Birch bark research and development

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This review will detail progress made in the previous decade on the chemistry and bioactivity of birch bark extractive products. Current and future applications of birch bark natural products in pharmaceuticals, cosmetics, and dietary supplements for the prevention and treatment of cancer, HIV, and other human pathogens are reviewed. Current developments in the technology of birch bark processing are discussed. New approaches for the synthesis of potentially valuable birch bark triterpenoid derivatives are also reviewed.

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Pavel Krasutsky

1 Introduction

The bark of the birch tree has been the subject of respect and admiration throughout prehistory[1,†] and history,[3,4] as well as the subject of curiosity of science and industry in the modern world.[5–14] The twentieth century has been a time of deep, fundamental study into the chemistry of birch bark products,[5–14] although during this time the application of this work was largely limited to traditional uses of NPs in the cosmetics industry.[15] The symbiosis of the birch tree and civilisation should now be reconsidered through the scientific vision of a new century. The last review on this subject was published in 1994;[8] this review addresses the achievements of the last decade, which have revealed remarkable biological and medical aspects of birch bark triterpenes and their derivatives. The most interesting of these are birch bark triterpenoids, which represent a new class of anti-cancer and anti-HIV[16] bioactives with a novel mechanism of action. The study of these NPs and their derivatives has already been developed beyond the framework of fundamental science, and ongoing clinical tests are currently approaching the level of new drug creation.[16,17] These developments in the area of birch bark products have stimulated parallel development of the technology of birch bark processing, as well as in the chemistry of triterpenoids, their synthesis and

† Among the possessions of the 5300-year-old iceman (found in 1991 in the Tyrolean Alps) were two birch bark bags and two walnut-sized birch fungi (*Piptoporus betulinus*), presumed to be a "medical kit". This fungus contains triterpenes with anti-bacterial and anti-cancer properties.[2]

selective derivatisation.‡ Birch bark extracts have found broad use in modern cosmetics and have the potential to be used as dietary supplements. Additionally, with the ever-increasing efficiency of birch bark processing and refinement technologies, outer birch bark and inner birch bark can now be regarded as another valuable source of natural chemicals (suberinic acids and tannins) with multiple uses. Although the chemistry of birch bark extracts is well established,[5–14] significant differences between the chemistry of Eurasian and North American birches are just now being recognised. Most notable are the chemical specifics of Alaskan birch trees that make them a source of unique composition. Recent research and development on birch bark processing makes all birch natural products accessible by quality and volume to any field that might find use for them. The goal of this review is to show that birch bark value-added products have a great potential in addition to the traditional uses of birch wood in the paper or forestry industries.

Table 1 Average chemical content (%) of birch bark extractive[a]

	B. pendula[9d,21]	*B. papyrifera*	*B. neoalaskana*
Betulin (**1**)	78.1	72.4	68.1
Betulinic acid (**2**)	4.3	5.4	12.5
Betulinic aldehyde (**3**)	1.2	1.3	1.4
Lupeol (**4**)	7.9	5.9	2.1
Oleanolic acid (**5**)	2.0	0.3	2.2
Oleanolic acid 3-acetate (**6**)	—	1.6	3.8
Betulin 3-caffeate (**7**)	0.5	6.2	6.1
Erithrodiol (**8**)	2.8	—	—
Other (minor)	3.2	6.9	3.8

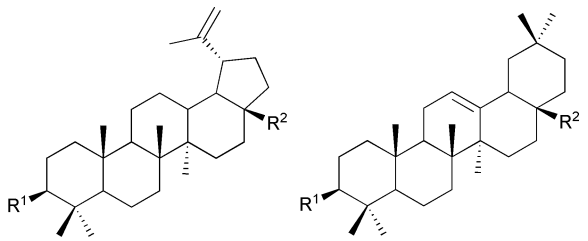
[a] Samples of outer birch bark of *Betula neoalaskana* were kindly transferred for extraction and GC/MS, NMR and HPLC analyses by the Professor of Forest Management, Edmond C. Packee (SNRAS Forest Science Department, University of Alaska, Fairbanks).

2 Bioactivities of birch bark products

2.1 Birch bark extractive

The chemistry of outer birch bark can be subdivided into the chemistry of the extractive and the chemistry of the natural polymer suberin.[8,9] The extractive includes a mixture of pentacyclic triterpenoids, lupanes (major) and oleananes (minor),[9] which are perhaps the most interesting for use as bioactive compounds (drugs, cosmetics, dietary supplements, biocides, bactericides, *etc.*). The chemical content of birch bark extracts from the 38 scientifically recognised *Betula* species[18] is similarly varied.[6–14] This variety makes it more practical to consider only extracts from the industrially and commercially managed *Betula* species (*B. pendula* and *B. pubescens*, Eurasia; *B. papyrifera*, Northern US and Canada) and the potentially interesting Alaskan birch, *B. neoalaskana*.[19] The average chemical content of extractives and formulas of triterpenoids for these three birch species, are presented in Table 1 and Scheme 1. The triterpenoid chemical content of species listed in Table 1 can also possess some variability[9c,20] within a specific species, and depends on the age of the tree and climatological conditions. Different methods of analysis and the lack of standard calibration procedures[6–14,20] may also be the reason for the reported variencies. Though minor components (see "Other" in Table 1), the following NPs should still be mentioned (Scheme 1): betulone (**9**); betulonic aldehyde (**10**); lupenone (**11**); betulonic acid (**12**); oleanolic aldehyde (**13**) and β-amyrin (**14**).[9d] The increased amount of betulinic acid (**2**) and betulin 3-caffeate (**7**) in N. American birch bark is an important difference, because these NPs are significant anti-cancer and anti-HIV ingredients. By reviewing the progress on the bioactivity of birch bark triterpenes and their derivatives over the past decade, the importance of these differences becomes more understandable.

‡ Efforts on birch bark product research and development by the Laboratory of Chemical Extractives (Natural Resources Research Institute, University of Minnesota–Duluth, USA, http://www.nrri.umn.edu/cartd/lce/) encouraged the creation of this review.



R^1	R^2		
OH	CH_3	Lupeol (**4**)	β-Amyrin (**14**)
OH	CH_2OH	Betulin (**1**)	Erithrodiol (**8**)
OH	CHO	Betulinic anhydride (**3**)	Oleanolic aldehyde (**13**)
OH	COOH	Betulinic acid (**2**)	Oleanolic acid (**5**)
O=	CH_3	Lupenone (**11**)	
O=	CH_2OH	Betulone (**9**)	
O=	CHO	Betulonic aldehyde (**10**)	
O=	COOH	Betulonic acid (**12**)	
AcO	COOH		Oleanolic acid 3-acetate (**6**)
$(HO)_2C_6H_3CH{=}CHCOO$	CH_2OH	Betulin 3-caffeate (**7**)	

Scheme 1 Triterpenes of the outer birch bark of *Betula pendula*, *B. papyrifera*, and *B. neoalaskana*.

Table 2 Anti-HIV activity for betulin derivatives **2**, **15–18** and AZT

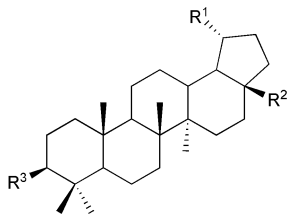
Compound	EC_{50}/μM	IC_{50}/μM	Therapeutic index
Betulinic acid (**2**)	1.4	12.9	9.2
3-*O*-(3′,3′-Dimethylsuccinyl)betulinic acid (**15**)	<0.00035	7.0	>20 000
3,28-*O*-(Di-3′,3′-dimethylglutaryl)betulin (**16**)	0.00066	14.2	21 515
3-*O*-(3′,3′-Dimethylsuccinyl)-28-*O*-(2′,2′-dimethylsuccinyl)betulin (**17**)	0.00087	36.9	42 400
3-*O*-Glutaryldihydrobetulin (**18**)	0.00002	23.59	1 120 000
AZT	0.045	1873	41 622

The total average yield of *B. pendula* and *B. pubescens* extractive (~27%)[9c] is higher than that of N. American *B. papyrifera* and *B. neoalaskana* (~22%).[20,22]

2.2 Birch bark triterpenoids

Triterpenoids are the most ubiquitous class of natural secondary metabolites (there are more than 4000 compounds in the terrestrial and marine flora). They have been widely studied, have been previously reviewed in this journal,[23] and have high potential as bioactive NPs.[24,25] Outer birch bark from boreal forests contains the highest quantity of triterpenoids of all plants (20–35%). It is generally believed that the physiological function of these NPs is defence (a plant's acquired resistance) against plant-pathogens.[26–28] This has led to the expectation that triterpenoids could also act against pathogens that cause human and animal diseases. However, the use of these NPs, including birch bark triterpenoids, has remained quite limited, in part because of their low solubility (<1 mg L^{-1} in water), high log *P* value (>9); and high molecular weight (>500 Daltons). This reduces their attractiveness as promising drug candidates through the formal concept of the *rule of five*,[29] rational drug discovery[29–31] and combinatorial chemistry.[32] In spite of the fact that these qualities have limited the interest in these NPs by the drug industry,[33–35] 21 drugs based on NPs have been launched on world markets between 1998 and 2004.[34] The three following triterpenoids are currently undergoing clinical trials at the US National Institute of Health: betulinic acid (**2**) as an anti-cancer compound,[17] a semi-synthetic derivative of betulinic acid (PA-457) as anti-HIV compound,[16] and the natural water-soluble triterpene glycoside (QS-21, an oleonolic acid derivative) as an adjuvant for vaccines.[34] The primary preference for the use of triterpenoids as bioactives is their established low toxicity. Native Americans and native Siberians used birch bark (*B. papyrifera* and *B. pendula* respectively) as a source of folk medicine. This historically recognised internal use of birch bark extractive,[3,4] coupled with the scientifically measured low toxicity of triterpenoids,[36] support the use of birch bark chemicals not only in drugs, but as dietary supplements, cosmetics, biocides, washing materials, agrichemicals, *etc.* The major birch bark NPs **1**, **2**, **4**, **5** and **7** merit special attention as potentially promising bioactive compounds or precursors to drug ingredients.

