 line is also notable [30–32]. An interesting dependence of biological activity on structure was found at a comparison of the effects of lupeol and lupenone (**II**) on the growth of B162F2 murine melanoma cells. An inhibition of the growth of B162F2 murine melanoma cells by lupenone was established to occur due to the induction of apoptosis [33].

High inhibiting activities of lupenone on the herpes simplex viruses HSV-1 and HSV-2 and also African swine fever virus (ASFV) were reported in [34].

Lupeol derivatives (**III**)–(**V**) containing functional groups in the ring B displayed a high inhibiting activity toward α-glucosidase and a moderate antibacterial activity [35]. Lupeol ester (**VI**) was found to display cytostatic activity against JB6 cells [36]. Compounds (**VII**) and (**VIII**) were shown to be weak cytostatics [37]. Ester (**IX**) (in a concentration range from 5.1 to 9.0 μg/ml) exhibited a marked inhibiting effect on KB, LNCaP, hTERT-RPE1, and HUVEc cells [38]. A rare lupane derivative, diol (**X**), found in *Camellia japonica* seeds, was observed to inhibit the induction of Epstein-Barr virus early antigen (EBV-EA). Its effectiveness was comparable to that of the reference inhibitor, β-carotene [39].

Lupeol and compounds (**XI**)–(**XIII**) were noted to possess antiinflammatory activity due to their effect on prostaglandin biosynthesis [40].

Ochraceolides (**XIV**)–(**XVII**), isolated from the bark of tree *Kokoona ochracea*, are effective cytostatic agents [41, 42]. Lactones (**XIV**)–(**XVI**) (at a concentration range of 0.26–0.53 μg/ml) inhibit propagation of P-388 cells. Compound (**XVII**) is active against both P-388 and KB-3 cell lines, but only at concentrations higher by one order of magnitude. Compounds (**XV**) and (**XVII**) also display a significant inhibitory effect on farnesyl protein kinase [$IC_{50}$ of 0.7–1.0 μg/ml) [41]. Lupen-3β,11α,15α-triol esters (**XVIII**)–(**XXII**), present in the bark of tree *Ulmus davidiana*, have been found to be highly effective protectors against glutamate-induced neurotoxicity [43]. The potential of these esters as means for neuron protection from glutamate-induced neurotoxicity is beyond question.

### *1.2. Betulin and Its Derivatives*

This class of compounds is represented by (**XXIII**)–(**XXIX**) with the hydroxymethyl or formyl group instead of 28-Me group and esters (**XXX**)–(**XXXIII**).

A study of cytotoxic activity of betulin (**XXIII**) allowed establishing its low activity toward LuC, KB, LNCaP, hTERT-RPE1, and HUVEC cells [20]. 3β-*O*-(*E*)-Coumaroyl betulin (**XXX**) proved to be much more active against the KB, LNCaP, and HUVEC cells. Sinapate (**XXXIII**) exhibited a marked activity toward all the cells [38]. A weak cytotoxicity of betulin and its diacetate is reported in [37].

Betulin can inhibit the activity of DNA Topo II at concentrations comparable with those of the well-known inhibitor etoposide [43]. As is well known that DNA topoisomerases play a crucial role in DNA metabolism events such as replication, transcription, recombination, and chromosome segregation at mitosis.

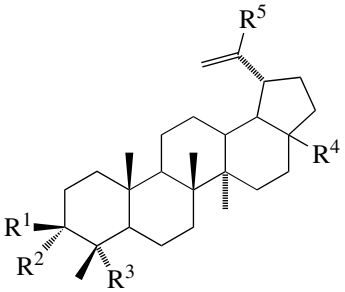
Betulin and its esters (**XXX**) and (**XXXI**) inhibit the growth of JB6 cells in agar in the presence of TPA [44]. A high activity of these esters allows their assignment to agents preventing tumor development.

