Betulin and Its Derivatives. Chemistry and Biological Activity

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Abstract

The data on natural sources of betulin and methods of its extraction are systematized in the review. Transformations of betulin and its available derivatives are considered. The data on the biological activity of betulin, its natural and synthetic analogs are presented. The promising character of the compounds based on betulin for creation of antiviral and antitumour agents.

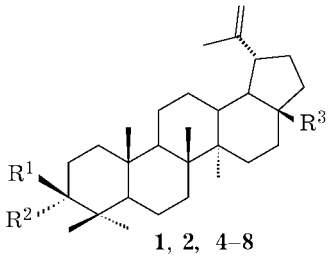
INTRODUCTION

Synthetic transformations of natural compounds for the purpose of developing biologically active agents have become the basis of the actively advancing scientific direction of perfect organic synthesis and medical chemistry. The greatest attention of researchers is attracted by native compounds with reliably established biological activity. An attractive factor is availability of natural metabolites due to frequent occurrence of the sources, and technological reasonableness of the methods of isolation of natural substances. Widely known examples of medically successful transformations of steroids, antibiotics of penicillane and cephallosporane groups, alkaloids of morphinane series have recently been supplemented with the modificants of cacrinostatic taxol, anti-glaucoma terpenoid forscoline, antiplasmodium medicine artemisinine and other preparations.

Compounds combining availability with valuable biological activity are frequent in the class of triterpenoids. Speaking of purposeful synthetic transformations of triterpenoids for medical chemistry, perhaps the most advanced object is the glycoside of licorice – glycyrrhizinic acid and its aglycones [1–6].

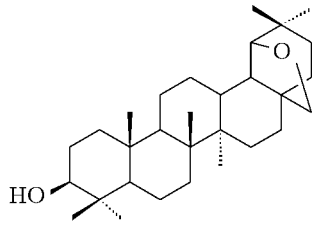
The recent two decades gave us grounds to expect that the preparations based on triterpenoids of lupane series could be involved in therapy of a number of diseases. The expectations are undoubtedly connected with betulin (3b,28-dixydroxy-20(29)-lupene) **1**, which is a triterpenoid surprisingly widespread in nature and easily available in almost any amount. The number of publications dealing with valuable biological activity of betulin and its natural and synthetic derivatives is increasingly growing.

The last review on the chemistry of betulin had been published 40 years ago as a chapter of the known monograph [7]. The present review includes the data of the transformations of betulin and its derivatives, such as betulic acid **2** and allobetulin **3**, which can easily be obtained from betulin and also occur in plants.



1, **2**, **4**–**8**

1 R^1 = OH, R^2 = H, R^3 = CH_2OH
2 R^1 = OH, R^2 = H, R^3 = COOH
4 R^1 = OH, R^2 = H, R^3 = CHO
5 R^1 = OH, R^2 = H, R^3 = COOMe
6 $R^1 + R^2$ = H, R^3 = CHO
7 $R^1 + R^2$ = O, R^3 = COOH
8 R^1 = OH, R^2 = H, R^3 = Me



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Among these triterpenoids, betulic acid is of special interest due to its significant biological activity exhibited both by the acid itself and by its numerous derivatives.

NATURAL SOURCES OF BETULIN AND THE METHODS OF ITS ISOLATION

The main source of betulin is birch bark. When the bark is heated, sublimation occurs. Lovits was the first to observe this in 1788. The name betulin was given to this compound by Mason in 1831. Later betulin was obtained by extraction. The birch bark has two clearly distinguished parts: external and internal. The external part of birch bark is especially rich in the extractives: their content reaches 40 %. The main component of almost all the extracts is betulin which determines the white colour of bark [8, 9]. The betulin content of the external part of bark varies within 10–35 % depending on birch species, site of ground and conditions, the age of a tree, and other factors.

Betulin was detected in pendent birch (*Betula verrucosa* Ehrh. = *Betula pendula* Roth.) and in downy birch (*B. pubescens* Ehrh.), the species widespread in Russia [10]. Betulin is also present in the bark of white birch (*Betula alba* L.) growing in Europe [11].

Along with betulin, extracts of the bark of these species contain its oxidized derivatives: betulic acid **2**, betulic aldehyde **4**, methyl ester of betulic acid **5**, betulonic aldehyde **6**, betulonic acid **7** [12].

A permanent satellite of betulin in birch bark is lupeol **8** (~10 % of betulin). Anomalously high content of lupeol close to the amount of betulin was described in [13]. Lupeol was isolated for the first time from sprouts of lupine (*Lupinus albus*), a fodder plant; later is was detected in other sources [14].

Along with lupane derivatives, birch bark contains triterpenes of oleanane and ursane series. In some birch species, for example in *B. dahurica* Pall., prevailing compounds are oleanic acid and its derivatives [10, 15]. Among other oleanane derivatives detected in the extracts of birch bark, noteworthy is a product of rearrangement of betulin, allobetulin **3**, which is easily obtained under the action of acid agents [7].

The qualitative composition of triterpenoids of the bark of black birch species *B. pubescens* and *B. pendula* is the same as that of white-bark birch, but their amount is 2–3 times smaller. The content of betulin in white-bark birch and in black-bark *Betula pubescens* is substantially lower than that in the bark of *B. pendula* [16].

Extracts of the internal part of bark contain small amounts of betulin; however, they can be used as sources of phenolic compounds [10].

Betulin, lupeol and the products of their metabolic hydroxylation were detected in at least twenty different plant species belonging to different genera and families. For example, betulin, lupeol and betulic acid were extracted from the bark of alder tree (*Aldus subcordata* L.) [17], zizyphys (*Zizi phus jujuba* M.) [18, 19] and from the top parts of thistle (*Atractylis carduus* L.) [20]. Betulic acid was detected in the leaves of plumeria (*Plumeria obtusa*) [21], triadenum (*Triadenum japonicum*) [22], in orchids (*Orchid Lusia indivisa*) [23] and other plants (*Dillenia papuana, Tryphyllum peltatum, Ancistrocladus heyneaus, Diospyros leucomelas, Tetracera boliviana, Sizyphus joazeiro, Syzigium claviflorum, Aerva javanica*) widespread in the tropics.

The methods of betulin extraction from birch bark and sulphate soap were widely discussed [8–10, 29–34]. Aliphatic and aromatic hydrocarbons, C_1–C_4 alcohols, acetone and

chlorinated solvents were proposed as extracting agents. The dependence of betulin extraction rate on the degree of birch bark grinding was described in [30]. More than 90 % of betulin present in birch bark passes into solution after 5 min long extraction of the raw material with particle size 0.15–1.5 mm. The process is almost independent of extragent type (hydrocarbon solvent LIAV-20, dichloromethane, methanol, isopropanol – water azeotrope, ethanol, 2-butanol – water azeotrope, acetone). With the raw material having larger particle size (0.8–4.0 mm), the rate and completeness of extraction depend on the kind of extracting agent increasing in the order: dichloromethane < isopropanol < 2-butanol = acetone < methanol < ethanol. At the same time, extraction of bark pieces in autoclave results in almost complete extraction of betulin [35].

The authors of [35] showed that preliminary activation of birch bark with water vapour in the presence of NaOH (240 °C, 1–3 min) allows one to decrease the time of extraction with ethanol and to extract up to 97 % of total betulin content. A detailed investigation of betulin crystallization, the key stage of purification of the raw triterpenoid, was carried out [30]. The solvents recognized as the most suitable agents for recrystallization are azeotropes of isopropanol and 2-butanol. The loss of betulin in the mother solution is 15–17 %. High-purity betulin can be obtained through 3,28-di-*O*-acetate [13]. Betulin extraction from bark of *Betula papyfera* birch by means of sublimation was discussed in [36].

TRANSFORMATIONS OF BETULIN AND ITS DERIVATIVES PROCEEDING WITHOUT CHANGES IN THE CARBON CARCASS

Synthesis of esters, amides, peptides and other related compounds

Different versions of esterification were proposed for betulin, betulic acid and their derivatives with the formation of mono and diacetylates of various acids [35, 37–52]. For instance, according to [50], high yields can be achieved obtaining mono- **9** and diesters **10** of betulin with succinic, phthalic, *O*-acetylsalicylic, nicotinic, cinnamic and *p*-methoxycinnamic acids, as well as with the acids of pyrethroid series [51]. Mono- **9**, **12**, **14**, **15**, di- **10**, **13** and triacylates **16** of betulin, 3a-hydroxyisobetulin, 3-ketobetulin, 2,3-dehydrobetulin, 30-hydroxybetulin and dihydrobetulinic acid were synthesized using the derivatives of succinic and glutaric acids, as well as campholic acid [46, 47, 53]. Acylates of 2,2-dimethylsuccinic acid turned out to be very promising anti-HIV agents, which stimulated the development of the methods of obtaining individual compounds **17–20** [46].

Esters of allobetulin **11** [50], betulic **12** [44] and 2-halogenobetulic [52] acids were described; 3-*O*-acylates of C_2–C_{10} alkyl esters of betulic acid with 2,2-dimethylsuccinic, 3,3-dimethylglutaric and 3-oxapentadicarboxylic acids of the type of **21** are being patented [48]. Cyanoethylation of betulin resulting in the formation of compounds **22**, **23** was described [54].

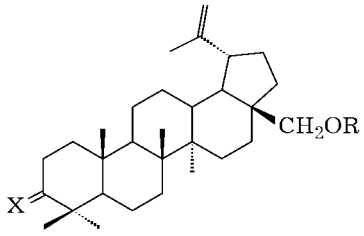
Much attention was paid to the synthesis of amides and peptides of betulic acid and its derivatives. The progress of these investigations was stimulated by the announcement [55] of high anti-HIV activity of the dipeptide of betulic acid **24** (RPR103611) followed by the patent publication [56] with the description of a numerous group of mono and dipeptides of 28-carboxylupane series. The main types of these compounds are represented by structures **25–33**.

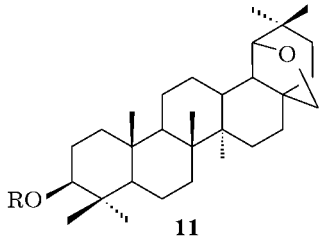
In the subsequent publication the authors of patent [57] paid attention to the synthesis of inverted peptides **34** according to the scheme providing the interaction between the chloroanhydride of 3-*O*-acetylbetulic acid with the excess of 1,7-diaminoheptane as the first stage.

The amides of 11-aminoundecanic acid with betulic and 3-desoxy-3b-aminobetulic acids **35** were described in [52].

A series of the amides of betulic acid with biogenic amino acids **36** [58], amides of betulonic acid with esters of amino acids **37** and with aliphatic and heterocyclic amines **38** was synthesized [44, 59, 60]. Mono- **39** and dipeptides **40** synthesized on the basis of betulonic acid revealed valuable biological activity [61].

Reducing amination of 28-(4-morpholin)-carboanhydride of betulonic acid **41** or reduction of its oxime was used to synthesize amides based on 3-aminobetulinic acid. Acylation of stereoisomeric 3-amines **42**, **43** with anhydrides





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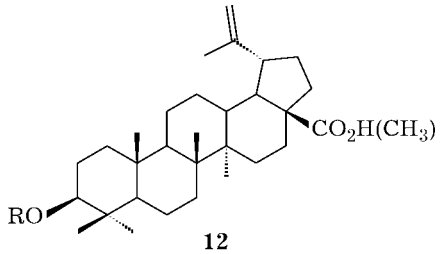

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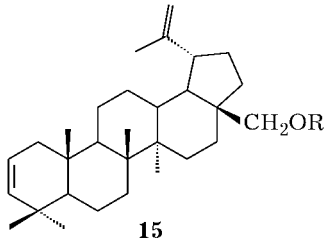
9 X = 

10 X = 

13 X = 

14 X = O



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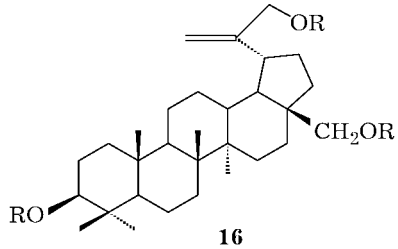

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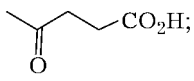
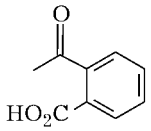
R = 



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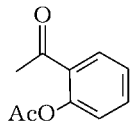

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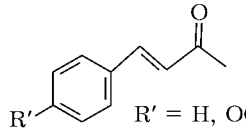

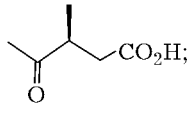

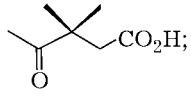

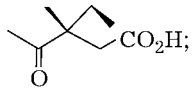

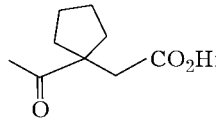

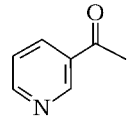

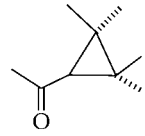

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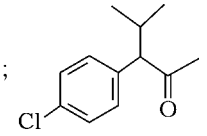

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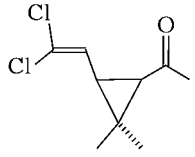

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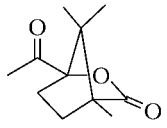
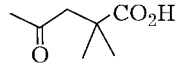
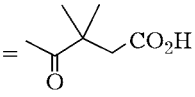
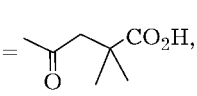
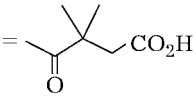
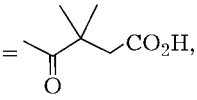


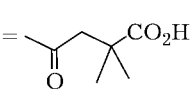
17 $R^1 = R^2 =$ 

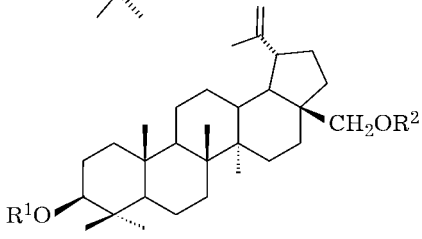
18 $R^1 = R^2 =$ 

19 $R^1 =$ 

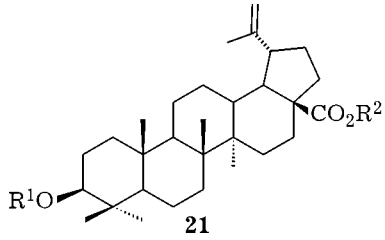
$R^2 =$ 

20 $R^1 =$ 

$R^2 =$ 



22 $R^1 = R^2 = (CH_2)_2CN$ **23** R^1 = H, $R^2 = (CH_2)_2CN$



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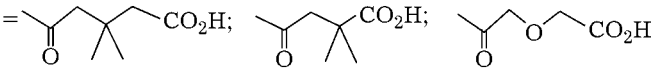
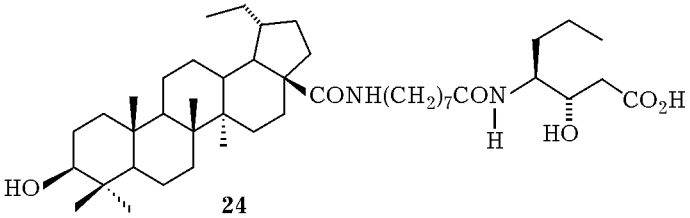
$R^1 =$ 