Betulin (**1**) is one of the oldest NPs, first isolated from birch bark and scientifically described in 1788.[37] Previous reviews[9,38,39] have cited betulin's moderate anti-cancer, anti-bacterial, anti-fungal, and anti-viral activity. Betulin and birch bark extractive have been proposed for use in cosmetics as an additive to shampoo,[40] skin-care,[41] dental-care[42] and hair-care[43] products. For these purposes, however, pure betulin in not usually used, but rather birch bark extract.[15] Fundamental research into the bioactivity of betulin (**1**) and betulin derivatives are ongoing. In most cases it has been shown that betulin and dihydrobetulin derivatives are usually more active than pure betulin as anti-cancer compounds[39,44–48] or anti-HIV compounds.[49–51] It has also been reported that 3- and 28-acylbetulin and 3,28-diacylbetulin derivatives have a fairly high level of anti-HIV activity *in vitro.* In particular, 3-*O*-glutaryldihydrobetulin (**18**)[52–56] was more active in an *in vitro* assay than the anti-HIV drug zidovudin (AZT) and all other triterpenoid derivatives studied (Table 2, Scheme 2),[53] including PA-457 [3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (**15**)].[16]



	R^1	R^2	R^3
2	$CH_3CH=CH_2$	COOH	OH
15	$CH_3CH=CH_2$	COOH	$HOOC\text{-}C(CH_3)_2CH_2COO$
16	$CH_3CH=CH_2$	$HOOC\text{-}CH_2C(CH_3)_2CH_2COOCH_2$	$HOOC\text{-}CH_2C(CH_3)_2CH_2COO$
17	$CH_3CH=CH_2$	$HOOC\text{-}CH_2C(CH_3)_2COOCH_2$	$HOOC\text{-}C(CH_3)_2CH_2COO$
18	*i*Pr	CH_2OH	$HOOC(CH_2)_3COO$

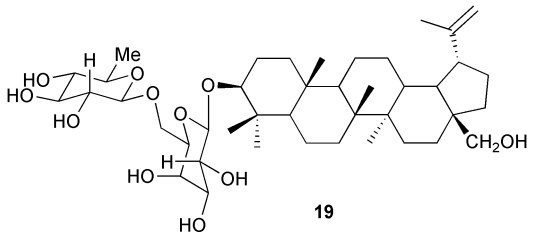
Scheme 2 Structures of the most active *in vitro* anti-HIV betulin derivatives (see Table 2).

The cytotoxicity of 3-*O*-phthalic betulin esters have been tested on tumour cell lines in MTT tests.[47] It was reported that hemiphthalic esters exhibited greater cytotoxicity than betulinic acid (**2**) or relatively inactive betulin (**1**). Betulin and other natural triterpenoids have been reported as selective catalytic inhibitors of human DNA topoisomerases with IC_{50} values in the range 10–39 μM.[57] DNA topoisomerases play important roles in replication, transcription, recombination, and chromosome segregation at mitosis.

Recio *et al.*[44] reported structural requirements for the anti-inflammatory activity of betulin and other natural triterpenoids of the lupane, oleanane, and ursane series. All triterpenoids displayed remarkable bioactivity against the oedema produced by phorbol 12-myristate 13-acetate (TPA). It was concluded that the basic hydrocarbon skeleton has no critical influence on activity, but the presence of polar anchors at C28 (hydroxylic or carboxylic) is of the highest importance.

Structural/functional properties of betulin and dihydrobetulin derivatives and their glycosides have been studied on Ehrlich tumour cells.[45] Hydrogenation of betulin and adding glucose to C3 both increased cytotoxic activity, but the presence of the two glucose residues at C3 and C28 significantly decreases anti-cancer

activity. Betulin 3-glycoside (**19**), which was isolated from the herb extract *Pulsatillae Radix*,[58] inhibits the rate of solid tumour growth in healthy male mice (S-180 cells) by 87–96% at dosages of 0.1–1.5 mg kg^{-1}.



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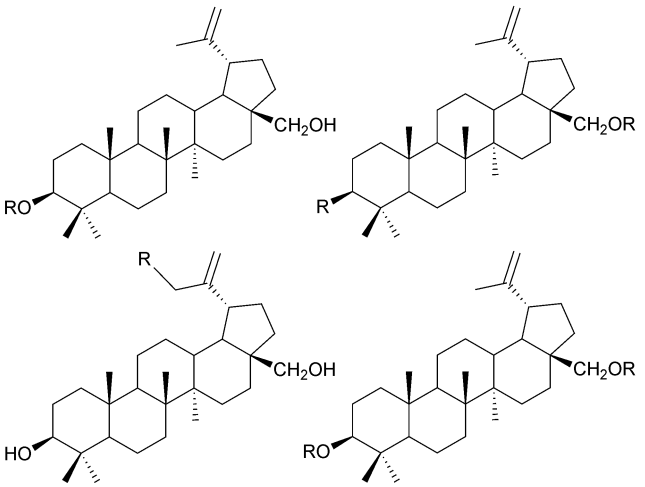
Flekhter *et al.*[59] and Baltina *et al.*[60] reported that a number of betulin 3,28-esters have a relevant hepatoprotective effect and influenza inhibition. Betulin has been modified at the C3 and C28 positions and the antiviral activity has been evaluated in *in vitro* assays. It was found that simple modifications to the parent structure of lupane triterpenoids produced agents that are effective against influenza-A and herpes simplex type-1 viruses. Betulin has also been proposed for the treatment of viral hepatitis-C.[61,62] These patents also claim that betulin has antiviral and immunomodulating properties. Hepatoprotective, anti-ulcer, anti-inflammatory, reparative, and anti-HIV activities were found for 3-*O*,28-*O*-dinicotinoylbetulin.[63] This betulin derivative also exhibits immunomodulatory activity. Herpes and Epstein–Barr virus inhibition by betulin and its derivatives has been reported by Amjad *et al.*[64,65] The anti-herpes activity of betulin and its derivatives have been a subject of numerous patents.[66–68] The anti-viral activity of some enveloped and non-enveloped viruses was reported for betulin (**1**), betulinic acid (**2**), and betulonic acid (**12**).[69] In addition to anti-viral activity, early research conducted by plant physiologists[28,70–72] indicated that either triterpene glycosides or saponins playing the major role in the self-protection of plants against fungi. In both cases the saponins themselves were not active against fungi or erythrocytes. The sugar portion of the glycoside molecule is merely the hydrophilic transporting functional group. The anti-fungal activity of these active forms was observed at concentrations $\geq$30 μg ml^{-1}. This is not a higher level of activity than commercial fungicides, but it is likely to be good enough for a plant's self-resistance against pathogenic fungi. This research hinted that betulin and its derivatives must display some level of anti-microbial activity. This activity was reported against *Fusarium oxysporum*,[73] *Staphylococcus aureus* (2–5 μg ml^{-1}),[74] the human pathogenic fungi *Microsporum canis* and *Trichophyton rubrum* (12.5 μg ml^{-1}),[75] plant fungi pathogens,[76] and other selected bacteria and fungi.[77] The anti-microbial properties of birch bark extract and betulin can be used in low-irritation cosmetics[78] and anti-pathogenic fungi cosmetics.[79] The anti-fungal use of betulin and some of its derivatives against plant pathogens have also been patented.[80–83] The potential bioactivity of triterpene glycosides led to two phases of research activity on their synthesis.[84,85] All of these efforts were limited to fundamental studies, probably because the industrial availability of betulin was limited. The methods for the synthesis of betulin glycosides are also rather complicated.[84,85] Analysis of the structural/functional properties of betulin derivatives led to the idea that the more amphiphilic characteristics that triterpenoids possess, the higher level of general bioactivity could be observed. Bioactive triterpenoids usually have a relatively polar structural fragment located at the ends of a non-polar triterpenoid nucleus. A review on a water-soluble triterpene glycoside adjuvant for vaccines supports this idea.[86] Selective inhibition of the catalytic sub-unit of rat liver cyclic AMP-dependent protein kinase (cAK) by amphiphilic triterpenoids, including betulin structures, show that it is necessary to include the lipophilic non-flexible triterpenoid fragment in the design of amphiphilic bioactives.[87] Such a notion is also supported by the recent synthesis (by Krasutsky *et al.*[88]) of betulin-based quaternary ammonium salts as bioactive cationic surfactants. The high level of anti-bacterial and anti-fungal activity of some water-soluble betulin-based quaternary salts makes them promising bioactive surfactants. Scheme 3 shows the general formulae of these betulin-based molecules.