Betulin demonstrates a moderate but clearly pronounced inhibiting effect on B162F2 cell proliferation accomplished by induction of apoptosis. However, aldehyde (**XXIV**) and keto aldehyde (**XXVII**) exhibit a substantially higher activity. It is likely that the presence of formyl group is necessary for the apoptosis initiation [33].

Aldehydes (**XXIV**), (**XXV**), and (**XXVII**)–(**XXIX**) containing formyl group at C-30 manifested themselves to be active inhibitors of NSGLG-N6 cell growth, with (**XXVIII**) and (**XXIX**) being particularly active [20]. Nevertheless, 3-epibetulonic aldehyde (**XXV**) showed a low activity toward A2780 cells [37]. The appearance of a hydroxyl group at C-28 has no essential effect on the cytostatic effect. Anyway, the effect of sorbikortal II (**XXVIII**) toward JB6 cells does not exceed that of betulin (**XXIII**) [44].

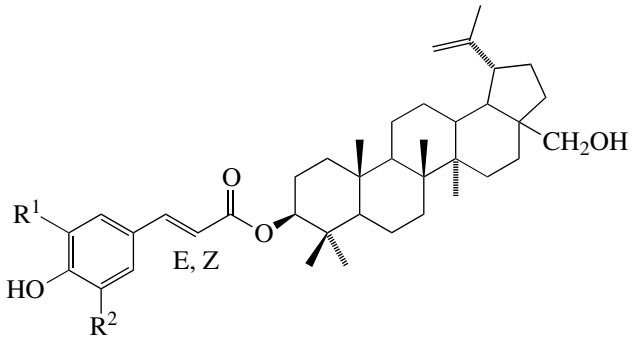
Several investigations are devoted to other manifestations of betulin biological activity. Betulin is a highly effective and selective inhibitor of prolyl endopeptidase [45]. The synergetic effect of betulin upon the simultaneous administration with acyclovir for suppression of HSV-1 and HSV-2 replication was reported. HSV-1 replication was shown to be blocked at acyclovir and betulin concentrations of 0.068 and 0.4 μg/ml, respectively. The suppression of HSV-2 replication required 0.4 and 8.4 μg/ml of these agents, respectively [46].

The betulin concentration of 6.1 μg/ml causes a 50% inhibition of HIV-1 replication at a selectivity index SI = 1.4. This result allowed the authors of [47] to consider betulin as an active anti-HIV agent.



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| (**XXIII**) | OH | H | $CH_3$ | $CH_2OH$ | $CH_3$ |
| (**XXIV**) | OH | H | $CH_3$ | CHO | $CH_3$ |
| (**XXV**) | OH | H | $CH_3$ | CHO | $CH_3$ |
| (**XXVI**) | OH | H | $CH_2OH$ | $CH_2OH$ | $CH_3$ |
| (**XXVII**) | – | O – | $CH_3$ | CHO | $CH_3$ |
| (**XXVIII**) | OH | H | $CH_3$ | $CH_2OH$ | CHO |
| (**XXIX**) | – | O – | $CH_3$ | $CH_2OH$ | CHO |

(**XXIII**)–(**XXIX**)



| | $R^1$ | $R^2$ | |
|---|---|---|---|
| (**XXX**) | H | H | (E) |
| (**XXXI**) | H | H | (Z) |
| (**XXXII**) | H | OH | (E) |
| (**XXXIII**) | $OCH_3$ | $OCH_3$ | (E) |

(**XXX**)–(**XXXIII**)

Betulin manifested itself as a moderately active inductor of the production of tumor necrosis factor α and cytokines [48].

Betulin was also found to display antitubercular activity, as it inhibited the growth of *Mycobacterium tuberculosis* [49, 50].

Unlike lupeol, betulin weakly inhibits the development of plasmodium [32].

### *1.3. Betulinic Acid and Its Derivatives*

This group includes natural triterpenoids (**XXXIV**)–(**LVI**).