R^2 = H, C_2–C_{10}–H or isoalkyl



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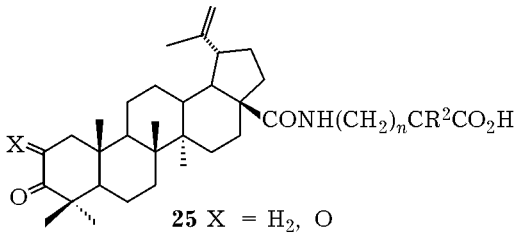
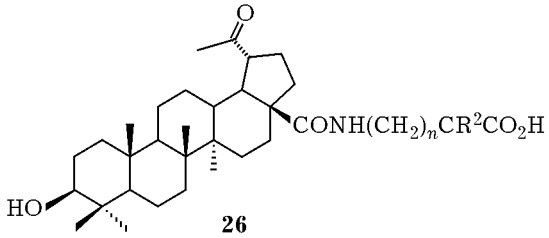


25 X = H_2, O



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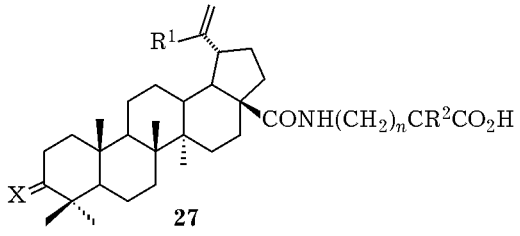
25–27 R^2 = H, n = 5–12, 16, 17; R^2 = Me, n = 9



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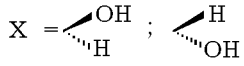
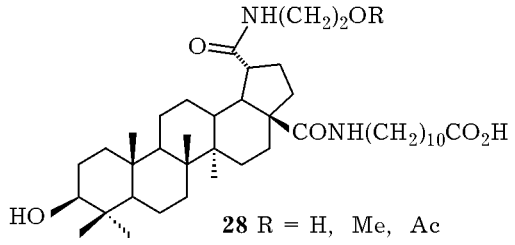
X = 

R^1 = CH_3, CH_2OH(Ac), CH_2NEt_2, $CH_2N(CH_2)_5$, $CH_2S(CH_2)_2OH$



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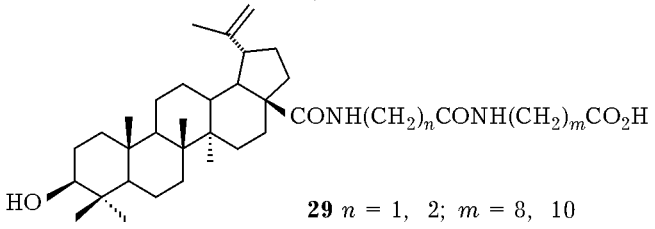


29 n = 1, 2; m = 8, 10

of the acids shown in the scheme and removal of protection resulted in N-acylates **44**, **45** [62].

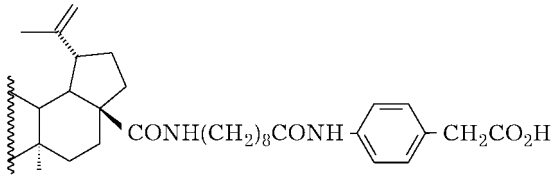
The synthesis of carbamates and carbamides based on 28-nor-17-amino derivatives of betulin and 3-ketobetulin proceeds smoothly. Azides of acids **46**, synthesized through chloroanhydrides, rearrange into isocyanates **47** forming carbamates **48** and carbamide derivatives **49**, **50** [44, 60, 63].

A large group of dipeptides of betulic acid of the type of **51** differing by the presence of the tetrazole fragment was patented as the means of antiviral action [64].

The synthesis of amides of betulic acid of the type of **52** modified by introducing functional groups at the C-30 atom was described [65].

A large group of mono and diconjugates of 3a-hydroxylup-20(29)ene-23,28-dicarboxyl acid **53** which was extracted from the leaves of a Vietnamese plant *Schefflera octophylla* (Lour.) Harms (Araliaceae), with *L*-amino acids was described in [66].

Similar conjugates **54** with the esters of amino acids were obtained on the basis of



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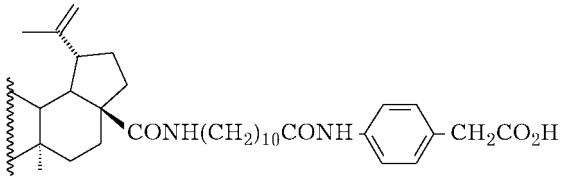

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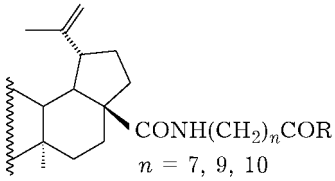
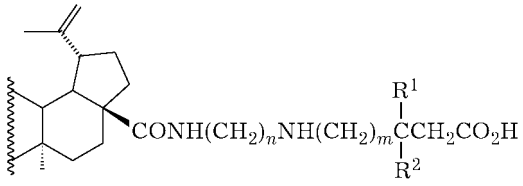
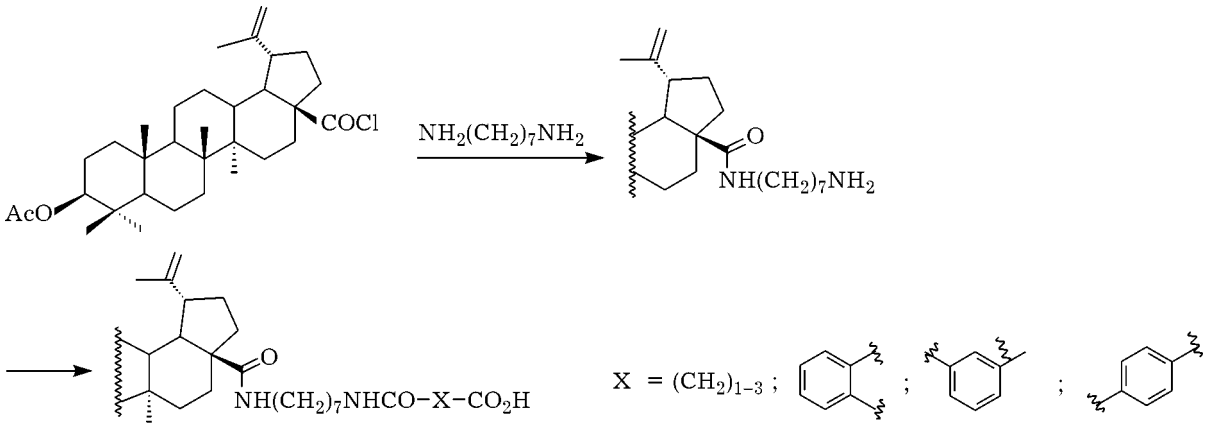


32

R = Gly, Ala, *L*-Asp, *L*-PhGly, *L*-Ala, *D*-Ser, *D*, *L*-i-Ser, *L*-Lys, *L*-Asp, *L*-Pro, *L*-PhAla, *L*-Val, *L*-Thre, *L*-Statine



33 R^1 = H, Me, Ph; R^2 = H, Me, Et



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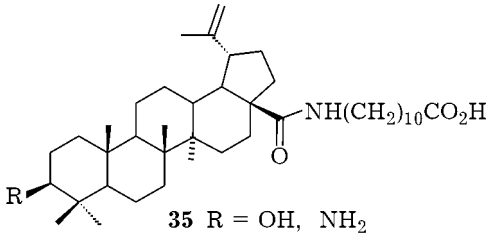
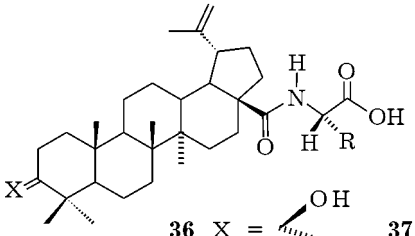
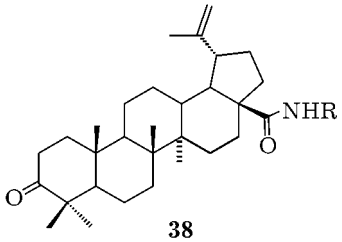


35 R = OH, NH_2



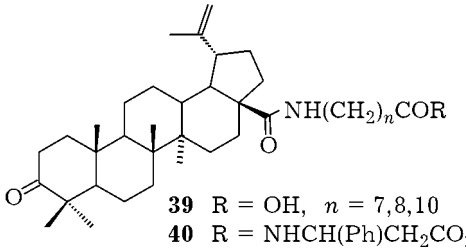
36 X = **37** X = O

R = $CH_2C_6H_5$, $CH_2(C_6H_4(4\text{-}OH))$, H, $CH_2C_2HSCH_3$, CH_2(3-indoline), $CH(CH_3)CH_2CH_3$, CH_2CO_2H, $CH_2CH(CH_3)_2$, $CH_2CH_2CO_2H$, CH_3, $NCH_2CH_2CH_2CHCO_2H$, $CH(CH_3)_2$



38

R = C_6H_{13}, $C_{16}H_{33}$, $C_{18}H_{35}$, $(CH_2)_2OH$, 2-pyridyl, 3-quinolyl, 6-quinolyl, 8-quinolyl



39 R = OH, n = 7,8,10
40 R = $NHCH(Ph)CH_2CO_2H$, n = 8

b-lactone and other structural analogues of 3a-hydroxylup-20(29)-ene-23,28-dicarboxylic acid [67].

Hydrazides **55** obtained using a usual procedure from betulic and betulonic acids, with substituted benzaldehydes, were transformed almost quantitatively into the corresponding N-benzalhydrazides **56** [44, 68].

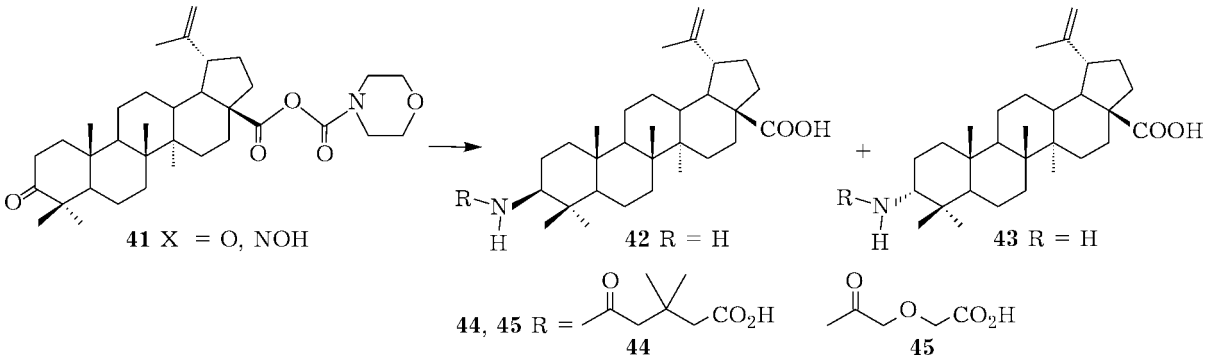
The synthesis of 3,28-disubstituted derivatives of betulic acid **57** was performed using the methods of peptide synthesis starting from amino acids fixed on a polymer substrate [69].

Synthesis of betulin glycosides

Glycosilation of betulin under the action of a-acetobromoglucose in the presence of $CdCO_3$ resulted in the formation of the expected diglycoside **58**; the yield was only 17 %. The major product is allobetuline glycoside **59**, which is formed as a result of rearrangement considered below. Glycosylation of betulin 3-*O*-acetate resulting in the formation of 28-*O*-glycoside **60** proceeds more smoothly. Anomeric glycosides **61** were obtained from 28-*O*-acetate [70].

The use of $Hg(CN)_2$ as a condensing agent in the reactions of 18,19-isobetulin and its epoxide with a-acetobromoglucose allowed obtaining mono- **62**, **63** and b-D-diglycosides **64**, **65** [71].

Condensation of 28-*O*-acetylbetulin and hepta-*O*-acetyl-a-D-cellobiosyl bromide under the conditions of Gelferich reaction ($Hg(CN)_2$, $MeNO_2$) carried our stereoselectively, followed by deacetylation, resulted in the formation of betulin 3-b-D-cellobioside **66**, which was then introduced as an acceptor into the reaction with



41 X = O, NOH

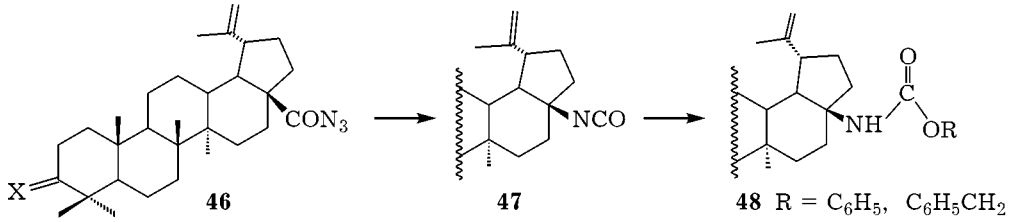
42 R = H

43 R = H

44, **45** R =

44

45

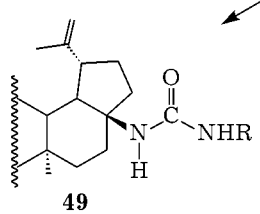


46

47

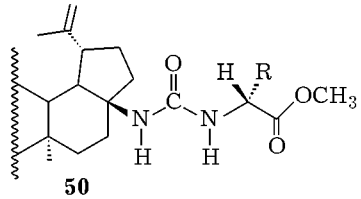
48 R = C_6H_5, $C_6H_5CH_2$

X = OH, H ,O, NOH



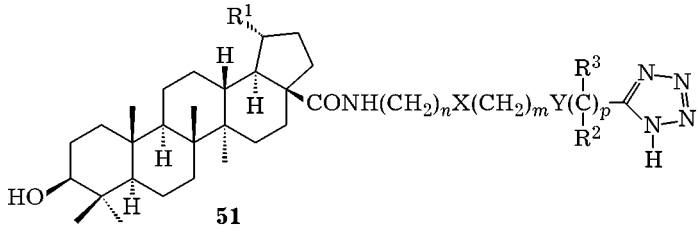
49

R = C_6H_{13}, $C_{10}H_{21}$, 2-pyridyl, 8-quinolyl



50

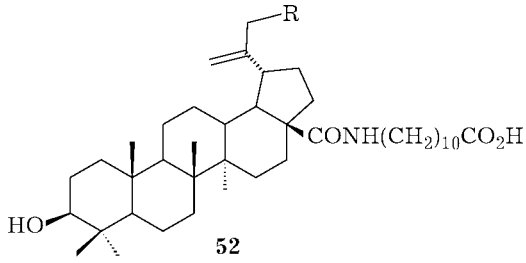
R = CH_3, $CHCH_3$ $CH_2CH(CH_3)_2$, $(CH_2)_2SCH_3$