R = $[COCHXNR^1R^2R^3]^+ Y^-$ or $[NR^1R^2R^3]^+ Y^-$
where
Y = Br, Cl
R^1, R^2, R^3 = H, Alk, Ar, *etc.*
X = H, Alk, Ar, *etc.*

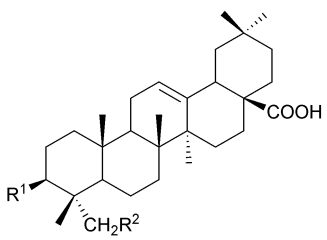
Scheme 3 Betulin-based amphiphilic water-soluble quaternary salts.

Betulin and birch bark extract are already used as a dietary supplement, Betual®, for active liver protection, prevention and treatment of acute alcoholic intoxication,[89] and as an additive to alcoholic beverages.[90] Clinical studies indicate that Betual® may reduce both alcoholic intoxication and hangover intensity.[89,90] Hepatoprotective effects of betulin and betulinic acid against ethanol-induced cytotoxicity in hepG2 cells have also been reported by Szuster-Ciesielska *et al.*[91] It is very important to note that *B. verucosa* birch bark extract (betulin 70%, betulinic acid 6% and lupeol 5%) did not display toxicity, either during clinical tests or in the three years since Betual® commercialisation.[92,93] Recently, betulin and birch bark extract have been patented as adaptogenic remedies,[94] interferon inducers,[95] antihypoxitic products,[96] hepatitis-C preventatives and treatments,[97] anti-influenza[98] and tuberculosis prophylactics,[99] and as additives in cosmetics, pet foods, lipase inhibitors, and foods containing triterpenes.[100]

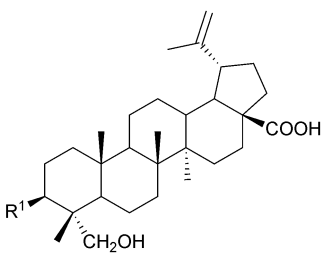
Betulin 3-caffeate (**7**) and other natural triterpene caffeates are among the less studied birch bark extract components. Ekman *et al.*[21] reported levels of compound **7** of up to 0.5% in European

B. verucosa through an indirect experimental approach. Table 1 includes direct analytical HPLC measurements for *B. papyrifera* and *B. neoalaskana*.[20] This work shows a higher content (6%+) of **7** in the N. American bark of *B. papyrifera* and *B. neoalaskana*. Kolomitsyn *et al.*[20] have reported the isolation of natural **7**, as well as a new method of its synthesis from betulin (**1**) (See Section 3.3). This work also reported the inhibition by **7** of the growth of P19, NT2/D1 and K1735-M2 cells, compared with a number of other birch bark triterpenes. Notably, caffeate **7** showed the highest level of anti-proliferative activity *in vitro* among all birch bark triterpenes, including betulinic acid (**2**).[20] This was the only explanation for the inhibitory activity of N. American birch bark extract being equivalent and higher to the level of anti-cancer activity of betulinic acid (**2**). The anti-cancer and anti-inflammatory activities of non-triterpene caffeates[101] as well of some extracts containing triterpene caffeates[102,103] has previously been reported.

The anti-HIV activity of non-triterpene caffeic esters and their immune modulation effect *in vivo* have also been reported.[104] The presence of betulin 3-caffeates also makes birch bark extract a good sun-block ingredient for cosmetics because of its good UV-absorption.[20] Thus, anti-melanoma bioactivity combined with UV-protective characteristics may lead to the creation of new cosmeceuticals from N. American birch bark extracts or betulin 3-caffeate (**7**) and other triterpene caffeates. Some birch bark extracts include oleanolic acid 3-caffeate (**23**) and 3β,23-dihydroxyolean-12-en-28-oic acid 3β-caffeate (**21**) from *Betula davurica*,[105] 3β,23-dihydroxyolean-12-en-28-oic acid 23-caffeate (**22**) and 3β,23-dihydroxylup-20(29)-en-28-oic acid 3β-caffeate (**23**) from *Betula pubescens*,[106] and betulinic acid 3-caffeate (**32**) from *Betula platyphylla* Sukatchev var. *japonica* Hara.[107]

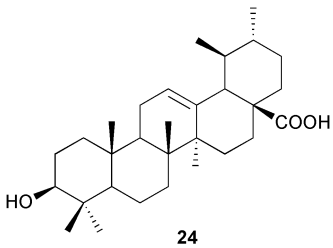


	R^1	R^2
20	3,4-$(HO)_2C_6H_3CH=CHCOO$	H
21	3,4-$(HO)_2C_6H_3CH=CHCOO$	OH
22	OH	3,4-$(HO)_2C_6H_3CH=CHCOO$



23 R^1 = 3,4-$(HO)_2C_6H_3CH=CHCOO$

Oleanolic acid (**5**),[108,109] betulinic acid (**2**),[82] and ursolic acid (**24**)[108,110] have been reported as anti-cancer, anti-inflammatory, anti-bacterial, and anti-viral bioactives previous to this review period. These findings triggered a surge of drug design activity focused on these commonly available NPs. The popularity of these compounds is reflected by the recent publication of six reviews of triterpenoid acid **2**, **5**, and **24**.[111–116] The moderate, but well recognised, level of anti-cancer bioactivity of acids **5** and **24**[108–110] stimulated drug design efforts to use these structures as a basis for the creation of highly efficient synthetic anti-cancer candidates. As a result, the design and synthesis of a highly active inhibitor of nitric oxide production in mouse macrophages, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (**25**), was achieved.[117] This synthetic oleanane triterpenoid (CDDO) has highly potent differentiating, antiproliferative, and anti-inflammatory activities,[118] and induces apoptosis of human myeloid leukaemia cells by a caspase-8-dependent mechanism.[119,120] Further development of different CDDO derivatives has led to new bioactive compounds that might be used for the prevention and treatment of certain cancers, arthritis, multiple sclerosis, Alzheimer's disease, and Parkinson's disease.[121]



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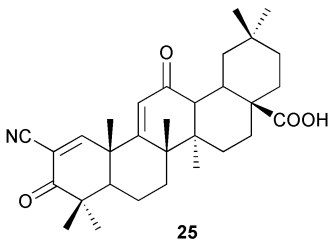

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The low content of oleanolic acid (**5**) and oleanolic acid acetate (**6**) (see Table 1) in birch bark extract does not make birch bark a good source for manufacturing these natural chemicals. However, the acidic fraction of birch bark (containing betulin 3-caffeate (**7**), betulinic acid (**2**), and oleanolic acid (**5**)), if separated, could be used as an anti-cancer composition.

It has been reported that plant extracts containing oleanolic (**5**) and betulinic (**2**) acids from *Pterocarya tonkinesis* (Franch.) Dode[122] and *Nerium oleander*,[123] and *epi*-oleanolic acid (**26**) from Korean mistletoe[124] manifest a high level of anti-carcinogenic activity.