**1.3.1. Biological activity of betulinic acid. Antitumor activity.** Betulinic acid (BA), being really wonderful compound, attracts close attention in the last ten years. BA (**XXXIV**) is produced by many plants [4]. The plants belonging to families of Betulaceae, Ebenaceae, and Peoniaceae are considered to be the most reliable BA sources. If one discusses the problem of BA isolation from plant raw material in Russia, the method based on the two-stage transformation of betulin still remains unrivalled. This approach is reported in a number of communications and includes oxidation of betulin to betulonic acid (**XXXVI**) by Cr(VI) salts and subsequent reduction of betulonic acid to BA.

Pharmacological properties of BA are discussed in reviews [14, 51, 52]. The BA anticancer activity is considered to be its most promising pharmacological characteristic. The BA cytostatic effect, for the first time reported in 1991 [53], is thoroughly investigated in a series of recent studies [54–62].

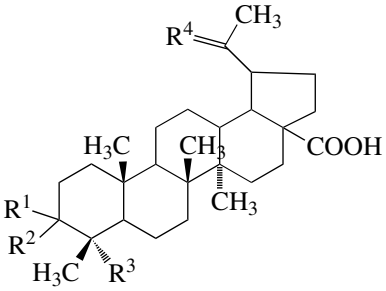
The study of BA cytotoxicity against cells of more than 35 tumor types allowed finding out that BA is the most effective as the inhibitor of growth of glioblastoma, medulloblstoma, melanoma, and some other cell lines. BA is low active ($IC_{50} > 20$ μg/ml) against breast cancer BC-1 cells, COL2, LNCaP, LU1, U373, KB tumors, and HT 1080 sarcoma cells [54]. The data on BA cytotoxicity are given in the table.

BA is highly selective against UISO-MEL-1, UISO-MEL-2, and UISO-MEL-4 cell lines obtained from metastases of lymph nodes, pleural liquid, and primary skin carcinoma, respectively [54].

BA was also found to selectively induce apoptosis in U373 human glioma cells [58]. The study of BA activity against nine neuroblastoma and melanoma cell lines allowed Schmidt *et al.* [56] to conclude that BA introduction induces the mechanism of apoptosis in these cells.

The BA activity toward the cell lines of neuroectodermal origin was demonstrated on medulloblastoma and glioblastoma cells [55, 63]. The induction of programmed cell death was shown to be independent of the synthesis of new mRNA; it requires protein synthesis [64].

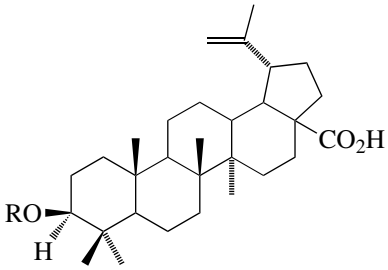
BA appeared to be active against head and neck squamous cellular carcinoma cell lines [65]. Cells of melanoma and ovarian, lung, and cervical carcinoma are sensitive to BA action [50]. Fibroblasts appeared to



(**XXXIV**)–(**XLIII**)

| | $R^1$ | | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|---|
| (**XXXIV**) | OH | | H | $CH_3$ | $CH_2$ |
| (**XXXV**) | H | | OH | $CH_3$ | $CH_2$ |
| (**XXXVI**) | – | O | – | $CH_3$ | $CH_2$ |
| (**XXXVII**) | OH | | H | $CH_3$ | O |
| (**XXXVIII**) | OH | | H | $CH_2OH$ | $CH_2$ |
| (**XXXIX**) | – | O | – | $CH_2OH$ | $CH_2$ |
| (**XL**) | $OR^6$ | | H | $CH_3$ | $CH_2$ |
| (**XLI**) | $OR^7$ | | H | $CH_3$ | $CH_2$ |
| (**XLII**) | $OR^8$ | | H | $CH_3$ | $CH_2$ |
| (**XLIII**) | $OR^9$ | | H | $CH_3$ | $CH_2$ |