51

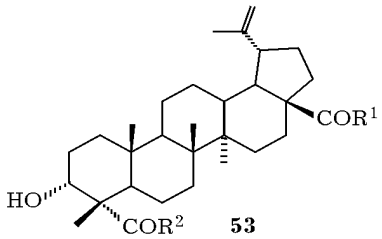
R^1 =

R^2, R^3 = H, CH_3; X = bond, carbamoyl, N-methylcarbamoyl, aminocarbonyl, N-methylaminocarbonyl;
Y = bond, *m*- or *p*-phenylene; m = 0–3; n = 6–12; p = 0–2; $m + n + p$ = 6–14



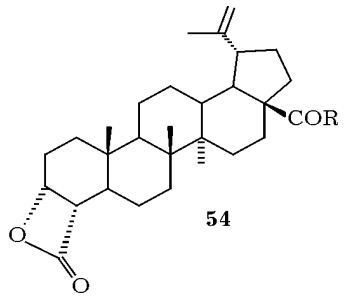
52

R = NH_2, OAc, NEt_2, $NH(CH_2)_2OH$,
$NH(CH_2)_2NEt_2$, $NHCH_2CO_2Me$,
$S(CH_2)_2OAc$, SCH_2CO_2Et,
$S(CH_2)_2NEt_2$, $SC_6H_4F(n)$



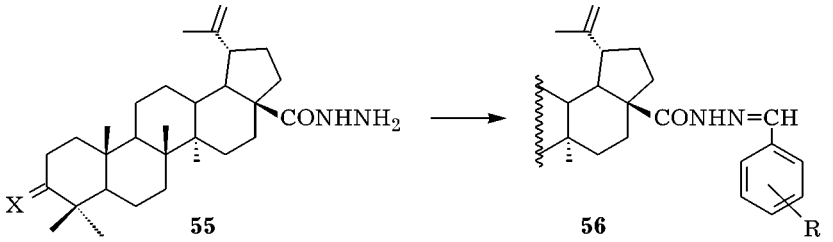
53

R^1, R^2 = *L*-Ala, *L*-Leu, *L*-Pro, *L*-Phe, *L*-Ser, *L*-Tyr, *L*-Trp, *L*-Glu, *L*-His, *L*-Met, *L*-Lys



54

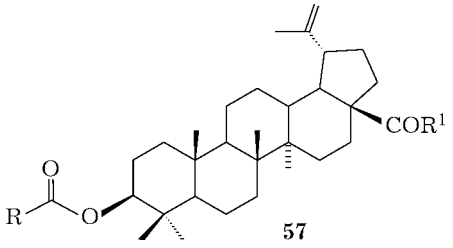
R = Gly-OEt, *L*-Ala-OMe, *L*-Leu-OMe, *L*-Phe-OEt, *L*-Ser-OMe, *L*-Tyr-OMe, *L*-Trp-OMe, *L*-Glu$(OMe)_2$, *L*-His-OMe, *L*-Met-OMe, *L*-Lys-OEt



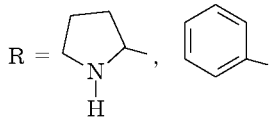
55 **56**

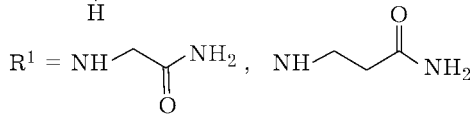
X =  , O, NOH

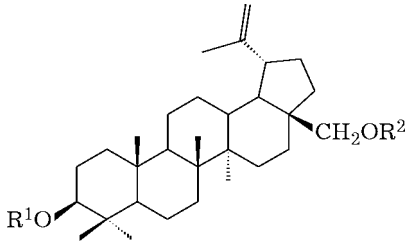
R = *o*, *m*-OCH_3, *p*-Cl, *m*-I, *p*-Br, *p*-OC_2H_5



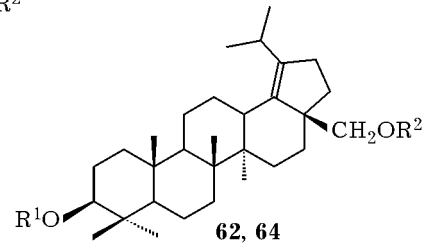
57

R = 

R^1 = 

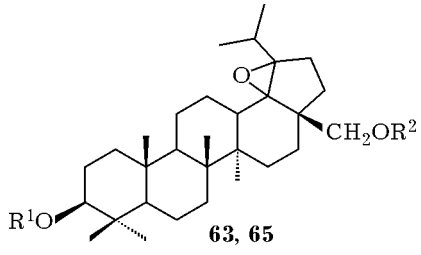


58 R^1 = R^2 = β-Glc$(OAc)_4$
60 R^1 = Ac, R^2 = β-Glc$(OAc)_4$
61 R^1 = α, β-Glc$(OAc)_4$, R^2 = Ac

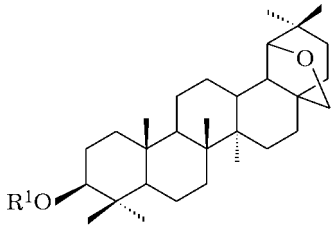


62, 64

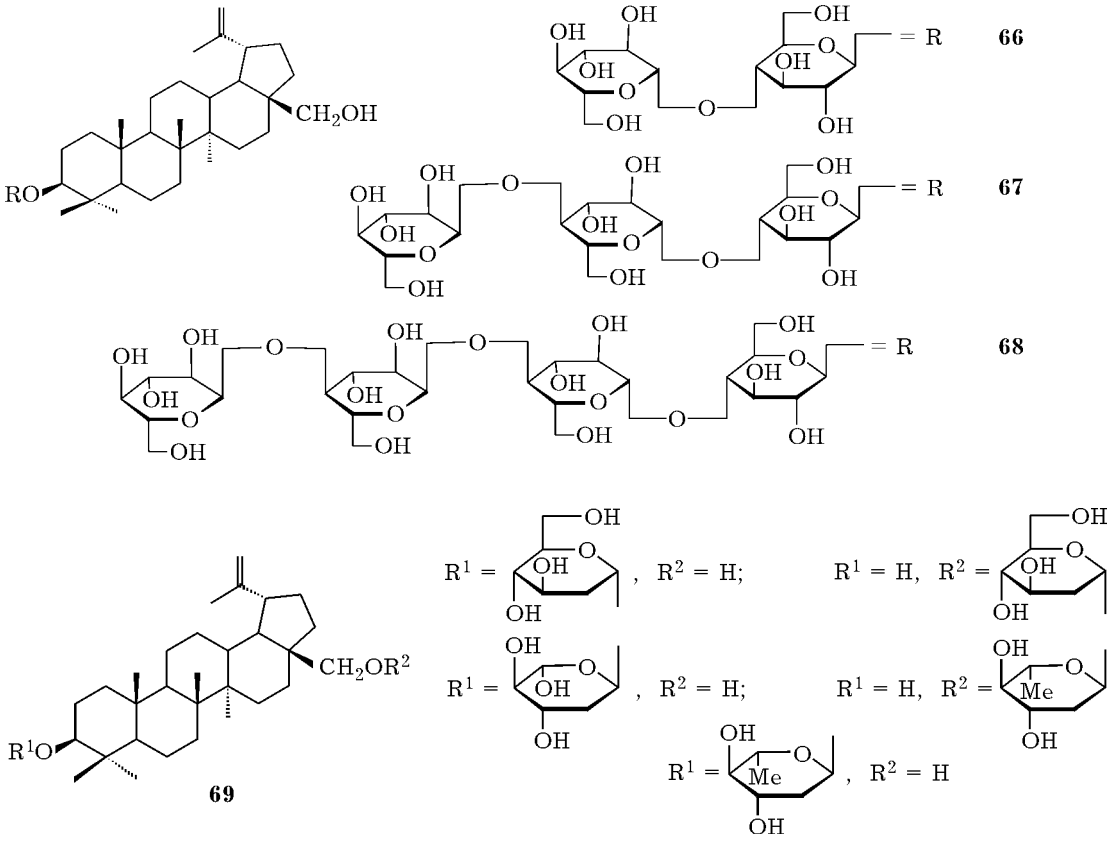
62, 63 R^1 = β−Glc$(OAc)_4$, R^2 = H
R^1 = H, R^2 = β-Glc$(OAc)_4$
64, 65 R^1 = R^2 = β-Glc$(OAc)_4$



63, 65



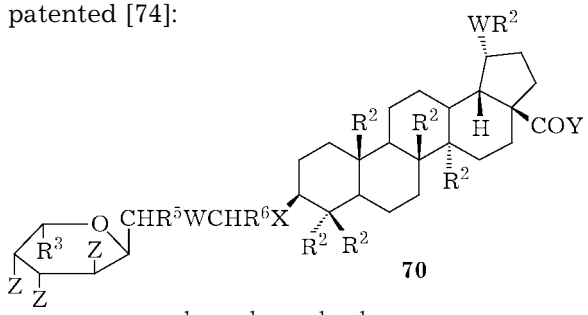
59 R^1 = β-Glc$(OAc)_4$



enzymatic transglycosilation with cyclodextringlycosyltransferase; the products were 3-b-D-(4-*O*-b-D-maltotriosyl) glycosides of betulin **67**, **68** [72].

Glycosilation of 3- and 28-monoacetates of betulin by glycal acetates under the conditions of acid catalysis resulted in the formation of 2-desoxy-a-D, 2-desoxy-a-L and 2,6-didesoxy-a-L-arabinohexopyranosides of betulin **69** [73].

A series of O-, S- and N-glycosides of betulic acid with the general formula **70** was patented [74]:



70

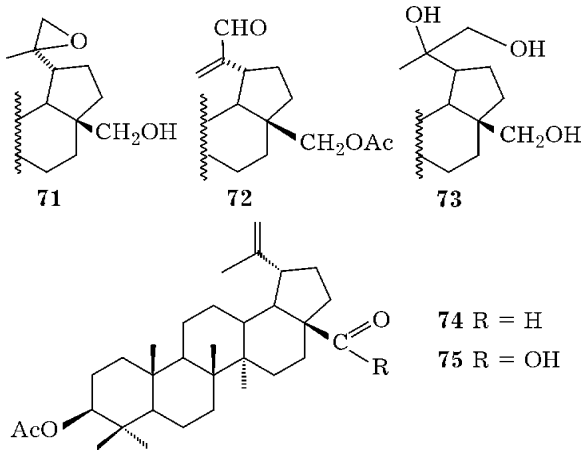
where Y = OR^1, NR^1_2, OM^1; R^1 = H, lower alkyls; M^1 = metal; $R^2 = CH_2OR^1$, lower alkyls, COY; Z = NR, H, OMe lower alkyls, O-Glc; X = O, S, NR^1, NR^1_2; W = C:O, $C:CR^1_2$, $CR^1CR^1_3$, CHR^4; R^4 = H, OH, OSO_3M^1, $NH(CH_2)_nNH_2$, $n = 1–8$, NR; R^5, R^6 = H, Me; $R^5 + R^6 = (CH_2)_5$, $(CH_2)_4$.

Oxidative transformations of betulin and its derivatives

In the works of Ruzhichka, carried out in the first half of the past century, the synthesis of betulin 20,29-epoxide **71** was carried out from betulin under the action of monoperphthalic acid [7], 3b,28-dixylroxylup-20(29)-ene-30al **72** was synthesized under oxidation with selenium dioxide, and 20,29-dihydroxybetulin **73** was obtained by treatment with osmium tetraoxide [75].

Betulonic aldehyde **6** and 3-*O*-acetylbetulinic aldehyde **74** were synthesized under the action of cromium tioxide on betulin and 3-*O*-acetylbetulin in acetone [76].

In order to obtain aldehdye **74** from 3-*O*-acetylbetulin, oxidation according to Swern was applied (DMSO ($COCl_2$) (yield: 93 %) [77, 78], and by oxidation with oxygen in the presence of the catalyst $Pd(OAc)_2$. During further



oxidation with oxygen of the air, aldehyde **73** is easily transformed into 3-*O*-acetylbetulinic acid **75** under the action of the source of radicals, N-hydroxyphthalimide, 2,2'-azobisisobutyronitrile, 2,2,6,6-tetramethyl-1-piperidinium-oxyl or Co(III) acetylacetonate [79]. Betulin oxidation according to Swern results in the formation of betulonic aldehyde **6** with a yield of 93 % [78].

The authors of [80] described selective oxidation of betulin into betulic aldehyde **4** with a selective oxidizing agent SHO with the regeneration of the latter *in situ*.

The reagents containing chromium (VI): pyridinium dichromate (PDC), pyridinium chlorochromate (PCC) – oxidize betulin in methylene chloride efficiently to form betulonic aldehyde **6** [81]. Oxidation of betulin with PDC in dimethylformamide results in a mixture of betulic **2** and betulonic **7** acids, betulic **4** and betulonic **6** aldehydes [82]. Selective oxidation of betulin with the reagents containing Cr(VI) in the presence of an interphase catalyst was described in [83].

A more selective process is oxidation of betulin by chromic anhydride in acetic acid, with betulonic acid **7** as the main product of reaction [84]. The reaction proceeds similarly with the standard Jones reagent as an oxidizer [85]. Interesting products were obtained by oxidation with the help of CrO_3, 3,28-di-*O*-acetyldihydrobetulin in acetic acid at 100 °C. The authors of [86, 87] succeeded in identifying the products of oxidation in cycle E **76–78**, including lactone of seco-acid **78** and the compounds with oxygen-containing functions in D ring **80–84**.

The methods of oxidation by the action of Cr(VI) were used for transformations due to the isopropenyl group, and also to carry out transformations of dihydrobetulin. For instance, oxidation of 3,28-di-*O*-acetylbetulin with CrO_3 in acetic acid at 70 °C results in a mixture of epimeric 20R- and 20S-lupan-29-carboxylic acids **85**, **86** [88]. Oxidation into nor-ketone **87** was also described [89].

Much attention has been paid to oxidation reactions involving the isopropenyl group. It should be stressed that almost in all the cases not only the products with intact C-3 fragment are formed but also nor-compounds. For example, if the action of *m*-chloroperbenzoic acid (*m*-CPBA) on 3,28-di-*O*-acetylbetulin is selective resulting in the formation of the expected epoxide **88** [80], perbenzoic acid transforms diacetate into a complicated mixture of compounds with prevailing allyl alcohol **89** and norterpenoid **90** [90].