From the experience of complementary medicine, it has been noticed that the presence of oleanoic acid (**5**) in plant extractives is often accompanied by anti-bacterial properties. Such bioactivity was reported for extractives from *Syzygium guineense* (against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*)[125] and *Lythrum salicaria* (against *Proteus mirabilis* and *Micrococcus luteus*).[126] The anti-bacterial properties of pure oleanoic acid have also been reported (*Streptococcus mutans* assay).[127] The anti-caries activity of oleanolic acid (**5**) with β-cyclodextrin has potential use as a corresponding extractive for dental care products.[128]

Oleanolic acid (**5**) has been found to be an active anti-HIV component in the following plant extractives: *Rosa woodsii*, *Prosopis glandulosa*, *Phoradendron juniperinum*, *Syzygium claviflorum*, *Hyptis capitata*, and *Ternstromia gymnanthera*.[129] Mengoni *et al.*[130] reported that oleanolic acid (**5**) inhibits the replication

of HIV-1 in all the cellular systems studied (EC_{50} range 22.7–57.4 μM). It was suggested that triterpenoid **5** inhibits HIV-1 protease activity. These results trigger the idea that oleanolic acid (**5**) may be a good basis for anti-HIV drug design. Intensive study of anti-HIV activity of oleanolic acid derivatives supports this possibility.[131–133]

The following pharmacological activities of oleanolic acid (**5**) and derivatives also merit special mention: prevention and treatment of anxiety and depression in mammals;[134] treatment of hyper-sensitivity and/or hyper-reactivity,[135] and treatment of non-insulin-dependent diabetes mellitus.[136] In addition, they have been found to promote antibody generation[137] and immunomodulatory activity,[138] function as vasodilators and restorative agents for endothelial dysfunction,[139] and exert gastroprotective effects.[140] The use of oleanoic acid (**5**) as a component of complementary and conventional medicines is common in China.[110a,141]

Oleanolic acid derivatives, such as QS-21,[34,86] derived from the bark of the S. American tree *Quillaja saponaria* (Rosaceae), have been found to be very efficient water-soluble triterpene glycoside adjuvants. QS-21 is an experimental adjuvant to vaccines (melanoma, malaria, HIV, breast cancer, prostate cancer, streptococcal pneumonia, influenza, herpes, hepatitis-B) being examined in Phase II and Phase III US-NIH trials. GP1-0100 (Saponimmune) is a derivative of *Quillaja saponaria saponine*, which was developed by Galenica Pharmaceuticals[142] as an adjuvant for vaccines.[143,144]

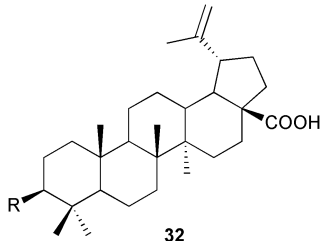
Betulinic acid (**2**) and its derivatives have been the most intensively studied group of birch bark triterpenoids during the previous decade because of the discovery of their unique anti-cancer and anti-HIV activities. Although recent reviews[111,112,145] cover the literature up to the end of 2003, it should now be worthwhile to consider betulinic acid from the perspective of advanced birch bark research and development. This is because birch bark seems to be the best source, industrially and commercially, for natural and semi-synthetic betulinic acid manufacturing (see Section 3) among all other possible and previously reported natural sources, which number more than 20.[111] Almost all natural extracts that contain betulinic acid have been historically known as complementary medicines and have been reported in fundamental studies as being active against tumours,[145,146] cancers,[147–149] inflammation,[150] bacterial pathogens,[151,152] and viruses.[153] The period of biological screening of natural extracts (the phytotherapy period) in the 1980s was transformed into a thorough fundamental study of the bioactivity of pure natural triterpenoid ingredients of extracts as possible chemotherapeutics. Yasukawa *et al.*[154] reported the relevant inhibitory effects (at 5 μM concentration) of pure betulinic acid (**2**) on TPA-induced inflammation as being roughly similar to its inhibitory activities against tumour promotion *in vitro*. The ensuing report of Pisha *et al.*[155] on betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis stimulated both fundamental resarch (into melanomas,[154,156–158] leukaemia,[159–161] brain-tumours,[162,163] human gliomas,[164] colon and prostate cancers,[165] the Ewing's sarcoma family,[166] and head and neck cancers[167]) and applied efforts (into the prevention and treatment of melanomas,[165,168–171] tumour-associated angiogenesis,[172] cancer and HIV,[173,174] liver, lung, colon, prostate, and breast cancers,[175] neuroectodermal tumours,[176] leukaemia, lymphomas, and lung, prostate and ovarian cancers.[177] The triggering of apoptotic activity by betulinic acid through a direct effect on mitochondria was reported by Fulda *et al.*[156,162] Galton *et al.*[178,179] reported that betulinic acid is an apoptosis inducer in skin cancer cells and causes differentiation in normal human keratinocytes. This research supports the application of betulinic acid not only for drugs but also for cosmetics. Cosmetics developers believe that betulinic acid (at 50–500 mg per gram of cosmetic) may prevent and help to treat UV-induced skin cancer,[171] reduce signs of cellulite and stimulate collagen synthesis for skin-care products,[180] prevent sunlight-caused signs of aging, wrinkles, and blotches,[181] and improve skin homogeneity and pigmentations.[182] Clinical tests of betulinic acid as a treatment for melanoma began in 1999.[17] Hata *et al.*[183] studied cytotoxicities of 11 lupane group triterpenoids (Table 3) against three human leukaemia cell lines, two melanoma cell lines, two neuroblastoma cell lines, and normal fibroblast cells. It was reported that only lupane triterpenes with a C28 carbonyl group (**2**, **3**, **10**, **29**, **30**) exhibited an inhibitory effect for cancer cell growth, in the concentration range 0.48–11.1 μM. The most active triterpenoids, betulinic acid (**2**), betulinic aldehyde (**3**) and betulonic aldehyde (**10**) markedly inhibited eukaryotic human topoisomerase-I at an IC_{50} level of ~5 μM.

Chowdhury *et al.*[184] reported that betulinic acid and its derivatives inhibit the catalytic activity of rat liver DNA topoisomerase-I in a dose-dependent manner with an efficacy as good as camptothecin. In a manner different from camptothecin, betulinic acid (**2**) and its derivatives interact directly with the enzyme

Table 3 IC_{50} values (μM) of lupane triterpenes against human cancer cell growth[183]

	Leukaemia			Melanoma		Neuroblastoma		Normal cells
Compound	HL60	U937	K562	G361	SK-MEL-28	GOTO	NB-1	WI38
Lupeol (**4**)	19.9	16.8	>20	>20	>20	>20	19.7	>20
Lupenone (**11**)	15.8	11.9	18.2	>20	>20	>20	>20	>20
Lupeol 3-acetate (**27**)	>20	>20	>20	>20	>20	>20	>20	>20
Betulin (**1**)	14.7	14.4	14.5	12.4	16.2	17.1	16.5	15.2
Betulone (**9**)	18.9	16.8	18.7	10.6	>20	>20	>20	16.4
Betulin 3,28-diacetate (**28**)	19.2	>20	>20	>20	>20	>20	>20	>20
Betulinic aldehyde (**3**)	1.1	3.7	4.9	9.6	10.6	7.5	8.8	18.5
Betulonic aldehyde (**10**)	0.48	1.5	1.8	9.4	9.3	5.2	5.8	17.3
Betulinic acid (**2**)	6.6	10.0	9.8	5.2	6.5	7.9	9.5	>20
Methyl betulinate (**29**)	10.8	11.1	8.8	8.7	4.8	6.8	6.3	19.3
Methyl betulonate (**30**)	7.8	8.8	10.9	8.5	7.4	9.4	9.6	>20

and inhibit the formation of the topoisomerase-I complex with the DNA. Notably, dihydrobetulinic acid (**31**) inhibits enzyme activity at a concentration of 1 μM, which is ten times more efficient than the activity of betulinic acid (**2**) at a concentration of 10 μM. Many efforts have been directed at the design of more efficient anti-cancer betulinic acid derivatives.[185–196] Shentsova *et al.*[185] reported that adding glucose to the C3-position of betulinic acid and dihydrobetulinic acid increases the cytotoxic activity. Symon *et al.*[186] revealed the high cytotoxicity of a betulinic acid cyclopropane derivative against human melanomas of the Colo 38 and Bro lines, and a human ovarian carcinoma of the CaOv line (IC_{50} 10 μM). It was discovered that the hemiphthalic ester of betulinic acid is more active than betulinic acid (**2**).[187] The activity of betulinic acid amides has been demonstrated against melanoma (at 0.25 μg ml^{-1}) and liposarcoma (at 0.3 μg ml^{-1}).[189] A number of betulinic acid derivatives (3-*O*-acyl, 3-hydrazine, 2-bromo, and 20,29-dibromo) have shown IC_{50} values <1 μg ml^{-1} on human cancer cell lines MOLT-4, JurkatE6.1, CEM.CM3, BRISTOL8, U937, DU145, PA-1, A549, and L132.[190] Ring A seco derivatives of betulinic acid manifested significant cytotoxic activity against the T-lymphoblastic leukaemia cell line CEM (4–6 μM).[191] Sarek *et al.*[197,198] and Urban *et al.*[191,199] reported broad efforts towards synthesis of ring A seco and E seco betulinines with cytotoxic pro-apoptotic activity on a wide diversity of cancer cells. Research into the structure–activity relationships for betulinic acid derivatives are underway, but natural betulinic acid (**2**) still remains a fairly plausible anti-cancer chemopreventive and chemotherapeutic candidate. This is due to its low toxicity, which has been accepted through its long-term use in complementary medicinal history, as well as its favourable therapeutic index, even at doses up to 500 mg kg^{-1} body weight.[200] Systemic side effects are not observed for betulinic acid at any dose.[145] However, the low solubility of betulinic acid in water and high hydrophobicity (log *P*)[201] does not portend good delivery of this chemical to targets. Therefore, research into the pharmacokinetics of betulinic acid,[145,200] creation of new pro-drugs[174,202,203] and formulation[204] seems appropriate. The efficient combined treatment and synergistic cytotoxicity of different chemotherapeutics with betulinic acid (**2**) have been observed[204] and claimed by patent.[175] Betulinic acid (**2**) and its derivatives induce growth inhibition in proliferative diseases other than cancer, such as inflammation.[44,205–207] Bernard *et al.*[208] suggested that betulinic acid from plant extracts is responsible for this activity by binding and inhibiting phospholipase A_2, with a binding energy of -90 kcal mol^{-1}. These ideas have been supported by other studies.[209,210] Among natural anti-inflammatory derivatives of betulinic acid, betulinic acid 3-caffeate (**32**, or pyracrenic acid), is worth special mention. All plant extracts which contain these triterpenoids, as well as oleanolic acid 3-caffeate (**33**) manifest marked anti-inflammatory properties.