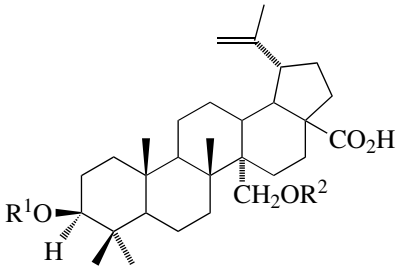
$R_6$, (*Z*)-*p*-coumaric acid residue
$R_7$, (*E*)-*p*-coumaric acid residue
$R_8$, (*Z*)-ferulic acid residue
$R_9$, (*E*)-ferulic acid residue



(**XLIV**), (**XLV**)

| | R |
|---|---|
| (**XLIV**) | a) |
| (**XLV**) | b) |

(a) coffeic acid residue
(b) cinnamic acid residue



(**XLVI**), (**XLVII**)

| | $R^1$ | $R^2$ |
|---|---|---|
| (**XLVI**) | H | a) |
| (**XLVII**) | H | b) |

(a) (*E*)-*p*-coumaric acid residue
(b) (*Z*)-*p*-coumaric acid residue

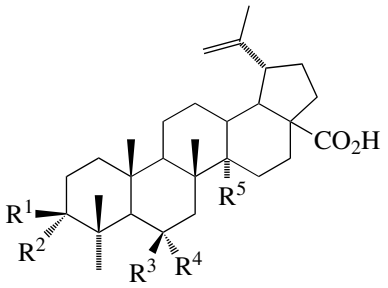
be much more stable than tumor cells, whereas peripheral lymphoblasts are completely insensitive to BA ($IC_{50} \geq 50$ μg/ml).

Screening of 60 human carcinoma cell lines reliably established the absence of selectivity in the BA activity [14]. All research groups confirmed the absence of BA activity toward nontumor nontransformed cells [57, 59, 63]. The absence of BA activity against normal cells is due to the difference in acidity of intracellular medium (pH = 7.3 for normal cells and 6.9 for malignant cells) [66].

BA activity against a number of model animal tumors was successfully detected using *in vivo* experiments. BA administration was shown to substantially decrease the effect of TPA on 7,12-dimethylbenz[a]anthracene-induced murine skin tumor [53]. BA successfully inhibited the growth of transplanted human melanoma in thymusless mice [54]. It is particularly remarkable that BA displayed no system toxicity even at such a high doze as 500 μg/ml (100 times higher than the therapeutic doze). The BA administration was found to cause an increase in lifespan of mice with the transplanted ovarian carcinoma [57].

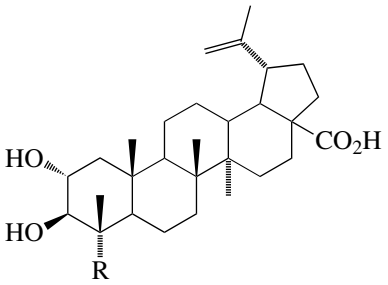
A number of recent studies are devoted to investigation of the mechanism of BA cytostatic effect. The induction of apoptosis is one of the main mechanisms of BA action. Apoptosis is characterized by a number of pronounced morphological and biochemical changes (DNA fragmentation, nucleus condensation, cell drying, formation of apoptotic bodies, and caspase activation) resulting in physiologically natural cell removal with the participation of phagocytes.