Oxidation by performic and peracetic acids resulted in aldehyde **72**, norketone **87** and epimeric noralcohol compounds **90** [90–92].

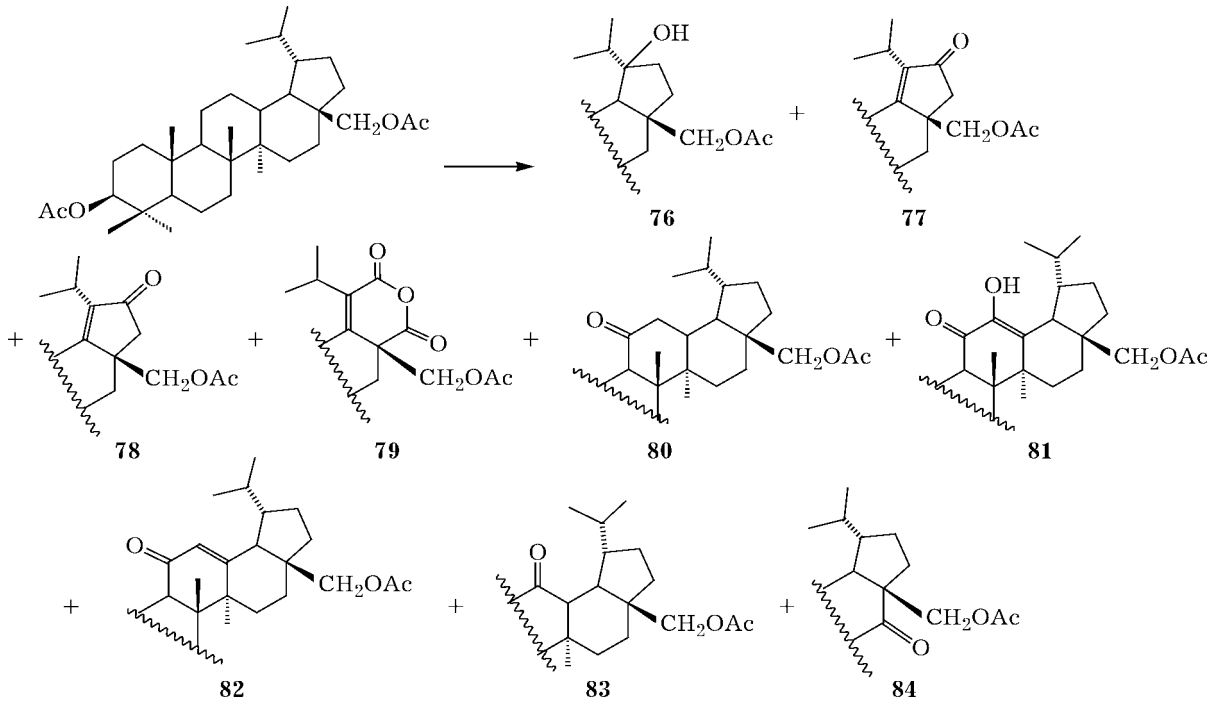
The action of *m*-CPBA on betulic acid and its 3-*O*-acetate is distinguished by complicacy. Thus, the action on betulic acid resulted in the formation of diol **91**, which is the product of epoxy ring opening, and aldehyde **92**, a product of isomerization of epoxide [93]. Oxidation of 3-*O*-acetate gave the products of isomerization of oxide **92**, **93**, and compounds **94** and **95** which are formed from aldehyde **92** and diol **91**, respectively [94].

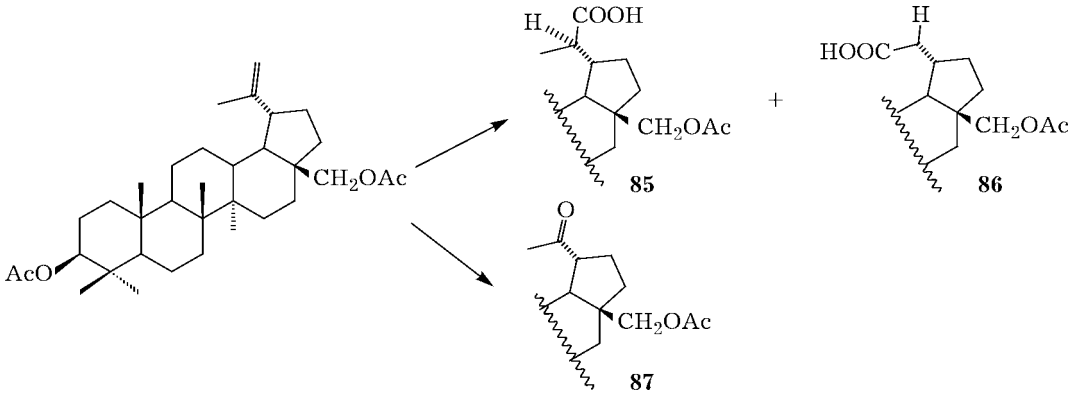
Oxidation of lupeol and its derivatives by peracids involving the isopropenyl group was described in [91, 95–98].

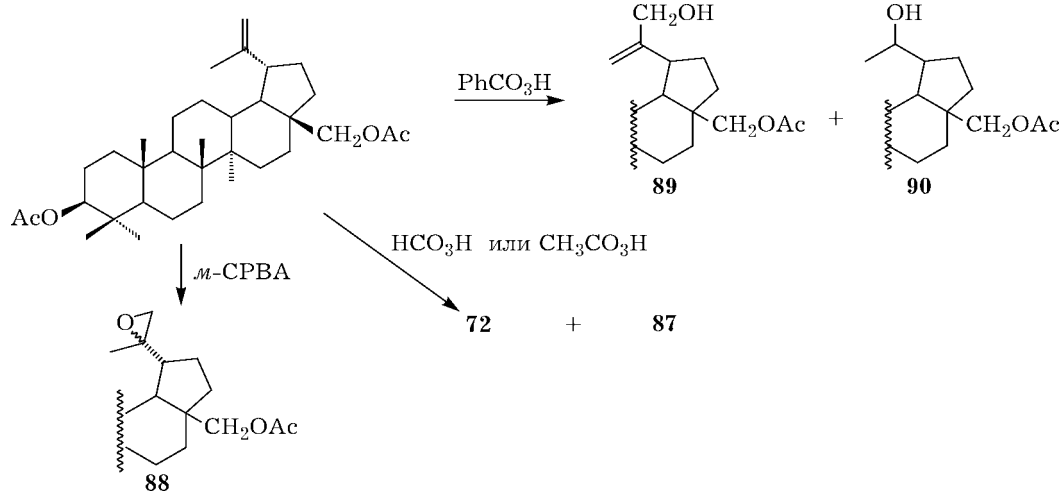
It is typical that *m*-CPBA interacting with dihydrobetulin 3,28-di-*O*-acetate **96** forms 19b-hydroxy derivative **97** [99].

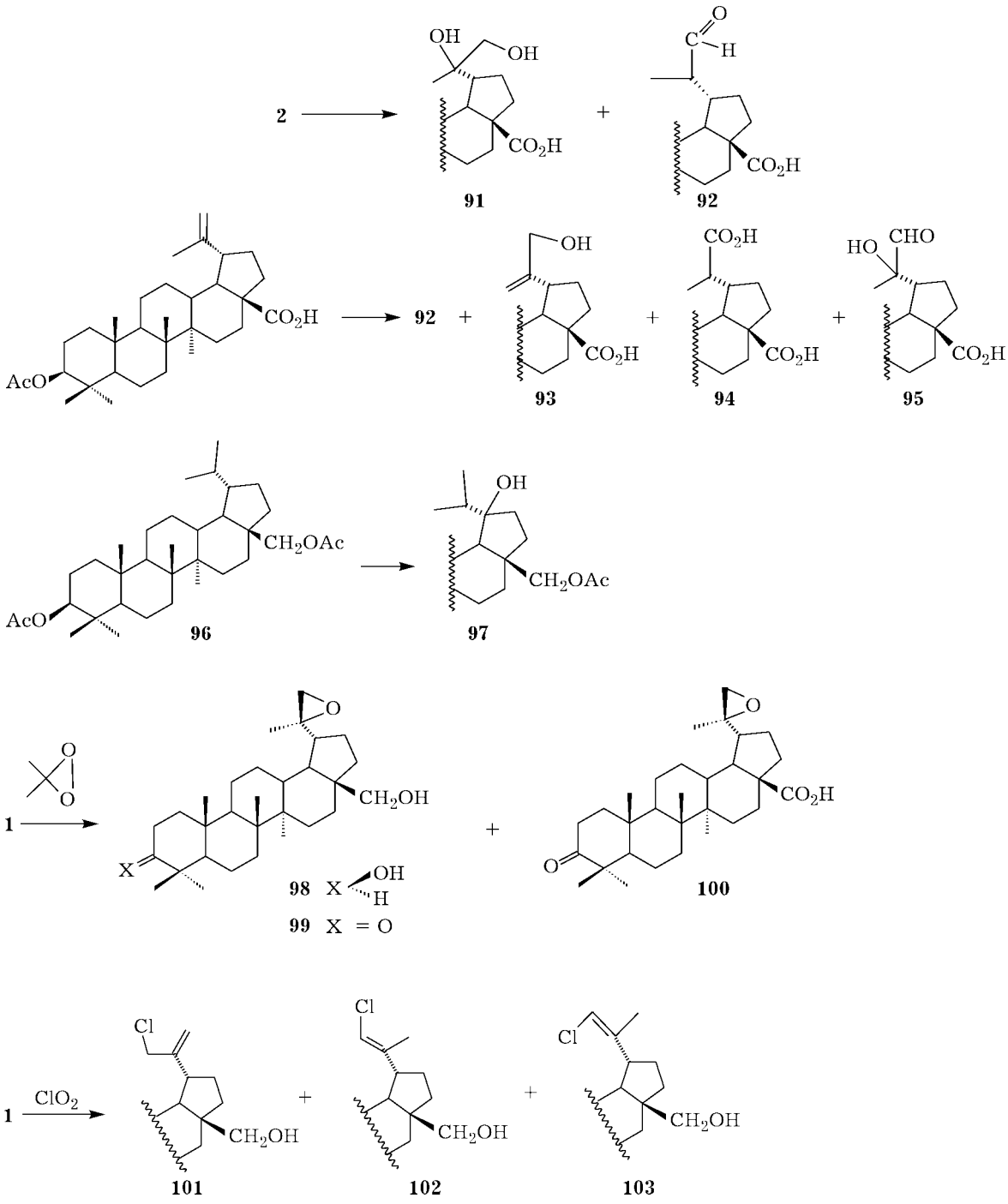
Oxidation of betulin with one, two and four equivalents of dimethyldioxyran allow obtaining 20,29-epoxybetulin **98**, 3-oxo-20,29-epoxybetulin **99** and 20,29-epoxybetulonic acid **100**, respectively [100].

It was established that betulin reacts quantitatively with ClO_2 giving a mixture of isomeric monochlorides **101–103** and an insignificant amount of dichlorinated products the structure of which has not been established yet [101].


AcO
CH2OAc
OH
O
76
77
78
79
80
81
82
83
84

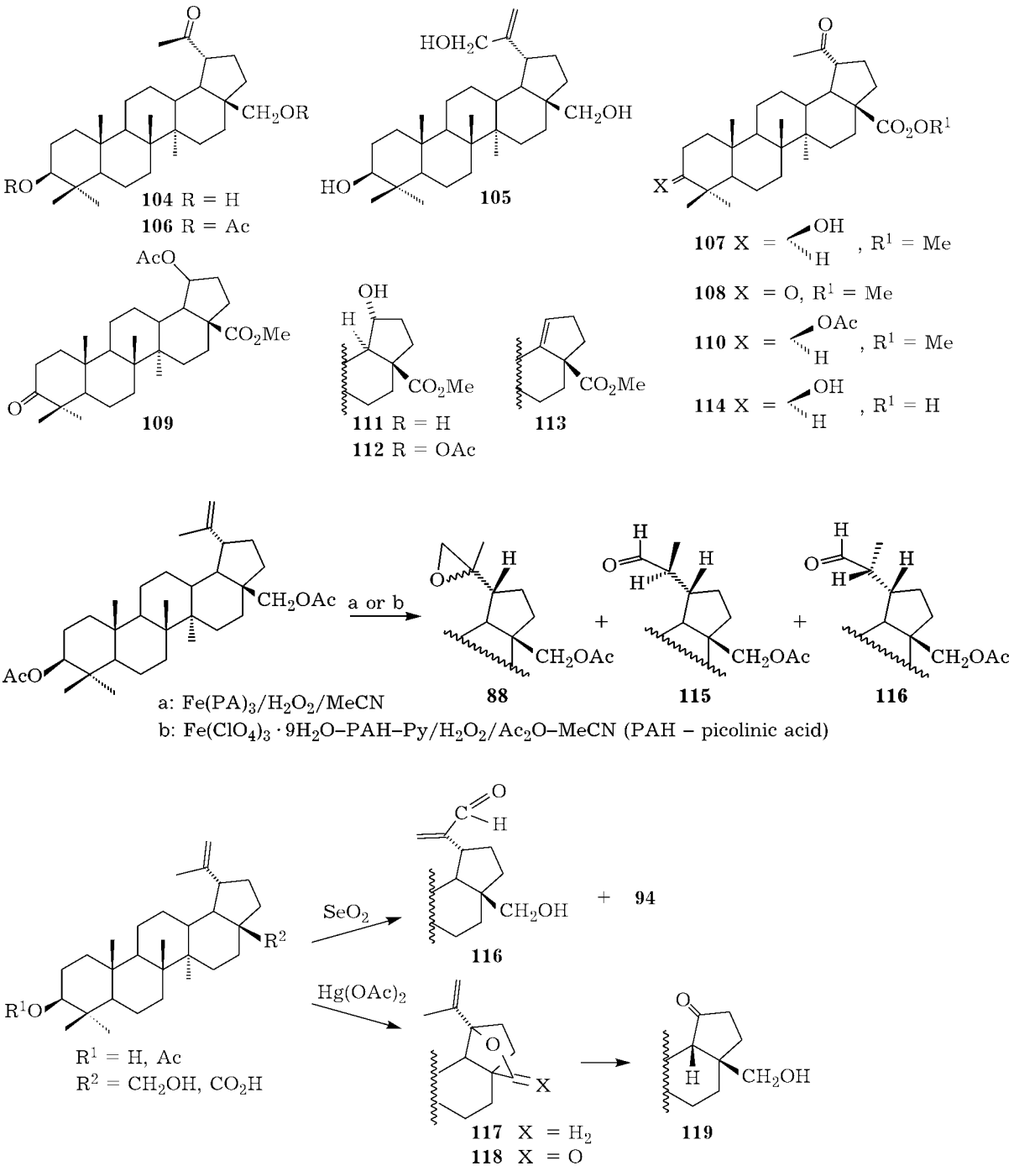

AcO
CH2OAc
H
COOH
HOOC
85
86
87


AcO
CH2OAc
PhCO3H
CH2OH
OH
89
90
м-CPBA
HCO3H или CH3CO3H
72
87
88



Ozonolysis of betulin gives norketone **104** which is identical with messagenine isolated from the plants of *Melilotus* genus, and triol **105** which is identical with the native compound from *Magtenus canarieusis* [102]. Ozonolysis of betulin 3,28-di-*O*-acetate resulted in the formation of norketone **106** with a high yield; methylbetulinate was transformed into methyl ester of platan acid **107**, and also 3-keto derivative of the latter acid **108** and the product of oxidation according to Bayer–Williger **109** [103, 104].