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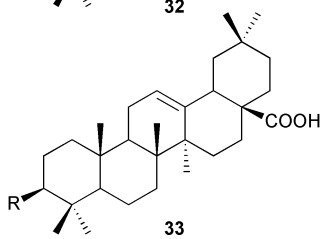


33

R = 3,4-$(HO)_2C_6H_3CH{=}CHCOO$

Caffeate **32** has been found in the bark of *Betula platyphylla* Sukatchev var. *japonica* Hara,[107] and **33** in the bark of *B. ermanii*,[211] *B. maximowicziana*,[212] *B. davuric*,[213] and *B. pubescens*.[214]

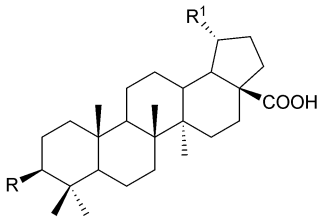
The anti-HIV activity of betulinic acid and its derivatives has been reported independently by Fujioka *et al.*[215] and Mayaux *et al.*[216] It was also reported independently that some plant extracts that contained betulinic acid or related triterpenoid acids showed anti-HIV activity.[217,218] This suggests that all triterpenoid structural analogues of betulinic acid (ursolic acid, oleanolic acid, platanic acid, moronic acid, *etc.*) have potential as anti-HIV chemotherapeutics. These NPs (betulinic acid (**2**),[215,219] oleanolic acid (**5**),[220] ursolic acid (**25**),[220] dihydrobetulinic acid (**31**)[215,219]) inhibit HIV-1 replication in acutely infected H9 cells and inhibit H9 cell growth at approximately the same level of bioactivity (Table 2 and Table 4). This level of bioactivity and toxicity was improved by studying the structure–activity relationship for betulinic acid derivatives.[219] It was observed that derivatives with C3 acyl groups are more active, especially if they have dimethyl groups in the C3′ position (see Table 2 and Scheme 4).

The anti-HIV parameter EC_{50} for betulinic acid (see Table 3) was improved ~4000-fold for betulinic acid derivative 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (**15**) or DBS (PA-457)[16]. At the

Table 4 Anti-HIV activities for triterpenoid acids[215,220] and their derivatives[219] **8–11** and AZT

Compound	EC_{50}/μM	IC_{50}/μM	Therapeutic index
Betulinic acid (**2**)	1.4	12.9	9.3
Oleanolic acid (**5**)[a]	3.7	47	12.7
Ursolic acid (**25**)[a]	4.3	14.2	3.3
Dihydrobetulinic acid (**31**)	0.9	12.6	14
3-*O*-(3′,3′-Dimethylsuccinyl)betulinic acid (**15**)	$<3.5 \times 10^{-4}$	7	>20 000
3-*O*-(3′,3′-Dimethylsuccinyl)dihydrobetulinic acid (**34**)	$<3.5 \times 10^{-4}$	4.9	>14 000
3-*O*-(3′,3′-Dimethylglutaryl)betulinic acid (**35**)	2.3×10^{-3}	4.5	1974
3-*O*-(3′,3′-Dimethylglutaryl)dihydrobetulinic acid (**36**)	5.7×10^{-3}	5.8	1017
AZT	0.15	1875	12 500

[a] IC_{50} and EC_{50} data in μg ml^{-1} (from ref. 220) were recalculated to μM ml^{-1}.

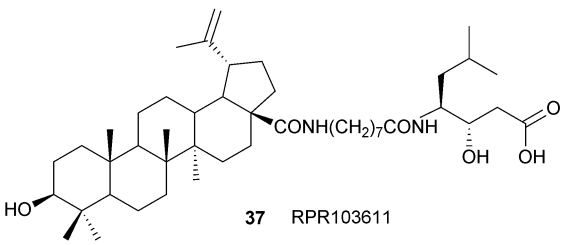


	R	R^1
15	$OCOCH_2C(CH_3)_2COOH$	$CH_3CH{=}CH_2$
34	$OCOCH_2C(CH_3)_2COOH$	$(CH_3)_2CH$
35	$OCOCH_2C(CH_3)_2CH_2COOH$	$CH_3CH{=}CH_2$
36	$COCH_2C(CH_3)_2CH_2COOH$	$(CH_3)_2CH$

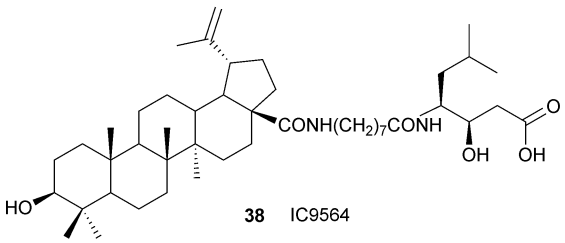
Scheme 4 Structures of the most active anti-HIV betulinic acid derivatives.

same time, the toxicity parameter IC_{50} was increased by only a factor of two. Thus, the average improvement in the therapeutic index is ~2000-fold.

Similar levels of improvements in the *in vitro* bioactivity through modification of triterpenes were also successfully shown with derivatives of betulin,[52–55] moronic acid,[221] oleanolic acid,[222] and ursolic acid.[223] It was shown that minor, but specific, changes in structures may lead to changes in activity. For example, dihydrobetulinic acid (**31**) is slightly more potent against HIV than betulinic acid (**2**);[215] 3α-epimers are less potent than 3β-triterpenoid acids;[222,224,225] 3-oxo derivatives, 3-amines and 3-ethers are less active than 3β-hydroxy derivatives;[216,224,226,227] and dehydration at C3 leads to non-active compounds.[224] Derivatisation of the C30 position also leads to less active compounds. Research on a series of betulinic acid amides[216,224,226,227] revealed structures (**37**, RPR103611) and (**38**, IC9564) which were active against HIV *in vitro* at a submicromolar level in a wide range of cell cultures (Scheme 5).



37 RPR103611



38 IC9564

Scheme 5 Structures of the most active anti-HIV betulinic acid amides – (3*S*,4*S*)-*N′*-(*N*-(3β-hydroxylup-20(29)-ene-28-oyl)-8-aminooctanoyl)-4-amino-3-hydroxy-6-methylheptanoic acid (**37**, RPR 103611) and (3*R*,4*S*)-*N′*-(*N*-(3β-hydroxylup-20(29)-ene-28-oyl)-8-aminooctanoyl)-4-amino-3-hydroxy-6-methylheptanoic acid (**38**, IC9564).

The mechanism of anti-HIV action is a very important factor for the introduction of anti-retroviral therapy and the prevention of disease development. An anti-HIV drug must be highly active against "wild" and mutant HIV, because resistance to new drugs can sometimes develop within days of treatment.