The induction of apoptosis under the effect of BA mainly occurs according to two ways: an external involving CD95/CD95L death receptors or the internal via caspase activation and subsequent alteration of



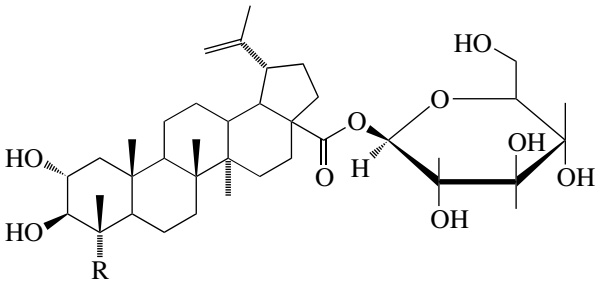
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| (**XLVIII**) | OH | H | H | H | $CH_2OH$ |
| (**XLIX**) | – O – | OH | H | | $CO_2H$ |
| (**L**) | – O – | H | OH | | $CO_2H$ |

(**XLVIII**)–(**L**)



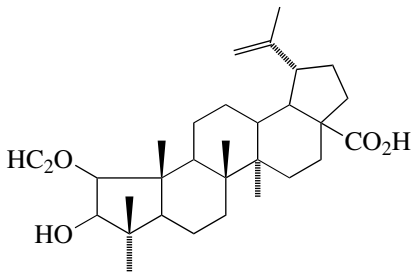
(**LI**), (**LII**)

| | R |
|---|---|
| (**LI**) | $CH_3$ |
| (**LII**) | $CH_2OH$ |



(**LIII**)–(**LV**)

| | R |
|---|---|
| (**LIII**) | $CH_3$ |
| (**LIV**) | $CH_2OH$ |
| (**LV**) | $CH_2OCOC_6H_2(OH)_3$ |



(**LVI**)

mitochondrial permeability. The system of CD95/CD95L death receptors was not found to be involved in the BA-induced cytotoxicity in neuroblastoma [58], glia [59], and melanoma [60] cells.

The treatment of MEL-2 melanoma cells with BA results in apoptotic events, such as morphological alterations and formation of 50-kbp DNA fragments. It was shown that, under the action of BA, a more marked DNA fragmentation and a faster inhibition of cell growth in the case of metastatic C8161 human melanoma cells occur than in the nonmetastatic C8161/neo6.3 cell line [60].

The experimental proofs of the fact that the BA-induced apoptosis is mediated by mitochondria with subsequent caspase activation, which is typical of the internal way of apoptosis, have been accumulated. For example, the BA-treated cells lose their membrane potential [58, 60–64], which results in release of proapoptotic mediators, cytochrome *c* and apoptosis-inducing factor [60]. BA was found to activate caspases-1 and -8 in neuroblastoma [53, 65], glial cells [59], and squamous human carcinoma cells [65].

There is a communication where an opposite opinion was stated that BA does not induce caspase activity in melanoma cells [64]. However, in this case, BA was administrated in the minimum concentration of <10 μg/ml, which is insufficient for caspase activation [58].

The participation of mitochondria in BA-induced apoptosis was confirmed in noncellular system: it was

Cytostatic activity of betulinic acid (**XXXIV**) *in vitro*

| Cell lines | Tumor type | $IC_{50}$, μg/ml | References |
|---|---|---|---|
| A172 | Glioblastoma | 8 | [61] |
| SK14 | ″ | 12 | [61] |
| SK17 | ″ | 11 | [61] |
| SK19 | ″ | 16 | [61] |
| SK22 | ″ | 8 | [61] |
| SK37 | ″ | 12 | [61] |
| SK51 | ″ | 7 | [61] |
| SK55 | ″ | 6 | [61] |
| SK60 | ″ | 10 | [61] |
| U 118 | ″ | 5 | [61] |
| U 138 | ″ | 9.5 | [61] |
| U 251 | ″ | 5 | [61] |
| U 343 | ″ | 5 | [61] |
| H 460 | NSCLG | 1.5 | [57] |
| POG B | SCLC | 4.2 | [57] |
| D283 | Medulloblastoma | 3 | [61] |
| D341 | ″ | 7.5 | [61] |
| MHU1 | ″ | 9 | [61] |
| MHU3 | ″ | 10 | [61] |
| MHUU | ″ | 4 | [57] |
| Me665/2/21 | Melanoma | 1.5 | [57] |
| Me665/2/60 | ″ | 1.6 | [57] |
| ULSO-MEL-1 | ″ | 1.1 | [54] |
| ULSO-MEL-2 | ″ | 2.0 | [54] |
| ULSO-MEL-4 | ″ | 4.8 | [54] |
| A-2780 | Ovaria | 1.8 | [57] |
| ICROV1 | ″ | 4.5 | [57] |
| OVCAR5 | ″ | 3.3 | [57] |