Ozonization of 3-*O*-acetylmethylbetulinate results in the formation of the expected 3-*O*-acetylmethylplatanate **110** and three minor products **111–113** [52, 103]. Platan acid **114** was obtained by oxidation of betulic acid with the system $NaIO_4/OsO_4$ [105]. The transformation of betulin into platan acid extracted from the

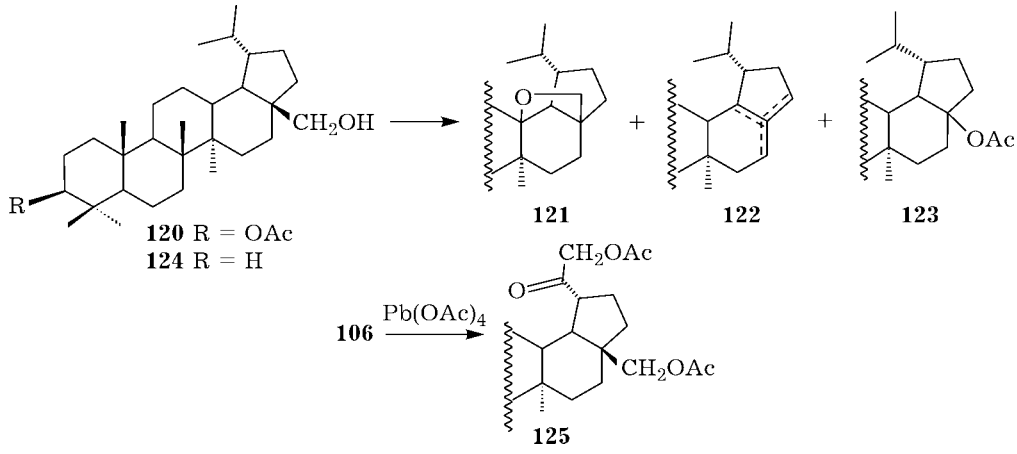


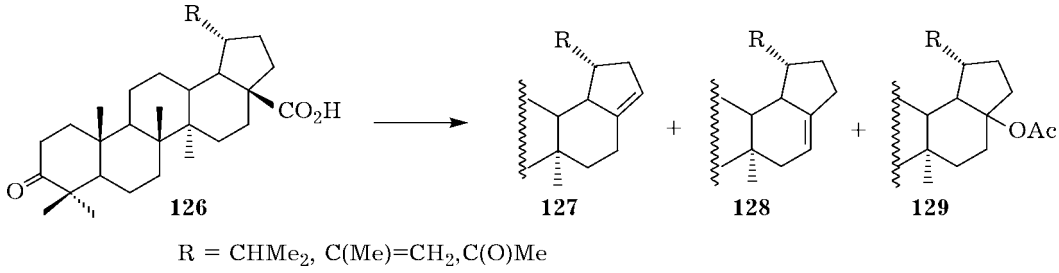
bark of *Platanus hypbrida* tree [28, 106] seems extremely important in connection with the discovery of high anti-HIV activity of this compound.

The action of ozone sorbed on SiO_2 (dry ozonolysis) on 3,28-di-*O*-acetyldihydrobetulin proceeds as an attack at C-19 with the formation of product **97** mentioned above [107].

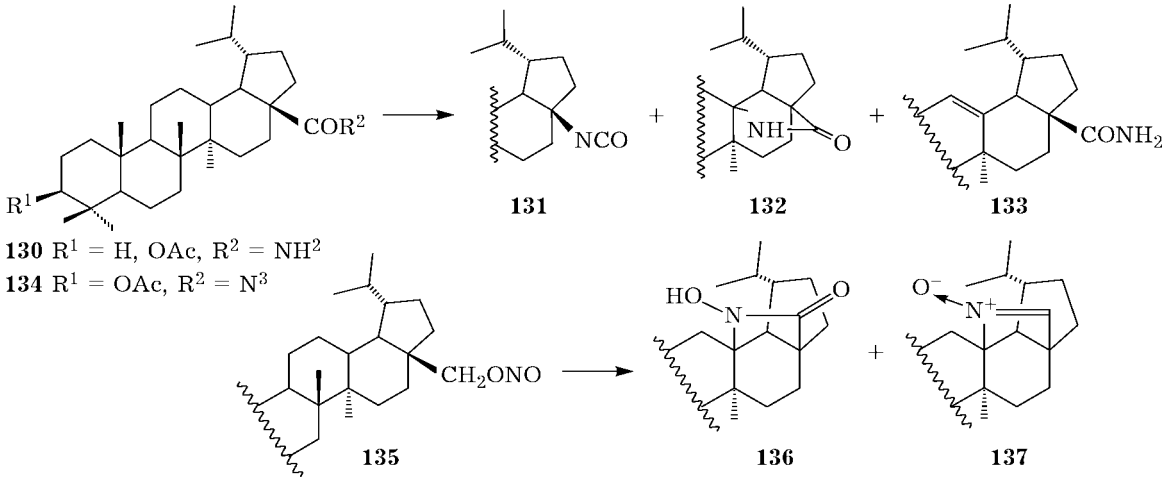
3,28-di-*O*-acetylbetulin gets oxidized under the action of H_2O_2 in the presence of Fe(III) picolinate or the complex of $Fe(ClO_4)_3$ with piconic acid giving epoxide **88** and the products of its isomerization, namely, stereoisomeric aldehydes **115**, **116** [108].

The allyl oxidation of lupane triterpenoids can be successfully carried out under the action of $Hg(OAc)_2$ on betulin or 3-*O*-acetylbetulin. The products of this reaction are 19,28-oxides **117** formed with high yields [111–113]. Under the same conditions, 3-*O*-acetylbetulic acid is





R = $CHMe_2$, $C(Me)=CH_2$, C(O)Me

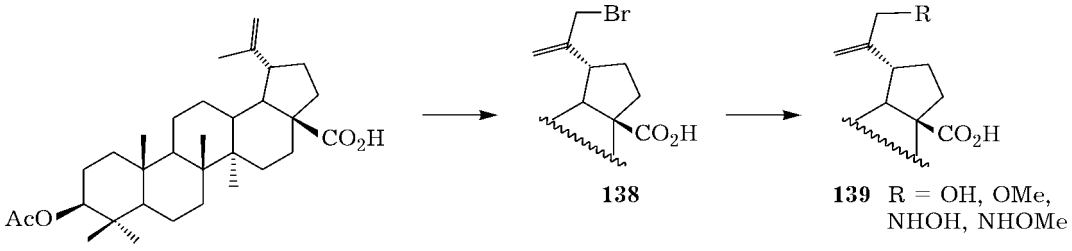


transformed into lactone **118** which was then used to obtain trisnorketone **119** [114].

Photooxidation of 3-*O*-acetyldihydrobetulin **120** proceeds with the formation of sole 13b,28-oxide **121** if I_2/HgO is used as the reagent. In the presence of $Pb(OAc)_4$ or $I_2/CaCO_3$ along with oxide **121**, 28-norterpenoids **122**, **123** are formed [115]. Oxidation of 28-lupanol **124** proceeds similarly to form oxide **121** and norterpenoids of the type of **122**, **123** [116]. Oxidation of norketone **106** with the help of $Pb(OAc)_4$ proceeds with the formation of acetoxyketone **125** [117].

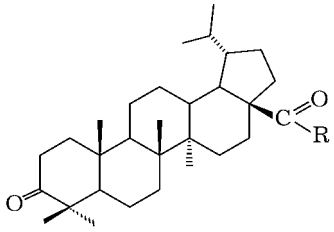
The derivatives of betulonic acid **126** undergo oxidative decarboxylation resulting in the formation of 28-norterpenoids **127–129** [117].

Amides of 3-*O*-acetyldihydrobetulic, 3-desoxybetulic acids **130** under the action of $Pb(OAc)_4$ form isocyanates **131** and lactams **132**. In the presence of I_2, some amount of unsaturated amide **133** is formed [118]. Isocyanate **131** was obtained by photolysis of the azide of dihydrobetulic acid **134**. Photolysis of 28-nitrite of dihydrobetulin **135** resutls in hydroxylactam **136** and nitrone **137** [119].

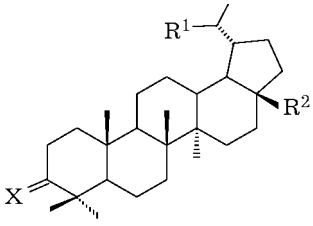


138

139 R = OH, OMe, NHOH, NHOMe



140 R = OMe
141 R = H



147 $R^1 = R^2 = CH_2OH$, X = 

148 $R^1 = CH_2OH$, $R^2 = CO_2Me$, X = 

149 $R^1 = CO_2H$, $R^2 = CO_2Me$, X = O

Allyl bromination involving the isopropenyl group was used to synthesize 30-substituted derivatives. Thus, bromination of 3-*O*-acetylbetulic acid with N-bromosuccinimide in CCl_4 proceeds with the quantitative formation of bromide **138**, which easily enters nucleophilic substitution resulting in 30-substituted products **139** [105].

Reactions of reduction and hydrobromination

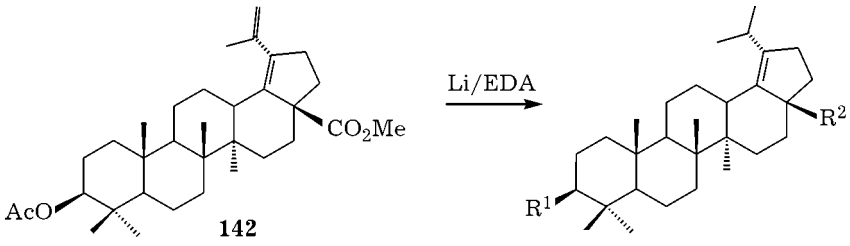
Hydrogenation of the double bond in betulin and betulic acid proceeds easily with nickel [9], palladium [120] and platinum [121] catalysts. Efficient hydrogenation catalyst is $RhCl[Ph_3P]_3$ [122]; lithium in ethylene diamine was also used [123]. Raney nickel in boiling *p*-cymene acts as a catalyst of disproportionation. Methylbetulinate is transformed with a high yield into methydilhydrobetulonate **140** [124, 125], while betulin gives dihydrobetulonic aldehyde **141** [126].

Reduction with lithium in ethylenediamine can be accompanied by such side reactions as reduction of carbomethoxy group, decarboxylation and deoxygenation. This is how the reaction of 3-*O*-acetyl-18,19-dehydromethylbetulinate **142** proceeds to form a mixture of four compounds **143–146** [127].

Hydroboration of betulin was used to synthesize 3b,28,30-trihydroxylupane **147** [128, 129], while methylbetulinate through the stage of dihydroxyester **148** was transformed into 3-keto-29-carboxylic acid **149** [93].

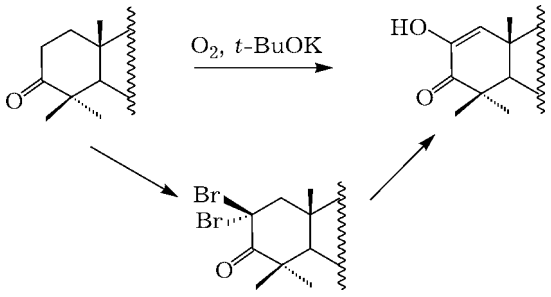
Reactions in cycle A

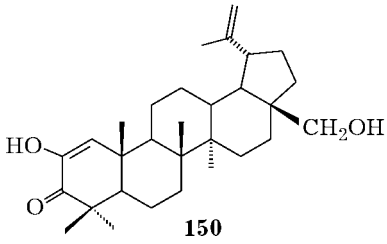
Oxidation of 3-ketolupanes with oxygen in the presence of *t*-BuOK proceeds smoothly. Diosphenols **150**, **151** were obtained from



142

143 $R^1 = OH$, $R^2 = CO_2H$
144 $R^1 = OH$, $R^2 = CH_2OH$
145 $R^1 = H$, $R^2 = CO_2H$
146 $R^1 = OH$, $R^2 = H$





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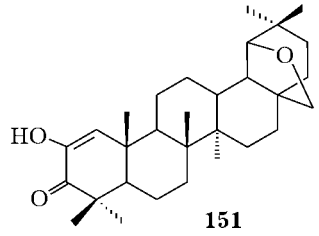

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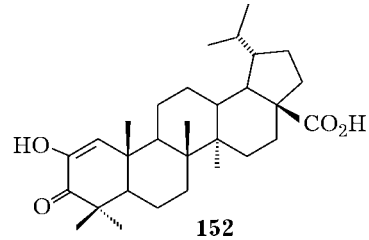

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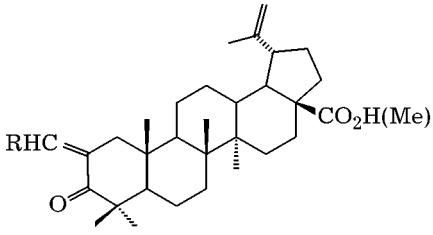
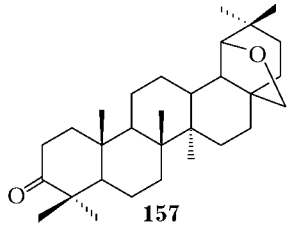


153 R = OH
154 R = $NHCONH_2$
155 R = $NHCSNH_2$
156 R = C_6H_5, $C_6H_4(OMe)$, $C_6H_4NO_2(o)$, $C_6H_4OEt(n)$, $C_6H_4Cl(n)$, $C_6H_4Br(n)$, $C_6H_4I(m)$, $C_6H_3(OMe)_2$ (2,3), α-furyl



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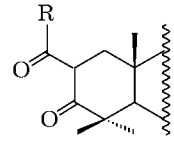

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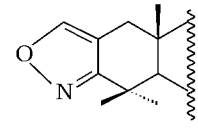

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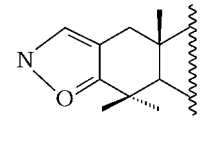
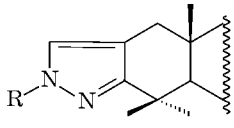


160

R = H, COOEt, CF_3

MeONa



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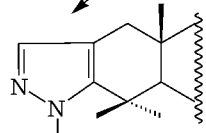

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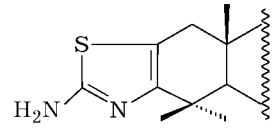

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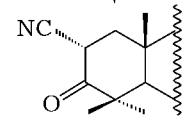
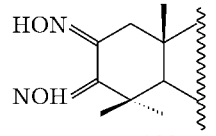


164

R = H, Me



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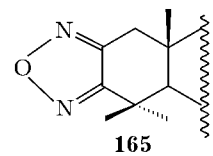

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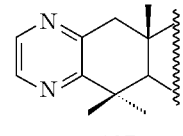

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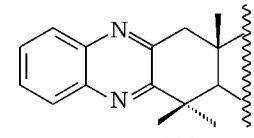


168

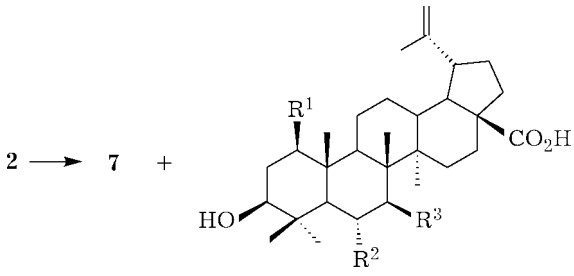
3-ketobetulin [130] and allobetulone [131], respectively. It is characteristic that under these conditions dihydrobetulic acid at first turns into 3-ketone, then into diosphenol **152** [132]. Diosphenols are easily obtained also by means of alkaline hydrolysis of 2,2-dibromo-3-ketones [133].