Of 20 anti-HIV drugs approved for use in the US, 11 are RT (reverse transcriptase) inhibitors (*e.g.*, Zidovudine, Azidothymidine, and AZT[227]), eight are PR (protease) inhibitors (*e.g.* Abacavir[228]), and one is a viral fusion inhibitor (Fuseon[229]).[230] Betulinic acid and other natural triterpenoids do manifest a moderate inhibitory effect on HIV-1 reverse transcriptase,[231–233] as well as on HIV-1 protease.[234–236] Akihisa *et al.*[232] reported the inhibitory effect of 55 triterpenoids, including birch bark lupane and oleanane groups, on a purified HIV-1 reverse transcriptase. The best inhibitory effect has been found for betulin 3,28-diacetate (1.3 μM), lupenone (**11**) (2.1 μM) and betulonic aldehyde (**10**) (3.4 μM). The inhibition activity of betulinic acid (**2**) was 7.9 μM. Quere *et al.*[236] provided a computational analysis for betulinic acid and other triterpenoids as potential dimerisation inhibitors of HIV-1 protease. This theoretical work was supported by experimental observations for natural triterpenoids.[235,237] Mayaux *et al.*[216] and Soler *et al.*[227] reported that betulinic acid amide (**37**, RPR103611) did not inhibit the *in vitro* activity of HIV-1 protease, reverse transcriptase and integrase, or the binding of gp120/CD4. These derivatives appeared to stop entry of HIV-1 at a post-binding, envelope-dependent virus–cell fusion process. Holz-Smith *et al.*[238] conducted tests of analogues of compound (**37**, RPR103611) betulinic acid derivative (**38**, IC9564) (Scheme 5). Results from a syncytium formation assay indicated that IC9564 blocked HIV type 1 (HIV-1) envelope-mediated membrane fusion. This research suggested that HIV-1 gp120 plays a key role in the anti-HIV-1 activity of IC9564. Sun *et al.*[226] reported that among a series of IC9564 derivatives the L-leucine derivative (EC_{50} 0.46 μM) is equally as promising as compound **38** itself (EC_{50} 0.33 μM) against HIV infection. The structure–activity relationship data also indicated that a double bond in IC9564 can be eliminated. Yuan *et al.*[239] confirmed that the HIV-1 envelope glycoprotein gp120 is the key determinant for the anti-HIV-1 entry activity of IC9564. To date, very few fusion inhibitors have been described.[230,240]

Another new mechanism for anti-HIV action was revealed for betulin and acylated derivatives of betulinic acid (see compounds from Tables 2 and 4). The most promising anti-HIV drug candidate DSB (3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid, **15**) was synthesised by Kashiwada *et al.*[219] It was shown that neither HIV-RT inhibition (in a concentration range 167–219 μM, $IC_{50} = 18$ μM), nor inhibitory activity against HIV-induced membrane fusion (in a concentration range 33–70 μM) could explain such a high level of HIV inhibition ($EC_{50} < 3.5 \times 10^{-4}$ μM). Kanamoto *et al.*[241] reported an unusual mechanism for such anti-HIV activity. In a p24 immunosorbent assay of culture supernatants, DBS inhibited virus expression 18 hours after infection. This suggests that DBS affects virion assembly step and/or budding of virions.[241] Further study of this mechanism by Li *et al.*[242] demonstrated that DBS (PA-457)[16] disrupts a late step in Gag processing. This blocks the conversion of the capsid protein (p25) to a mature capsid protein (p24). It has been shown that *in vitro* mutations of the DSB-resistant virus map to the p25 to p24 cleavage site. The resulting virions from DBS-treated cultures were non-infectious. Thus, the mechanism of the anti-HIV action of DBS (PA-457)[16] and other acylated triterpenoids suggests new drug targets for

AIDS suppression. This new class of HIV bioactives are termed maturation inhibitors.

It seems that there is no connection between this described mechanism and the mechanism of the HIV budding process.[243,244] The budding and maturation processes are the last events in the HIV infection cycle. During these events, the HIV-1 assembly forms enveloped particles in a cell membrane that will bud from the cell. The Gag protein is incapable of breaking a cell's membrane, and therefore, through its p6 domain, the Gag protein uses cellular proteins Tsg101 for membrane cleavage. Triterpenoid molecules, like **15–18** (see Table 2) or **15**, **34–36** (see Table 4), could interfere with the interaction between Tsg 101 and p6, and ubiquitin, through a tetrapeptide motif (PTAP) within the p6 domain. However, it has been reported that DBS (PA-457) did not disrupt the Gag–Tsg101 interaction.[242] Nevertheless, this target still seems very attractive for drug design on the basis of triterpenoid structures for HIV therapies.

Huang *et al.*[245] combined the idea of complex modification of betulinic acid at C3 (for inhibiting HIV-1 maturation) and at C28 (for blocking HIV-1 entry). As a result the most potent compound ([(*N*-[3β-*O*-(3′,3′-dimethylsuccinyl)lup-20(29)-en-28-oyl]-7-aminoheptyl)carbamoyl]methane) inhibited HIV-1 at an EC_{50} of 0.0026 μM and was at least 20 times more efficient than either the anti-maturation lead compound **15** (DSB, PA-457) or the anti-entry lead compound **38** (IC9564). This bifunctional betulinic acid derivative is active against both HIV entry and maturation.

The anti-HIV activity of triterpenoids can be summarised thus: a) natural triterpenoids, especially of the lupane and oleanane groups, function as moderately active anti-HIV agents at micromolar concentrations through inhibition of HIV reverse transcriptase and protease and/or inhibiting the maturation process; b) specific derivatisation of triterpenoids (C3 or C3,C28-acylation or C17–COOH amidation) lead to significant increases in anti-HIV activity to submicromolar concentrations and encompass new types of virus inhibitory mechanisms; c) acylation of betulin, betulinic acid, oleanolic acid, ursolic acid, moronic acid and platanic acid leads to compounds blocking viral maturation at nanomolar concentrations (maturation inhibitors); d) C17–COOH amidation of betulinic acid leads to compounds that block entry of HIV into cells (fusion inhibitors); and e) polyfunctionalisation of triterpenoid molecules with certain pharmacophoric groups could lead to the design of anti-HIV bioactives with combined mechanisms of action (inhibiting maturation, blocking virus entry, fusion inhibition, *etc.*). Fundamental research into the anti-HIV action of betulinic acid and its derivatives has resulted in an intensive patenting process in this direction.[174,246–253] Other than anti-cancer or anti-HIV bioactivity the following directions of potential betulinic acid use should be mentioned: food additives to control obesity,[254] immunomodulatory activity,[255–257] anti-malarial activity,[258] anti-aging cosmetics,[259] anti-wrinkle cosmetics,[260] and anthelmintic activity.[261]

Lupeol (**4**) is a well documented fruit-, vegetable-, and bark-based NP found in olives, figs, mangoes, and other fruits and medicinal herbs.[46,262,263] It is also the most lipophilic triterpenoid component from outer birch bark extract. Lupeol (**4**) and its derivatives have been found as the principal active ingredient in the following folk medicine plants: *Pimenta racemosa* var. *ozua* (Myrtaceae),[264] *Alstonia boonei* root bark,[265] *Crateva nurvala* (Hindi: *Varuna*),[266] the leaves of *Ixora coccinea* L.,[267] *C. religiosa* bark,[268] *Dendropanax* sf. *querceti*,[269] the leaves of *Teclea nobilis*,[270] the bark of *Bombax ceiba*,[271] the roots of *Strobilanthus callosus* and *Strobilanthus ixiocephala*,[272] *Vernonia scorpioides* (Asteraceae),[273] *Lactuca indica*,[274] *Holarrhena floribunda*,[275] and the birch bark extractive of almost all *Betula* species.[6–14,18] The spectrum of lupeol (**4**) bioactivity is rather broad, but different from the above-reviewed birch bark triterpenoids. Anti-proliferative (anti-inflammatory and anti-arthritis) activity for lupeol (**4**) is reported more frequently than for betulin (**1**) or betulinic acid (**2**). Fernandez *et al.*[264] reported that the extract of *Pimenta racemosa* var. *osua* containing lupeol (**4**) has a high level of activity against two experimental models of acute inflammation (paw oedema in rats and ear oedema in mice). The reduction of myeloperoxidase activity suggested that the mechanism is likely related to the neutrophil migration. A patent application[276] has claimed lupeol and its fatty acid esters to be useful anti-inflammatory and anti-arthritis agents. Kweifio-Okai *et al.*[265] reported the anti-arthritic effect of lupeol acetate. Isolated from *Alstonia boonei*, this NP was studied for its anti-arthrititic effect in CFA-induced arthritic rats. Oral treatment resulted in an increase in spleen weight and the reduction in serum alkaline phosphatase return to non-arthritic control values. Anti-arthritic mechanisms of lupeol derivatives[277] were studied with tests on the release of collagenase by rat osteosarcoma cells, the release of five lipoxygenase inflammatory products by human neutrophils, and on CCl_4-induced hepatotoxicity in rats. These tests explained the relative anti-arthritic action of triterpenoids (lupeol 3-linoleate > lupeol 3-palmitate > lupeol). The triterpenoids studied equally reduced LDL release and accelerated hepatic cell regeneration. Significant anti-inflammatory and anti-arthritis effects were revealed for lupeol and 19α*H*-lupeol isolated from *Strobilanthus callosus* and *Strobilanthus ixiocephala* roots.[272] Singh *et al.*[268] reported that lupeol had a significant dose-dependent effect on an acute and chronic inflammatory processes ($LD_{50} > 2$ g kg^{-1} in rats), but did not show any analgesic or anti-pyretic properties. A similar result was reported by Geetha *et al.*[278] on lupeol and lupeol 3-linoleate anti-inflammatory activity in comparison with the non-steroidal drug Indomethacin. Latha *et al.*[279] have reported the bioactivity of lupeol 3-eicosapentaenoate against adjuvant-induced arthritis in rats. The activation of glycoproteins and lysosomal enzymes and related inhibition of collagen in arthritic animals were significantly changed, nearly reaching the control level. A review on the inflammatory activity of plants and plant extracts, listing the principal chemical ingredients that cause this bioactivity, was published in 2003.[280] Lupeol is included in the list of bioactives responsible for the potency of these plant extracts.