shown that, after the treatment with BA, isolated mitochondria are capable of apoptosis initiation via the activation of caspase cascade [60, 63]. Therefore, one can presume that mitochondria play the central role in the BA-induced apoptosis. The exhaustion of ATP pool in cells is another important proof of the participation of mitochondria in the BA-induced apoptosis [14].

The generation of reactive oxygen species (ROS) is likely to be one of the phenomena occurring in various BA-treated cells and mentioned by various researchers. The cells of both types, gliomas [59] and melanomas [64], generate free radicals after the treatment with BA. The production of ROS results in the activation of proapoptotic p38MAPK and stress activated protein kinase/c-Jun $NH_2$-terminal kinase (SAP/JNK) [64]. This activation can be prevented by preliminary incubation (before the BA introduction) of cells with antioxidants [64]. In addition, BA effectively inhibits melanoma cell growth, when pH of the intracellular medium is lower than 6.8 [66, 67].

Additional details of BA cytostatic effect are considered in other papers. BA was shown to suppress the activity of DNA topoisomerase I (Topo I) by the disturbance in the interaction between enzyme and DNA-substrate rather than by the generation of the cleavable complex or the Topo I-mediated DNA degradation [68].

BA has demonstrated an *in vitro* inhibition of enzymatic activity of amino peptidase N ($IC_{50}$ = 7.3 ± 1.4 μM) [61, 69]. Amino peptidase N is closely related to the components of extracellular matrix, and its inhibition prevents melanoma invasion into the main membrane and, therefore, metastatic spreading [70].

Finally, it was shown that the cell treatment with BA results in substantial inhibition of the activation of the tumor necrosis factor NF-κB induced by various carcinogens and inflammatory agents [71]. This inhibitory effect is mediated by the suppression of IκBα kinase responsible for the phosphorylation of IκBα, a NF-κB inhibitor. The inhibition of IκBα can be another possible explanation of BA ability to cause apoptosis.

Recent publications added valuable information concerning the BA antitumor activity. Experiments on human breast (SKBR3) and colon (Colo-205) carcinoma cell lines showed that, during apoptosis, BA affects the trigger mechanisms connected with mitochondrial signaling pathways (MSP). Activation of caspase-3 occurs simultaneously with the appearance of polypeptide p17. BA was shown to effectively inhibit the growth of cells of these two types in a doze-dependent manner [72]. The BA-induced apoptosis of Elwing's sarcoma cells was not interrupted by hydrophilic antioxidants, like ascorbic acid [73]; however, racemic α-tocopherol eliminates the BA effect as an lipophylic antioxidant.

The study of antitumor effect of BA combination with vincristine on the transplantable B16F10 melanoma *in vitro* and *in vivo* has been reported [74]. An addition of BA to vincristine enhanced the inhibition of experimental lung metastases in melanoma cells. The results allowed an important conclusion that BA is an efficient supplement drug for increasing the effect of chemotherapy on melanomas. The combination of BA with TNFα induces the apoptosis of various tumor cells [75]. A combination involving BA, ceramide, and TNF-related apoptosis inducing ligand (TRAIL) was suggested as a potent supplementary agent for the treatment of glioblastoma multiforme (WHO Grade IV, GBM) along with cytostatic agents and radiotherapy [76].