Reactions involving C-2 condensation of betulonic acid with allobetulone were carried out. For example, 2-oxymethylene-3-ketones **153** react with carbamide and thiocarbamide forming compounds **154**, **155** [43, 134]. The formation of unsaturated ketones like **156** proceeds smoothly by the interaction with substituted benzaldehydes and furfural [43, 135].

The synthesis of nitrogen- and sulphur-containing heterocycles was carried out on the basis of 3-ketones using the classical methods. In particular, allobetulone **157** was condensed with athylformiate, diethyloxalate and ethyltrifluoroacetate forming b-dicarbonyl compounds **158**; their treatment with hydrazine, methylhydrazine, hydroxylamine, thiocarbamide resulted in the formation of the corresponding heterocycles **159–163** [136]. The formation of isomeric isoxazols **159**, **160** and pyrazols **161**, **162** is demonstrative; the differences between them are exhibited both in the spectral characteristics and in the reactivity. For example, isoxazole **160** treated with MeONa forms 2a-cyanoallobetulone **164** [137]. Oxadiazole **165** was obtained from dioxime **166** [133]. The derivatives of pyrazine **167** and quinoxaline **168** were synthesized from betulin and allobetulin through the stages of diosphenols using the standard procedures [138, 139]. The synthesis of pyrazoles and pyrimidines based on lupenone and 2-oxymethylene-betulonate was described in [140, 141]. According to Hard–Mowry reaction, 1,2,3-thiadiazole was synthesized from 3-semicarbazone of betulic acid [142].

Microbiological oxidation of betulin and its derivatives

It is known from the chemistry of steroids [143] and higher terpenoids [144] that microbiological oxidation often results in the formation of compounds which can hardly be obtained using the standard synthetic methods.



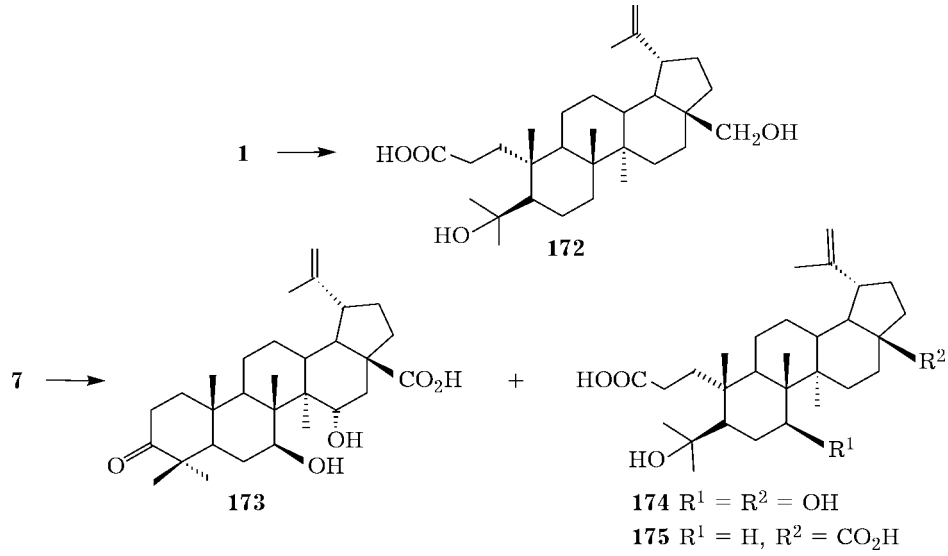
169 $R^1 = R^2 = H$, $R^3 = OH$
170 $R^1 = H$, $R^2 = R^3 = OH$
171 $R^1 = R^3 = OH$, $R^2 = H$

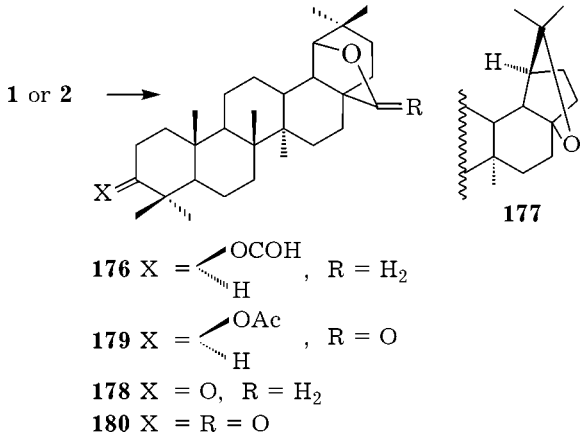
High anti-cancer activity of betulic acid was an incentive for the investigation of its microbiological transformation. According to [145], *Bacillus megaterium* ATSS 14581 culture transforms betulic acid into the known betulonic, 7b-hydroxybetulic **169** and 6a,7b-dixydroxybetulic **170** acids. The *Cunninghamella elegans* ATSS 9244 culture forms 1b,7b-dihydroxyacid **171**, while incubation with *Mucor mucedo* UI-4605 results in the formation of metabolite **169**. Oxidation of betulic acid by *Bacillus megaterium* ATSS 13368 culture proceeded with the formation of four metabolites: betulonic, 1b-hydroxy- and 11a-hydroxybetulonic acids [146].

Oxidation of betulin and betulic acid by the culture of *Chaetomium longirostre* fungi proceeds in a more complicated manner [147]. Betulin is selectively transformed into A-secoacid **172**. Betulonic acid gave unique compounds: 7a,15a-dihydroxy-3-ketoacid **173** and 28-norterpenoid **174**, as well as dicarboxylic acid **175**.

REACTIONS OF BETULIN AND ITS DERIVATIVES LEADING TO CHANGES IN THE CARBON FRAMEWORK

Isomerization of the derivatives of 20(29)-lupene containing oxygenated functional groups at C-28 atom under the action of acid agents results in the formation of oleanane derivatives. A classical reaction is the transformation of betulin into allobetuline 3-*O*-formiate **176** under the action of concentrated HCO_2H. The action of HBr in $CHCl_3$ solution leads to the formation of allobetulin [148, 149]. Transformation of betulin into allobetulin also occurs under the action of a mixture of glacial acetic acid and

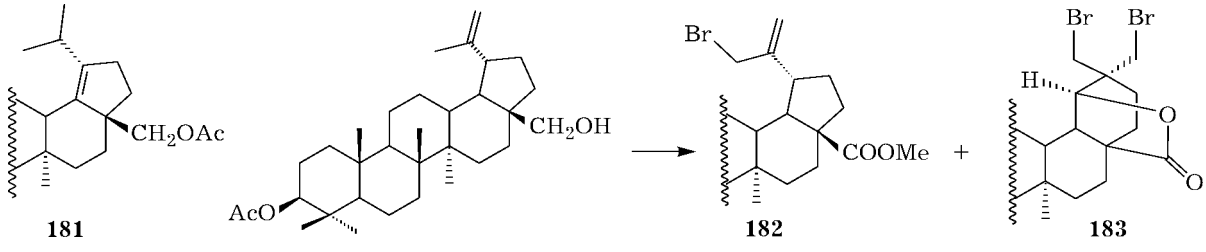


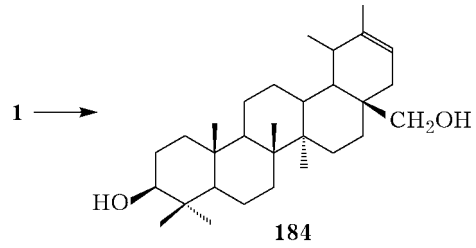


concentrated sulphuric acid [150], and under the action of concentrated hydrochloric acid in ethanol [151]. In the latter case, 20,28-epoxy-19a(H)-lupane-3b-ol **177** is formed as a side product with an yield of about 20 % [152]. One should also mention the transformations of betulin under the action of dimethylsulphate, toluene sulphonic acid and the acids deposited onto solid supports [153, 154], and under the action of trifluoroacetic acid [155].

Transformation of betulin into allobetulin proceeds efficiently also under the action of $Fe(NO_3)_3$ or $FeCl_3$ adsorbed on silica gel or aluminium oxide [156]. In addition, when reaction is carried out for a longer time in the presence of $Fe(NO_3)_3$ deposited on silica gel, betulin is transformed into allobetulon **178** in a single stage. Derivatives of betulic acid (3-*O*-acetate- and 3-keto-) form lactones **179**, **180** under the action of CF_3CO_2H [94]. It is typical that the protection by means of acetylation prevents skeleton isomerization. For instance, 3,28-di-*O*-acetylbetulin under the action of HBr in the solution of AcOH and Ac_2O is transformed into 18,19-isobetulin diacetate **181** [158]. A noteworthy allyl bromination reaction was observed under the action of N-bromosuccinimide in dimethylsulphoxide on 3-*O*-acetylmethylbetulinate; along with brominated ester **182**, a product of allobetulin rearrangement **183** was observed; it was formed as a result of bromination of the bromoester followed by lactonization [159].

As it has already been mentioned, allobetulin rearrangement occurs during glycosilation of betulin [70]. The possibility of betulin transformation into the derivatives of ursane





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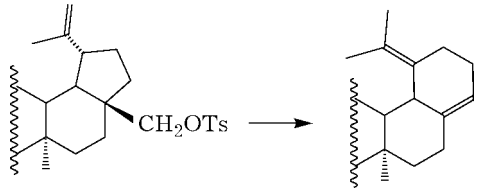
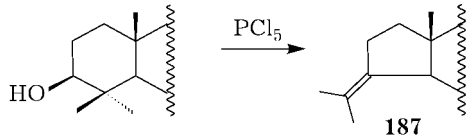


185 **186**



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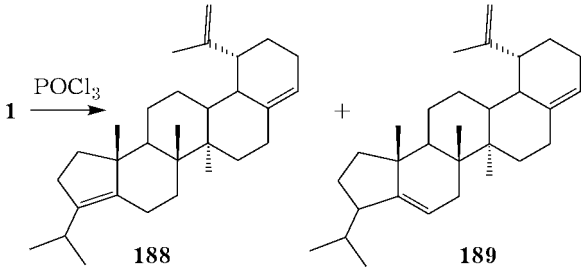


188 **189**

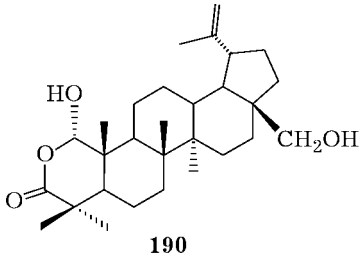
series **184** was demonstrated for the transformation of allobetulin under the action of $C_6H_5COCl_2$ at 150 °C [160]. Solvolysis of betulin 28-monotosylate **185** under the action of sodium acetate in acetic acid results in diene **186** [161].

As a result of tosylation followed by elimination of 3-*O*-acetylbetulic acid, 2,3-dehydroacid was obtained [52].

A typical reaction of 3b-oxyterpenes is Wagner–Meerwein rearrangement proceeding under the action of PCl_5 [1, 5, 7, 162]. Lupane derivatives are not exceptions; they form the corresponding isopropylidene derivatives **187**. Boiling betulin with $POCl_3$ in pyridine results in isomeric compounds **188**, **189** [7, 163].

Transformations of cycle A occur under oxidative action. For instance, when obtaining the above-mentioned betulin diosphenol **150**, hydroxylactone **190** is also formed. The action of H_2O_2 on diosphenol results in the formation of 2,3-secoacid **191** [130]. Diosphenolof betulonic acid **152** under the action of $Ba(OH)_2$ forms hydroxyacid **192** [132].