Lupeol (**4**) exhibits moderate but specific anti-cancer activity against androgen-sensitive prostate cancer cells,[281] B16 2F2 melanoma cells (inhibition of the migration of malignant melanoma cells by disassembling the actin cytosleleton),[282,283] and pancreatic adenocarcinoma cells (inhibition of the Ras signaling pathway),[284] and possesses anti-tumour-promoting effects in a mouse skin tumourigenesis model (modulates NF-κB and PI3 K/Akt pathways and inhibits skin cancer in CD-1 mice),[285] and is cytoprotective against free radical toxicity.[266] The potential use of lupeol as a preventive anti-cancer component of dietary supplements is an important aspect of the above-referenced studies. Lupeol (**4**) and its derivatives have been suggested to be of use for the prevention and treatment of skin disorders,

skin cancer, prostate cancer and pancreatic cancer.[286] The nearest NP derivative to lupeol (**4**), lupenone (**11**), exhibits a fairly high inhibitory effect on a purified HIV-1 reverse transcriptase ($IC_{50} = 2.1\ \mu M$).[232]

Lupeol (**4**) has also been reported as an anti-oxaluric and anti-calciuric NP in several studies.[266,287–289] The anti-urolithiatic activity of lupeol was assessed in rats by observing the weight of stones, by biochemical analysis of serum and urine, and by histopathology of the bladder and kidney. Lupeol prevented the formation of vesical calculi and reduced the size of preformed stones.[266] Malini *et al.*[288,289] studied the effect of lupeol (**4**) on urinary enzymes in hyperoxaluric rats. Lupeol treatment (25 mg kg^{-1} body-weight day^{-1}) significantly reduced the renal excretion of oxalate. Renal tubular damage was also reduced, as made evident by the decreased level of the urinary marker enzymes. Lactate dehydrogenase, inorganic pyrophosphatase, alkaline phosphatase, γ-glutamyl transferase, β-glucuronidase and *N*-acetyl-β-D-glucosaminidase were found to be elevated. This process lowers the stone-forming constituents in the kidney. The protective effect of triterpenoids on calcium oxalate crystal-inducing peroxidative changes in experimental urolithiasis was studied by Malini *et al.*[289] Lupeol (**4**) and betulin (**1**) have been found to be efficient at reducing the risk of stone formation in animals through preventing crystal-induced tissue damage and dilution of urinary stone-forming constituents. It is believed[288,289] that the mechanism of this activity may involve the inhibition of calcium oxalate crystal aggregation and enhancement of the animal's defence systems.

Anti-hypercholesterolemia action may be another potential use of lupeol (**4**). Sudhahar *et al.*[290] reported the role of lupeol and lupeol linoleate on lipemic-oxidative stress in experimental hypercholesterolemia. The oxidative tissue damage in hypercholesterolemic rats was manifested through elevation of the cardiac marker, serum CPK, and a decline in its action in the heart. Lupeol (**4**) and lupeol linoleate treatment reduces the LPO levels and increases enzymic and non-enzymic antioxidants. These observations emphasise the positive effects of lupeol and its linoleate derivative for reducing the lipidemic-oxidative abnormalities in the early stage of hypercholesterolemic atherosclerosis. The anti-hypercholesterolemic action of betulinic acid[291] and other triterpenoids[292] have been reported and claimed for use in food and beverages for vascular disorders or diseases.

The widespread availability of lupeol (**4**) in natural sources that have been broadly used as human food products throughout history makes this NP especially promising as an additive to food and cosmetics (*e.g.* shea butter,[263] stimulation of the synthesis of stress proteins,[293] compositions that promote melanin formation,[294] melanogenesis regulators for hair care products,[295] low-irritation cosmetics,[296] and cosmetics containing lupeol that prevent skin aging[297]).

Section 2 can be summarised as follows: in recent studies the extractives of outer birch bark and birch bark triterpenoids, as well as separated pure NPs, have shown a remarkably broad range of positive biological activities against the most dangerous human viral, bacterial and proliferative pathogens. The historically and scientifically understood low toxicity of these NPs gives them high potential in drug design. Birch bark extract, with its natural complement of triterpenoids, has still not exhausted its potential in dietary supplements or cosmetics.

3 Birch bark processing

Processing birch bark into the variety of NPs reviewed here has never been achieved on an industrial scale. At the same time, the wood processing industry (paper mills, veneer mills, and lumber mills) can be considered to be the best high-volume source of birch bark. This is probably because currently only low volumes of birch bark extracts are used as ingredients for cosmetics, shampoos,[15] and rarely as dietary supplements.[89,90,92,93] For these uses, it is sufficient to have low-volume sources of birch bark and pilot-scale extraction equipment, and to follow the extraction methods in the literature,[8,9,298] as a substantial market for pure birch bark triterpenoids has not yet been developed. Their uses are still rather limited, and do not require high-scale manufacturing or serious R & D support. The market for triterpenoids (betulin, betulinic acid, lupeol, oleanolic acid) as fine chemicals[299] is usually satisfied by small-scale chemical extractive laboratories (0.1–10 kg per year). At the same time, the necessity to provide research and development on processing birch bark into the corresponding NPs is growing in parallel with the growing potential for use of these products. Satisfying the use/needs for specific industrial R & D efforts is proceeding in the following directions: 1) Refining and formulation of outer birch bark to an appropriate standardised level from raw material after debarking; 2) Optimisation of the technology of processing outer birch bark into NPs; 3) Development of industrially viable ways of synthesising potentially interesting products. The Chemical Extractive Program of the University of Minnesota (US) and the Laboratory of Chemical Extractives (UMD, NRRI) is currently accomplishing this mission in cooperation with an industrial partner, NaturNorth Technologies, LLC (US).[300]

3.1 Refining and formulation of outer birch bark

The average paper mill or veneer plant that uses boreal birch wood (Canada, US – commercial birch tree *Betula papyrifera*; Finland, Russia (Karelia, Siberia) and China – *B. pendula* and *B. pubescens*) produces ~40 tons of crude birch bark (outer birch bark ~15%) daily, which represents ~12% of birch wood biomass.[7] Unfortunately, the only current high-scale usage of this bark is as a cheap fuel ($5.0–7.0 ton^{-1}, 7–11 MJ kg^{-1}) used by the manufacturers to save energy.[301] This means that average plant burns ~6 tons of outer birch bark daily, including the following major birch bark NPs: ~5 tons of betulin (**1**), ~250 kg of betulinic acid (**2**) and ~470 kg of lupeol (**4**) + minor components (see Table 1, data on *B. pendula* extract and Scheme 1). Theoretically, this also means that from an average manufacturer it would be possible to produce annually ~1800 tons of betulin (**1**), ~75 tons of betulinic acid (**1**) and ~150 tons of lupeol (**4**). These quantities of triterpenes exceed the current demands of the drug or cosmetics industry, but could satisfy the requirements for biocides, fungicides, insecticides, emulsifiers, adhesives, washing materials, shampoos, *etc.* These quantities of triterpenoids would also exceed demand by as yet undeveloped markets. Essentially, natural birch bark sources are virtually unlimited, and any prospective market could be satisfied. It is clear, however, that yields of triterpenoids produced from birch bark depend very much on the industrial R & D of outer birch bark extract processing (see Section 3.2). The crude birch bark resulting from birch wood debarking

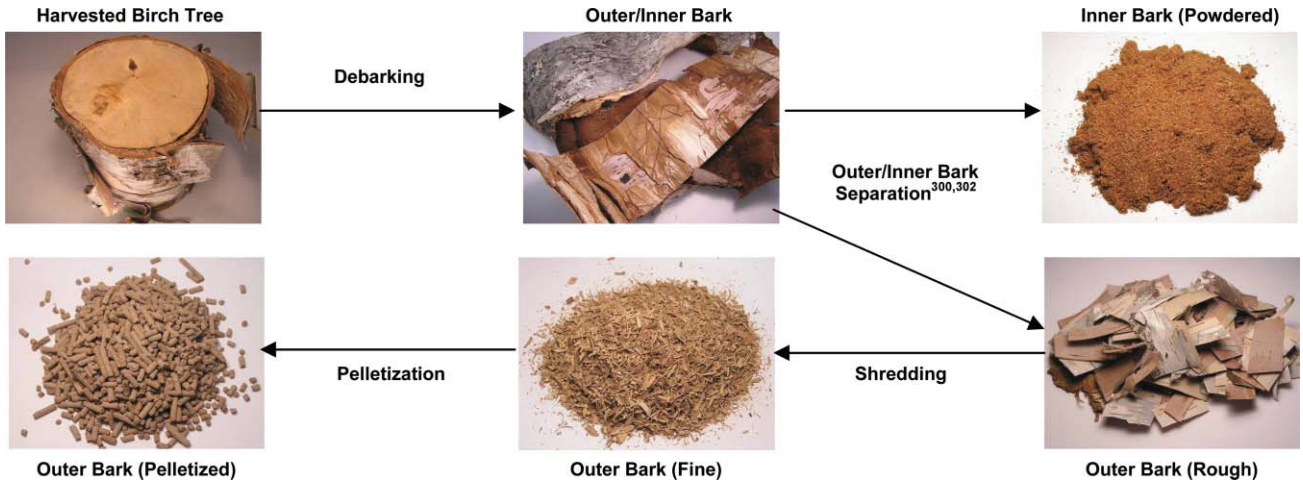


Fig. 1 Processing inner and outer birch bark.