**Antiviral activity.** BA was found to inhibit the HIV-1 replication in lymphoid cells ($EC_{50}$ = 1.4 μM) [77]. These results were confirmed in a recent study [78], in which BA was considered as a clinically prospective anti-HIV agent. BA exhibits a pronounced inhibitory effect against HSV-1 and ECHO6 virus [79, 80]. The latter is a representative of picornavirus family, which

involves nonenvelope RNA-based viruses, poliovirus, rhinoviruses, aseptic meningitis virus, and viral agents causing a number of respiratory diseases. The antiviral (anti-ECHO6) activity of BA is characterized by a low calculated value of $EC_{50}$ (0.007 μM) and a high (≥4000) maximal tolerated concentration/$EC_{50}$ ratio. These parameters of BA exceed those of pleconaril, the preparation suggested for the treatment of picornavirus-induced diseases [81, 82]. These results confirm the advances of the search of new drugs for the therapy of herpes and picornavirus-induced infections among lupane triterpenoids.

**Antibacterial activity.** BA displays antibacterial activity against Gram-positive and Gram-negative microorganisms [83] and fungi [84, 85].

**Anti-inflammatory activity** of BA was demonstrated on the experimental models of carragenin- and serotonin-induced inflammation in mice [86, 87]. An inhibitory effect of BA on phospholipase $A_2$ playing an important role in the modulation of inflammatory processes was observed [88, 89]. BA manifests properties of an cyclooxygenase I inhibitor and a moderate cyclooxygenase II inhibitor [90]. It was shown that BA exhibits its antiinflammatory effect through the induction of macrophages and modulation of the immune response [91].

**Bronchovasodilator activity** of a high level was displayed by BA: it inhibits hypotension, bradycardia, and carbocholine-induced bronchoconstriction. This activity is connected with the BA mediated effect on $Ca^{2+}$ ion flows [92].

**Analgesic activity** of BA was shown in the writhing test and formalin test in mice [93].

**Antiplasmodium activity.** BA is a moderate antiplasmodium agent [94, 95]. **Antihelminthic activity.** BA is also characterized with a marked antihelminthic activity; it causes helminth death in 98% cases, whereas the well-known preparation piperazine causes the helminth death only in 95% cases [96].

**Receptor binding.** BA is inactive against 16 receptor types, including $\alpha_1$-, $\alpha_2$-, and β-adrenoreceptors; adenosine $A_1$, dopamine $DA_1$ and $DA_2$, serotonin 5-$HT_{1/1A}$ and 5-$HT_2$, histamine $H_1$, benzodiazepine, and opiate receptors; GABAergic $GABA_A$ and $GABA_B$ receptors; and also $Na^+/K^+$ ATPase and DHP $Ca^{2+}$ channels. BA displays a weak affinity for muscarinic receptors [97].

Communication [98] is devoted to the development of BA officinal drugs; the authors insist on the advantages of BA solubilization with liposomes. This approach is more preferable than the use of synthetic polymers, since lipids included in liposomes are the natural components of human organism. Liposomal preparations could appear to be especially effective in the case of interaction with perforated capillaries usually supplying tumors with blood.

**1.3.2.Biological activity of natural derivatives of betulinic acid.** 3-Epibetulinic acid (epi-BA) (**XXXV**) is one of the natural BA derivatives. It exhibits no enhanced cytotoxicity against melanoma MS cells as compared with that of BA, but, at the same time, it is cytotoxic against melanoma Bro cells stable to BA [99].

Betulonic acid (**XXXVI**) is an important BA derivative, which can easily be obtained from betulin in one stage. Betulonic acid demonstrated a high activity against MEL-2 melanoma cells comparable with that of BA ($ED_{50}$ of 0.9 and 1.2 μg/ml, respectively). Unlike BA, keto acid (**XXXVI**) is a potent inhibitor of KB epidermal carcinoma cells ($ED_{50}$ = 2.5 μg/ml) [100]. Betulonic acid was found to display cytostatic activity toward A549, B16(F10), K562, LOX-IMVI, PC-3, and SK-MEL-2 tumor cell lines [101]. Inhibition of the Epstein–Barr virus replication by betulonic acid was also revealed [102].