Beckmann rearrangement of 3-ketone oximes was used to carry out modifications in cycle A. For example, allobetulon oxime forms a mixture of lactam **193** and seconitrile **194** [164]. According to [165], methyl ester of



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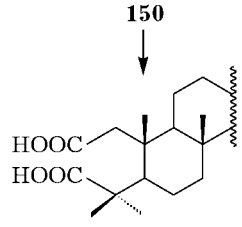

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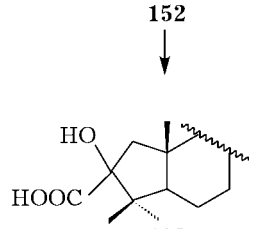

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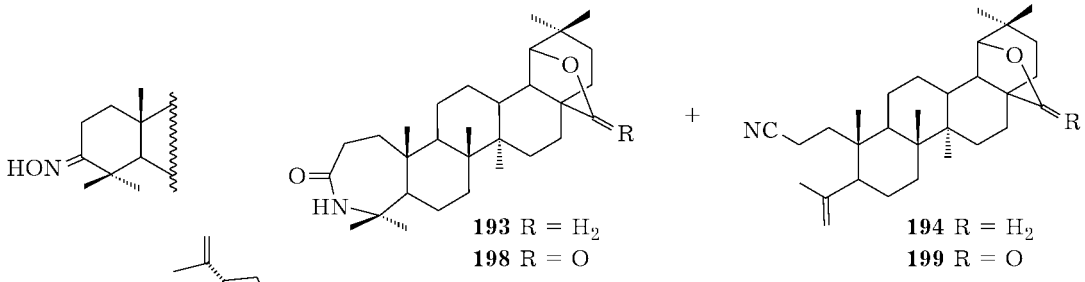
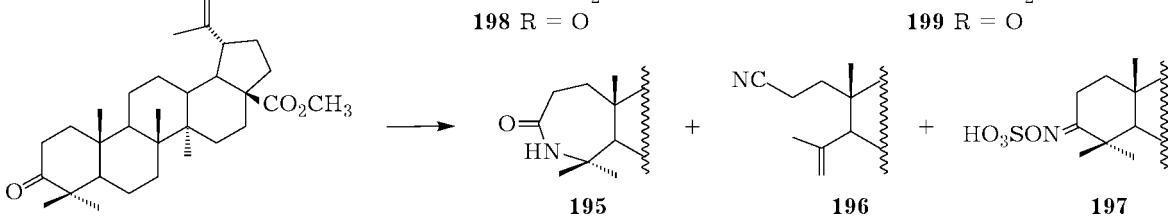
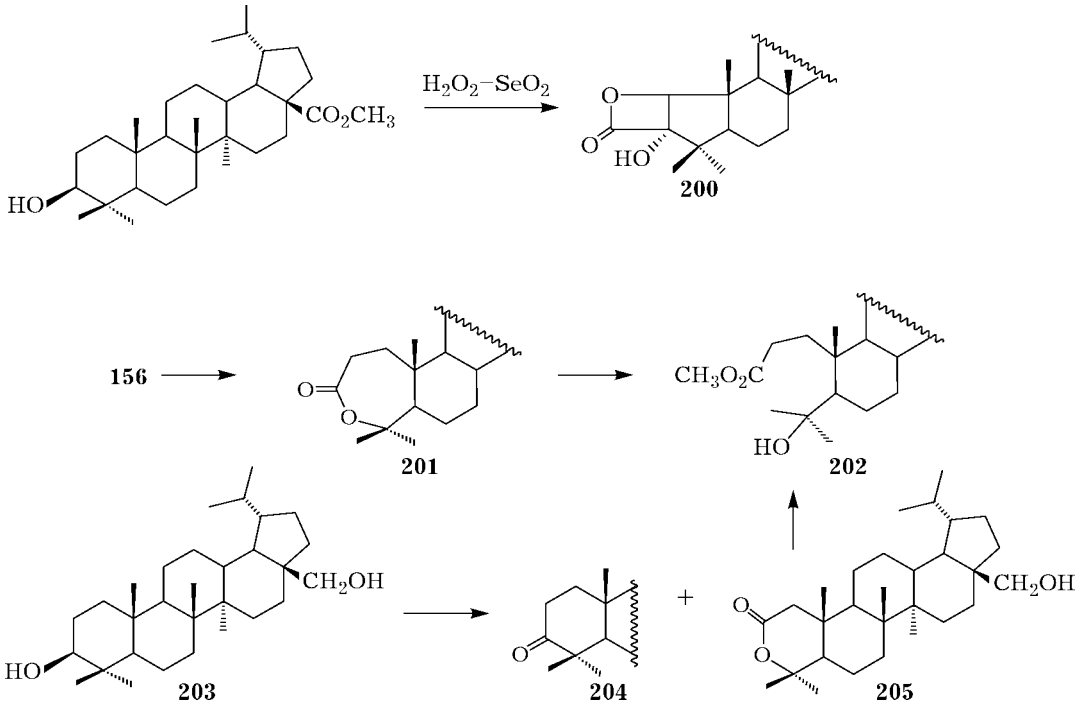


193 R = H_2
198 R = O

194 R = H_2
199 R = O



195 **196** **197**



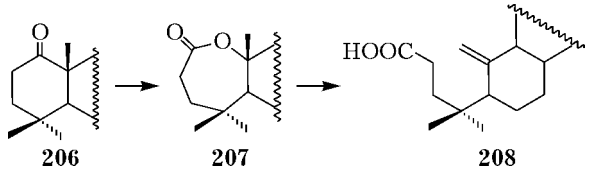
betulonic acid transforms mainly into lactam **195** druing heating with hydroxylamine-*O*-sulphonic acid in the solution of HCO_2H. The side products are seconitrile **196** and *O*-sulphonooxide **197**. Under treatment with a mixture of H_2SO_4–AcOH, the oxime of 3-ketobetulonate forms lactam **198** and seconitrile **199** of oleanane series [120].

An interesting rearrangeemnt is observed in cycle A of the methyl ester of dihydrobetulic acid under oxidation with a mixture of H_2O_2 and SeO_2. The product of this reaction is lactone **200** [166].

The opening of ring A was performed with the help of Bayer–Williger reaction. For instance, allobetulon is oxidized with peracetic acid into lacton **201**; its methanolysis results in the methyl ester of secoacid **202** [131, 167]. Dihydrobetulin **203** under the action of *m*-CPBA in boiling chloroform forms dihydrobetulon **204**, lactone **205** transformed into a secoacid of the type of **202** [99].

Lacton **207** was obtained by oxidation of 1-ketobetulin **206** with *m*-CPBA. Under the action of H_2SO_4 the lactone is transformed into 1,10-secoacid **208** [167].

The opening of cycle A was also carried out in a photo-initiated reaction of 28-monoacetate

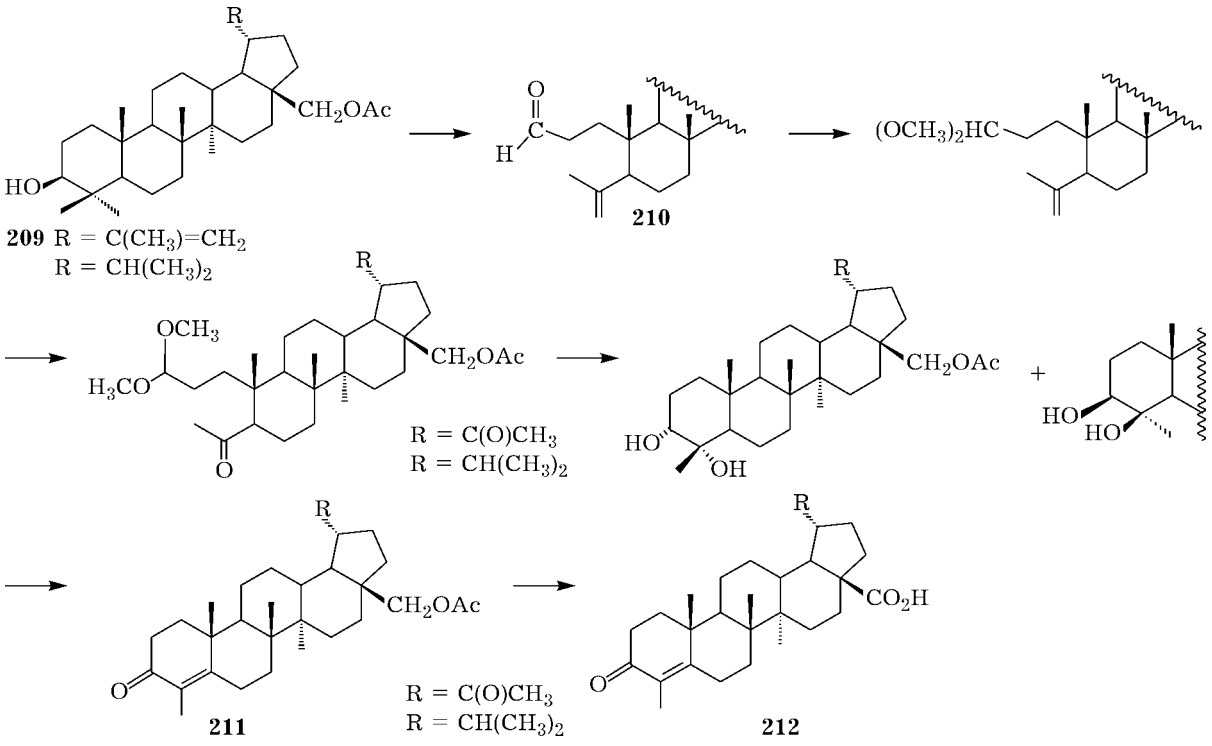


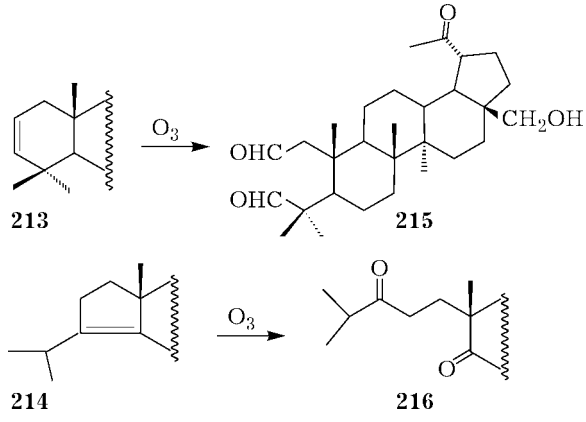
of betulin and of dihydrobetulin **209** with I_2-$PhI(OAc)_2$. The resulting secoaldehydes **210** were used to synthesize 4-desmethyl derivatives of betulin **211** [168]. Ketoacetate **211** was further successfully transformed into 2-oxydienone **212**.

The authors of [169] described the opening of ring A during the ozonolysis of 2,3-dehydrobetulin **213** and compound **214** with the formation of ketodialdehyde **215** and triketone **216**, respectively.

The opening of cycle E was carried out by oxidation of the derivatives of 18,19-isobetulin **181**. For example, its diacetate forms diketone **217** under the action of RuO_2 [158], while 18,19-isobetulin forms oxydiketone **218** which is a product of decarboxylation of 28-carboxylic acid formed by oxidation of the oxymethyl group [170].

If oxidation with RuO_4 is carried out in the presence of CF_3CO_2H, the products of degradation of cycles D and E are formed. For

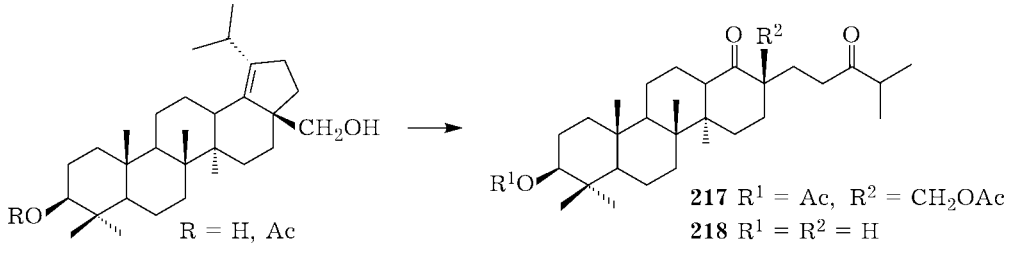


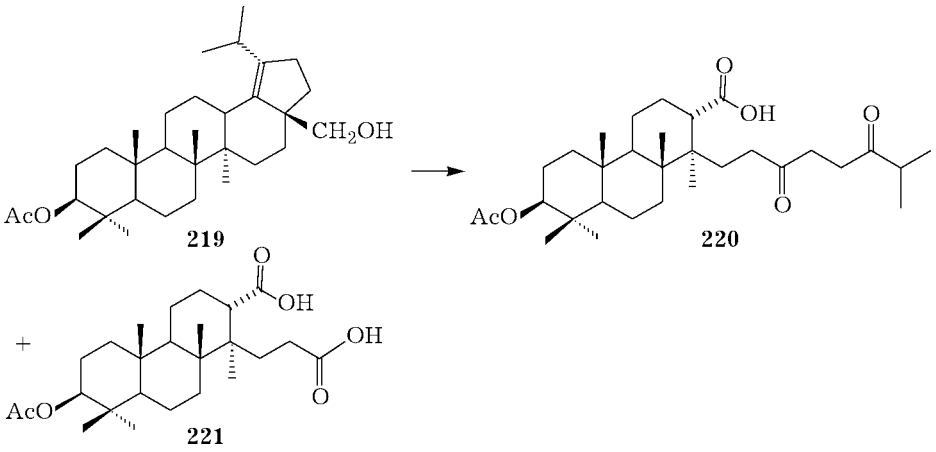


instance, 3-*O*-acetate of 18,19-isobetulin **219** was transformed into diketoacid **220** and dicarboxylic acid **221** [171].

BIOLOGICAL ACTIVITY OF BETULIN, ITS NATURAL AND SYNTHETIC DERIVATIVES

Biological characteristics of lupane triterpenoids were known as long ago as in the 19th century. In 1899, Wiler pointed to antiseptic properties of betulin, due to which this compound began to be used as a plaster for sterilization of wounds, cuts [172]; antirachitic action of betulin was reported in 1926 [173]. Extracts of birch bark, the main component of





which is betulin, exhibit wound *healing activity, cholesterol lowering action, anti-inflammatory, choleretic and liver-protecting activity* [174]. It is also known that the plant extracts containing lupeol, betulin, betulic acid exhibit *antitumour* action [175].

Betulin and betulic acid are of interest for medicine as the basis for the development of new antiviral agents. In 1994, anti-HIV activity of betulic acid **5** and related platan acid **114** were extracted from the leaves of *Zyzygium claviflorum* [26]; this served as a powerful incentive for the synthesis of its derivatives. Betulic acid suppresses reproduction of HIV-1 in the cell culture of H9 lymphocytes with EC_{50} = 1.4 mM [52]. Chemical modification of betulic acid resulted in obtaining its analogues possessing in some cases even higher anti-HIV-1 activity. On the basis of a large number of experimental studies, encouraging data on the interconnections between structure and activity were obtained. For example, higher anti-HIV-1 activity of dihydrobetulic acid was discovered (EC_{50} = 0.9 mM) [26, 52]. 3-Oxobetulic and 3-oxodihydrobetulic acids possess high activity as inhibitors of virus replication in the cells of H9 lymphocytes but they turned out to be toxic for cells [176]. Betulin and 20,29-dihydro-derivatives of betulin turned out to be less active inhibitors of HIV-1; this confirms the importance of the presence of carboxylic group at C-28 [46, 81]. 2-Hydroxybetulic acid (alphytolic acid) extracted from *Rosa woodsii* plant exhibits lower anti-HIV activity (EC_{50} = 42.3 nM) [52]. Betulin 3,28-di-*O*-nicotinate exhibited medium anti-HIV-1 activity with respect to suppression of the accumulation of virus-specific protein p24 (EC_{50} = 0.02 mg/ml, TI > 1000) but did not have any protective action on the cells [177]. a-, b-3-Alkylamido-3-desoxy derivatives of betulic acid did not possess anti-HIV activity [62]. So, the ester group in C-3 position is an important substituent for the manifestation of activity. Esterification of C-3 hydroxyl group with acetyl, benzoyl, crotonyl, sulphonyl and succinyl groups did not result in the formation of active compounds either. However, *tert*-butyl, (S)-(−)-camphonoyl and succinyl esters of betulic and dihydrobetulic acids turned out to be active inhibitors of HIV infection. The most promising compounds with respect to inhibition of HIV replication in the cells of H9 lymphocytes were dimethylsuccinyl derivatives of betulin **17–20**; among them, 3,28-di-*O*-acyl derivatives were more active than C-3-mono or C-28-mono esters [46]. The compounds are active in nanomolar concentrations; for example, for 3-*O*-(3',3'-dimethylsuccinate) of betulic acid, EC_{50} < 0.00035 mM, TI > 20 000; for 3-*O*-(3',3'-dimethyldimethylsuccinate-28-*O*-(2',2'-dimethylsuccinate of betulin EC_{50} = 0.00087 mM, TI = 42 400 [46, 81].