Betulonic acid exhibited antioxidant activity at a doze of 50 mg/kg (1/100 of $LD_{50}$) in the model $CCl_4$-induced toxic hepatitis, decreasing the level of malonic aldehyde in rat liver by 30–40% [103–105].

Betulonic acid also demonstrated an enhancement of the activity of liver microsomal metabolism upon oxidation of such substrates as amidopyrine, aniline, and erythromycin on the model of thermal ischemia of liver tissue [106]. The same model was used for the detection of antioxidant effect of betulonic acid, which was expressed as stabilization of lysosome membranes, decrease in the release of lysosomal hydrolases, and inhibition of proteolytic processes. The membrane-protective effect of betulonic acid exceeds that of natural antioxidant α-tocopherol [107].

Triterpenoid acids are the active components of Brazilian propolis characterized with a high anti-HIV activity. One of these compounds was identified as 20,28-dihydrobetulonic acid [108].

Platanic acid (**XXXVII**) produced by a number of plants [85] is a promising but insufficiently studied compound. The approaches to its synthesis starting from betulin and BA have been suggested [109, 110]. Platanic acid is regarded as a highly effective anti-HIV agent with the effectiveness comparable with that of the most active synthetic derivatives of BA [111].

The BA derivatives (**XXXVIII**) and (**XXXIX**), containing a hydroxymethyl group in position 4 and isolated from Chinese herb *Pulsatilla chinensis* (Bge) *Regel*, are cytotoxic against B16 melanoma cells at concentrations $IC_{50}$ of 32 and 22.5 μg/ml, respectively [112]. Biological testing of a series of natural BA esters and its derivatives has been carried out. For example, *Z*- and *E*-coumarates (**XL**) and (**XLI**) and *Z*- and *E*-ferulates (**XLII**) and (**XLIII**) were shown to display moderate anti-HIV activity ($IC_{50}$ 3–7 μg/ml) [113].

Esters of cinnamic, *p*-coumaric, and coffeic acids (**XLIV**)–(**XLVII**) displayed interesting properties. Cinnamate (**XLV**) inhibited the growth of HT29 human carcinoma at a concentration of 8 μg/ml [114], whereas *E*- and *Z*-coumarates (**XLVI**) and (**XLVII**) displayed

high cytostatic activities against A549, B16(F10), KB62, LOX_IMVI, PC-3, and SK-MEL-2 cell lines [101]. It has been proposed that the acidic moiety of the esters play an important role in the observed cytostatic effect. Betulinic acid acylated at 3-hydroxy group with a residue of coffeic acid, pyracrenic acid (**XLIV**), is a potent inhibitor of tissue granulation, and, therefore, is a promising antiinflammatory agent [115].

Methyl 27-hydroxy-3-epibetulinate (**XLVIII**) is an active Topo II inhibitor [116]. The dependence of activity on the configuration of the only one chiral center can be seen from the following example: 6β-isomer (**XLIX**) exhibits a high activity against NUGC cells of human stomach carcinoma, whereas 6α-isomer (**L**) is completely inactive [117].

The derivatives of betulinic acid with hydroxyl groups in positions 2, 3, and 4 (**LI**) and (**LII**) and their esters with carbohydrates (**LIII**) and (**LIV**) were isolated from seeds of *Combretum quadrangulare*. The four derivatives possessed high hepatoprotective effect, which was demonstrated in experiments with murine hepatocytes [118, 119]. The same effect has been found for the ester of gallic acid and BA (**LV**) [119]. Ceanothic acid, a product of the ring A rearrangement, demonstrated a cytostatic activity [120].

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