High anti-HIV activity was discovered in amides and peptides of betulic and betulonic acids [52, 57, 81, 178–180]. The amide of betulic acid with methyl ester of methionine exhibited anti-HIV-1 activity in MOLT-418 cell culture [59]. The series of w-aminoalkane and amino acid derivatives of betulic acid exhibited anti-HIV-1 activity in MT-4 and CEM-4 cell cultures in the region of nanomolar concentrations [56, 57]. A systematic investigation was

carried out to determine an optimal length of the amino alkane chain of the peptide. A chain of methylene links C_7–C_{11} is optimal for manifestation of anti-HIV activity. A peptide of the type of **25** containing 10 methylene links was chosen for modification. It was discovered that 3b-hydroxy substituent is optimal, while 3-desoxy, 3b-methoxy, 3b-amino and 2,3-dihydroxy derivatives of the indicated peptide turned out to be inactive. The transformation of isopropylidene group into acetyl (compound **27**) also gives a decrease in the activity, while the introduction of amide substituent (compound **28**) increases the activity [57]. Conversion of the amide bond results in the formation of even more active compounds (compounds of the type of **34**, EC_{50} from 44 to 150 nM) [56, 66]. The introduction of the second amino alkane chain results in the formation of compounds with higher anti-HIV activity; for the first chain, an optimal number of methylene links is equal to 7, while for the second chain it is 2–4 [181, 182]. Dipeptide **24** and its (S)-diastereo isomer with respect to the hydroxyl group of the peptide fragment demonstrated the best activity (EC_{50} is 50 nM in SEM cell culture and 40–44 nM in MT-4 cells). The derivatives of betulic acid with amide bond were patented by the Rhone-Poulenc Rorer company as the means to cure HIV [183–185].

When investigating anti-HIV activity of amides of betulic acid, very promising properties of peptide **32** were revealed; it causes a 50 % inhibition of virus reproduction when taken in the concentration of 2.6 nM. In addition, this peptide provides efficient protection of cells from the virus-induced cytostatic action [178–180].

According to the mechanism of action, the derivatives of betulic acid belong to the class of specific inhibitors of HIV-1 [81]. It is known that betulic acid and its derivatives are inactive towards HIV-2 [55, 57]. The mechanism of anti-HIV effect of betulic acid and its derivatives is connected with prohibition of the stage of fusion of the external viral shell with the cell membrane, that is, betulic acid is active at early stages of HIV replication cycle [55, 186, 187]; this circumstance comprises an advantageous difference of this compound from the substances of nucleoside nature which directly affect replication processes of the viral nucleic acid.

Betulic acid is an inhibitor of dimerization of HIV-1 protease, which consists of two identical semi-molecules; an active molecule is formed as a result of their fusion [188]. Investigation of the mechanism of action of betulic acid esters showed that these compounds inhibit the stage of virus penetration into a cell, but they do not inhibit reverse transcriptase and integrase of HIV-1 [57, 81, 187]. According to the data reported in [188], 3-*O*-(3',3'-dimethyl)-succinate of betulic acid inhibits HIV-1 protease (IC_{50} = 4 mM). Investigation of the mechanism of action of the peptides of betulic acid shows that, in addition to prohibition of the early stage of viral reproduction (with HeLa P4 cells) [189–191], the compounds of the indicated type are able to inhibit HIV reproduction when added after adsorption of virus. The amide-type derivatives of betulic and betulonic acids turned out to be inhibitors of reverse transcriptase of HIV [52, 191]. The authors of [192] presented the data on inhibition of the integration of HIV-1 into the genome of host cell by the derivatives of betulonic acid.

According to [193, 194], betulin and its derivatives should be considered as promising inhibitors of shell-free RNA-containing ECHO6 virus related to the pathogens causing poliomyelitis, rhinovirus infection, febrile and respiratory diseases. The highest efficiency was exhibited by betulic acid (EC_{50} = 0.07 mM, TI > 4000); it has a number of advantages in comparison with plecanorhyl preparation which is recommended in the USA to cure the diseases caused by picornavirus [194].

Highly active inhibitors of herpes virus of the simple type I were discovered among the derivatives of betulin and betulic acid [180, 193, 195]. Highly active compounds were discovered among amides, ureides and substituted benzalhydrazides [59, 63, 68].

The authors of [193] described the activity of betulin and its derivatives with respect to the influenza virus of type A within a broad concentration range. The most active compounds were 3-oxime and amide of betulonic acid [59]. The introduction of additional CONH and NHCONH groups at the C-28 position of lupane acid molecules potentiates their antiviral activity [59, 63]. Moderately expressed inhibit-

ing activity of allobetulin towards the influenza virus of type B [196].

The antiviral activity of derivatives of lupane triterpenoids is combined with high immunostimulating action [35, 44, 180]. Betulin bishemiphthalate and betulin dinicotinate stimulate the production of antibody-forming cells in mouse spleen 1.3 and 1.8 times more actively in comparison with the reference. Betulin dinicotinate weakens the slowed hypersensitivity to sheep erythrocytes. In addition, the indicated compounds prevent death of animals from acute radiation sickness [197].

Betulonic acid and its peptide **32** exhibited clear immunostimulating action exceeding the effect of incomplete Freud adjuvant [178–180]. The mechanism of this action can be connected with stimulation of the lymphocytes of different classes due to the influence of terpenoids on the synthesis of cytokines, for example interleukine-Z.

Anticancer activity

Cytotoxicity of the derivatives of lupane triterpenoids was investigated with respect to different kinds of cancer cells. Betulic acid possesses the most clearly exhibited anti-tumour activity among lupane triterpenoids. It was discovered in 1995 that betulic acid is a selective inhibitor of the growth of human black cancer cells [198]; later it was patented as an inhibitor of the growth of cancer cells [199]. It was reported that betulic acid is active against other cancer cells of neuroectodermal origin [200] and the cells of malignant brain tumours by inducing the apoptosis mechanism (programmed cell death), both *in vitro* and *in vivo* [201–207]. Recently, high efficiency of betulic acid against neuroblastoma, medulloblastoma, glioblastoma and Eving sarcoma, the most widespread group of malignant brain tumours in children, was discovered [208, 209].

Betulic acid is active in vitro against melanoma, lung and ovarian carcinoma within a narrow concentration range (1.5–4.5 mg/ml) [27]. Betulic acid turned out to be efficient against ovarian carcinoma in mice in the experiments *in vivo* [27]. It is interesting that antiproliferative activity of betulic acid is 10 times as high as the activity of the known antineoblastic preparation doxorubomycine with respect to human black cancer cells [27]. So, betulic acid is of interest for pre-clinical and clinical investigations against a broad range of malignant neoplasms. At present, betulic acid is involved in pre-clinical investigation in the USA as a medicine and prophylactic means against malignant black cancer for which mortality is 50 % of the number of patients revealed [198].

A number of derivatives was obtained by means of transformations of betulic acid at C-3, C-20 and C-28 positions; the anti-tumour activity of these compounds against human black cancer cells and oral fibrosarcoma was investigated [82]. Modification of betulic acid with amino acids increases its solubility in water, decreases its toxicity with the conservation of cytostatic activity [58]. Carcinostatic activity was discovered in a number of betulin acylates [210]. The derivatives of betulic acid modified at the C-28 position were also proposed as selective inhibitors of malignant black cancer [211]. 3-*O*-Methanesulphonate of betulic acid and its 2-halogenated derivatives are cytotoxic with respect to human ovarian carcinoma cells and other tumours [53]. It was established that the presence of a free carboxyl group at C-28 is necessary for the anti-tumour activity to be exhibited. The derivatives of dihydrobetulic acid are more active than the native compounds. The introduction of nitrogenous substituents into C-20 position causes an increase in cytotoxicity [82]. Oxidation of the double bond C-20(29) to norketone is accompanied by a decrease in cytostatic activity [105]. Betulonic acid possesses high cytotoxicity with respect to human black cancer cells (MEL-2) (ID_{50} = 1.6 mg/ml) and prostate adenocarcinoma (PC-3) (ID_{50} = 1.3 mg/ml) [105].

Metabolites of betulic acid **169–171** exhibited lower cytostatic activity with respect to black cancer cells than the initial acid did [145]. The products of microbiological oxidation of betulic acid **172–175** are active inhibitors of reproduction of Epstein–Barr virus which induces skin tumours [147]. The native 23-hydroxybetulic acid extracted from the roots of *Pulsatilla chinensis* (Bunge) Regel exhibits cytotoxicity similar to that of betulic acid towards human leukemia K-562 cells and human HeLa cells [212, 213]. Clearly expressed anti-leukemia ac-

tivity was discovered in betulonic aldehyde [214]. The authors of [215] investigated the anti-tumour activity of the derivatives of betulin and betulic acid obtained by further oxidative transformations of des-E-lupane derivatives of the type of **212**, **217**, **218**. It was shown that the compounds containing conjugated ketone or diketone function in ring E possess high activity *in vitro* with respect to leukemia cells.

The derivatives of betulic acid **57** exhibit activity against malaria four times as high as that of betulic acid itself. At the same time, *in vivo* experiments with the NK65 (*P. Berghei*) model of malaria revelaed that betulic acid turned out to be inactive and even toxic at the dose of 250 mg/kg per day. No anti-malaria activity was discovered for betulin at the dose of 500 mg/ml in K1 and T9-96 models *in vitro* [216].

The antibacterial activity of C-3 substituted derivatives of betulin with respect to a number of bacteria (*Staphylococcus aureus, Staphylococcus faecalis, Staphylococcus beta haemolyticus*) was described [217]. For betulic acid, rather low antibacterial activity was demonstrated towards *Bacillus subtilis* and *Escherichia coli* [218]. It was discovered that betulic acid, its methyl ester, 3-*O*-acetate, 3-*O*-allylate and 3-*O*-cinnamates exhibit antifydant activity (with respect to *Spodoptera litura* F. and *Achoca janata* caterpillar larvae); the activity of cinnamates is essential [219].

Interesting data were obtained on liver-protecting activity of betulin derivatives [35, 50, 134, 220]. Liver-protecting action (the effect on the functional status of rat liver) of a number of betulic acylates exceeds the activity of betulin and silibor towards liver affection in rats caused by CCl_4, tetracycline and ethanol [35, 50]. The highest liver-protecting activity was exhibited by betulin bishemiphthalate at a dose of 20 mg/kg; its activity exceeded that of the known liver-protecting medicine carsil [50, 220]. The introduction of the former compound into rats causes a decrease in the indices of intoxication and recovery of the functions of hepatocytes. Betulin dinicotinate promotes recovery of the level of marker enzymes of blood serum, alkaline phosphatase and bilirubin. In addition, this compound decreases the intensity of peroxide oxidation of lipids by a factor of ~1.8 and thus exhibits antioxidant properties [35]. High liver-protecting properties which exceed the activity of the officially permitted Silibinin preparation (IC_{50} = 29.9 nM), but are inferior to the activity of glycyrrhizinic acid were discovered in the native 2a,6b-dihydroxybetulic acid extracted from the seeds of *Combretum quadrangulare* [221]. Betulin exhibits protective action against the toxic effect of cadmium salts [222]. The authors of [223] showed that peptide and dipeptide derivatives of betulonic acid and its methyl ester exhibit antioxidant properties *in vitro* for the models of initiated oxidation of methyloleate. The antioxidant action of betulonic acid, its methyl ester and amides was investigated for the single and curative prophylactic application with the model of toxic hepatitis [224]. It was discovered that the curative and prophylactic introduction of the derivatives of betulonic acid decreases hepatotoxic action of CCl_4, decreases the intensity of peroxide oxidation of lipids in blood and in liver. The cytotoxic action on rats in the model of polychemotherapy was carried out by means of a single-time intraperitoneal introduction of a complex of cytostatics at a dose of 1/5 LD_{50} – cyclophosphan (21 mg/kg), doxorubicine (2.1 mg/kg), vincristin (0.04 mg/kg), prednisolone (2.1 mg/kg) [225]. It was established as a result of morphological investigation that the amides of betulonic acid exhibit antioxidant and liver-protecting effects against toxic hepatitis and also decrease hepato- and nephrotoxic action of polychemotherapy in the post-cytostatic period.

Betulic acid exhibits anti-inflammatory activity [28, 226]. The derivatives of betulic acid are of interest as anti-inflammatory means and inhibitors of lipoxygenase [74]. Betulonic acid exhibited antiulcer action exceeding that of Venter preparation for the models if affection of mucous coat of stomach in rats caused by indometacin and aspirin with the dose of 50 mg/kg [157]. Betulin dinicotinate and betulin bishemiphthalate exhibit anti-inflammatory action similar to that of orthofen for the carragenine and formalin models of inflammation of mouse legs; the doses are 12 and 20 mg/kg, respectively [35, 227]. The anti-inflammatory activity of these betulin esters is combined with clear antiulcer action observed with the models of acute and chronic stomach ulcer in rats affected by indometacin, aspirin, ethanol and liquid nitrogen [35]. The antiulcer effect of betu-

lin esters is similar to the activity of "Venter" and "Omez" preparations, doses of 12 and 20 mg/kg. Bis-tetramethylcyclopropane ester of betulin and 2-(4-chlorophenyl)-3-methylbutyric ester of allobetulin exhibited anti-inflammatory action at a dose of 50 mg/kg after oral introduction into mice [51]. Clear anti-inflammatory activity was observed in 2-substituted derivatives of methylbetulonate **154** and **155** [134]. Compound **155** exceeds the known preparations "Omez" and "Venter" in antiulcer action. With the models of affection of mucous coat of rat stomach caused by indometacin and acetylsalicylic acid, 3,28-dioximebetulonic aldehyde exhibited antiulcer action similar to that of Venter preparation [78].

2-a-L-C-Methylfuropiranosylpropane derivative of betulic acid inhibits inflammation caused by arachidonic acid [74]. High anti-inflammatory properties were discovered in pyrocrenic acid (3b-(3,4-dihydroxycinnamoyl)-oxylup-20(29)-ene-28ic acid) [228] and some other derivatives of lupane triterpenoids [229, 230].

The esters of lupeol with palmitic and linoleic acids exhibit anti-arthritis action [37]. It was established that betulin acetates possess hypolipidemic properties [72]. Betulin glycosides introduced into lecithin liposomes can enhance the effect of liposomal preparations decreasing the level of cholesterol in blood for the experimental hypercholesterolemia [231].

Cosmetic compositions with betulic acid active against wrinkles, cellulitiskin flabbiness were proposed [232]. Compositions containing lupeol exhibit rejuvenating action [233]. Betulic acid stimulates the synthesis of collagen in skin to a higher extent than ascorbic acid does [234].

Acknowledgements

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