(see Fig. 1) is not an appropriate natural material for high-scale outer birch bark processing, for two main reasons: 1) Outer birch bark must be separated from inner birch bark, woody material and soil. A specially designed process of bark shredding and screening gives outer birch bark of good quality[302]; 2) The low bulk density of outer birch bark (~0.1 g ml^{-1}) makes this raw material expensive to ship and inefficient for extraction. The process of outer birch bark extrusion designed by Krasutsky *et al.*[300,302] produces birch bark pellets with an appropriate bulk density (0.5–0.7 g ml^{-1}) for transportation and easy loading into an extraction apparatus. The world's first facility for manufacturing outer birch bark pellets is being launched in Two Harbors, Minnesota[303–305] in accordance with patented technology.[302] This could be considered to be the beginning of an industrial period of birch bark processing.

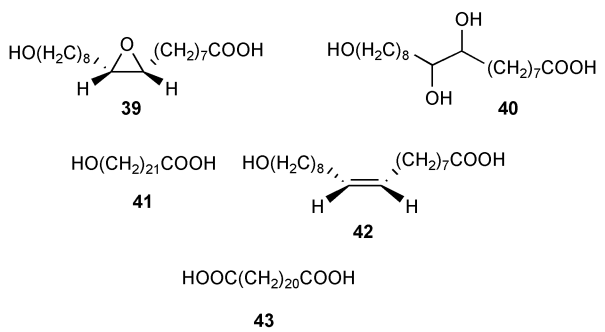
3.2 Manufacturing of birch bark NPs

This review will not discuss the numerous studies on solvents and methods of birch bark extraction conducted prior to 1994; a broad analysis of that period has been done by Kislitsyn.[8] All previous efforts were focused mainly on different methods for manufacturing birch bark extract. The use of different (polar and non-polar) solvents and methods depends very much on the market and customers' demands.

At this point it is useful to formulate current goals for industrial processing, which are somewhat different from just birch bark extract manufacturing. Pure birch bark triterpenoids obviously have higher value as active ingredients or precursors for drugs, special health care cosmetics, and dietary supplements. In accordance with this imperative, it is easy to identify commercially viable NPs from industrially available sources of outer birch bark (see Table 1). Three major triterpenoids, betulin (**1**), betulinic acid (**2**) and lupeol (**4**), seem to be the most plausible targets for research and technology development, but it is necessary to find the most appropriate solvent that could fulfil the multiple roles of a solvent for extraction and for the separation of these major components. However, recent research in this direction has been focused mostly on the separation of one product (betulin). Kuznetsov *et al.*[306–308] have found that the process of betulin extraction and separation can be improved by hot steam activation of birch bark. The preliminary activation of birch bark by an auto-hydrolysis method increased the yield of betulin and suberin by 25–40% compared to conventional extraction procedures. In order to intensify the extraction process and to increase the betulin yield, it was suggested to use short-time activation of bark by superheated steam in the presence of NaOH.[309] Kuznetsov *et al.*[310] improved betulin production by using acoustic pulses with alkali hydrolysis and extraction of wood-processing waste products. It was also shown that treatment of plant material with ultrasound improves the process of triterpene dissolution and extraction.[311] Pakdel *et al.*[312] reported a method for the separation of betulin from the outer bark of *B. papyrifera* by sublimation in a batch vacuum pyrolysis reactor. This process, which was studied in the temperature range 250–300 °C and under a total pressure of 0.7 kPa, gave a yield of betulin of 9.5% on the basis of the anhydrous bark used. A vacuum and atmospheric sublimation technique was also proposed by Guidoin *et al.*[313] Roshchin *et al.*[314] claimed a method of preparing betulin from the bark of *B. pendula* by extraction with petroleum ether. The yield of the extract, which had a 90–95% content of betulin (**1**), was 16–25% of absolutely dry outer birch bark. Polar solvents have also been used for the extraction of betulin.[315] Zhang *et al.*[316] reported extraction of betulin (**1**) from the bark of *B. platyphylla* by extraction with supercritical CO_2. Levdanskii *et al.*[317] proposed a rather complicated sequence of solvents (hexane, ethyl acetate, isopropyl alcohol, and water) for birch bark extraction. A claim was made on a process for obtaining highly pure crystalline betulin by extraction from birch bark with a high-boiling, water-immiscible solvent.[318,319] According to this method, the water-immiscible solvent extract is dissolved washed with dilute aqueous base, and the aqueous phase separated off. The yield of pure betulin (essentially free of betulinic acid) by this method did not exceed 4% with respect to crude birch bark. The extraction of lupeol or betulinic acid was not proposed by this method. Another invention[320] claims the separation of betulin and lupeol by boiling with hexane the product obtained by extraction of birch bark with methyl *tert*-butyl ether and treatment with alkali solution. After removing the solvent, the hexane-soluble lupeol is recrystallised once from ethyl acetate. The hexane-insoluble residue is practically pure (95%) betulin.

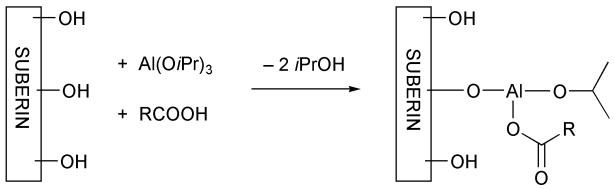
Methods invented by Krasutsky *et al.*[321–327] have described the separation and purification of all three major birch bark triterpenoids (betulin (**1**), betulinic acid (**2**), and lupeol (**4**)). A process of selective extraction was claimed with supercritical CO_2 (at a pressure between 3000 psi and 10 000 psi and a temperature between about 50 °C and 100 °C) to provide lupeol (**4**), betulin (**1**), and betulinic acid (**2**). These inventions also describe a method for the hydrolysis and separation of the birch bark suberinic acids 9*R*,10*S*-epoxy-18-hydroxyoctadecanoic acid (**39**), and *threo*-9,10,18-trihydroxyoctadecanoic acid/or phloionolic acid (**40**). The key benefit of these procedures is that the mild conditions of hydrolysis and separation allow the preservation of the oxirane ring in ω-hydroxy acid **39**.

Major suberinic acids that can be separated from birch bark are presented in Scheme 6. There are no principal technical limits for the industrial separation of these very important birch bark chemicals. The total yield of these acids from the bark of *B. paperifera* is 25–30%, which means that an average facility using boreal birch wood could produce 1.5–2.0 tons of suberinic acids daily. Comparable amounts of suberinic acids in the bark of *B. verrucosa* were previously reported by Ekman.[9b] Natural ω-hydroxy fatty acids (C_{18}-cutin monomers) are very interesting NPs for their possible use as plant protectants (by inducing plant resistance),[328,329] for the selective synthesis of the *E* and *Z* isomers of ambrettolide,[330] as precursors of skin-protecting ceramides,[331,332] as anti-cancer agents and perfumes, for their use in consumables containing ω-hydroxy fatty acids,[333] and for film-forming materials and polyesters.[334–336] The high application potential of birch bark suberinic fatty acids seems still undervalued by industry and the marketplace. The selective process for extracting acidic and non-acidic NPs from plants has been claimed by patent.[337] The main idea of this invention (by Krasutsky *et al.*) is the binding of acidic components of plant tissue (birch bark) by treatment with aluminum alcoholates (specifically with aluminum isopropoxide) or another basic reagent. Consequently, any traces of fatty acids or betulinic acid will remain stuck to the plant tissue or precipitate, while other neutral components (betulin, lupeol, *etc.*, see Scheme 7) could be selectively extracted with non-polar solvents. Acidic components can be extracted from the bark after extraction of all neutral components with any slightly acidified polar solvents. This approach enables the selective separation of lupeol (**4**), betulin (**1**), and betulinic acid (**2**) from birch bark.



Scheme 6 Birch bark suberinic acids.

It is likely that the presence of slightly acidic aromatic hydroxy-groups on the matrix plant polymer suberin supports the process of betulinic acid adherence to the plant tissue.[336,338] The general



Scheme 7 Binding of acids to birch bark suberin with $Al(OiPr)_3$.

character of the invention claims the use of such a procedure[337] for any extraction process when it is necessary to separate acidic components from neutral components.

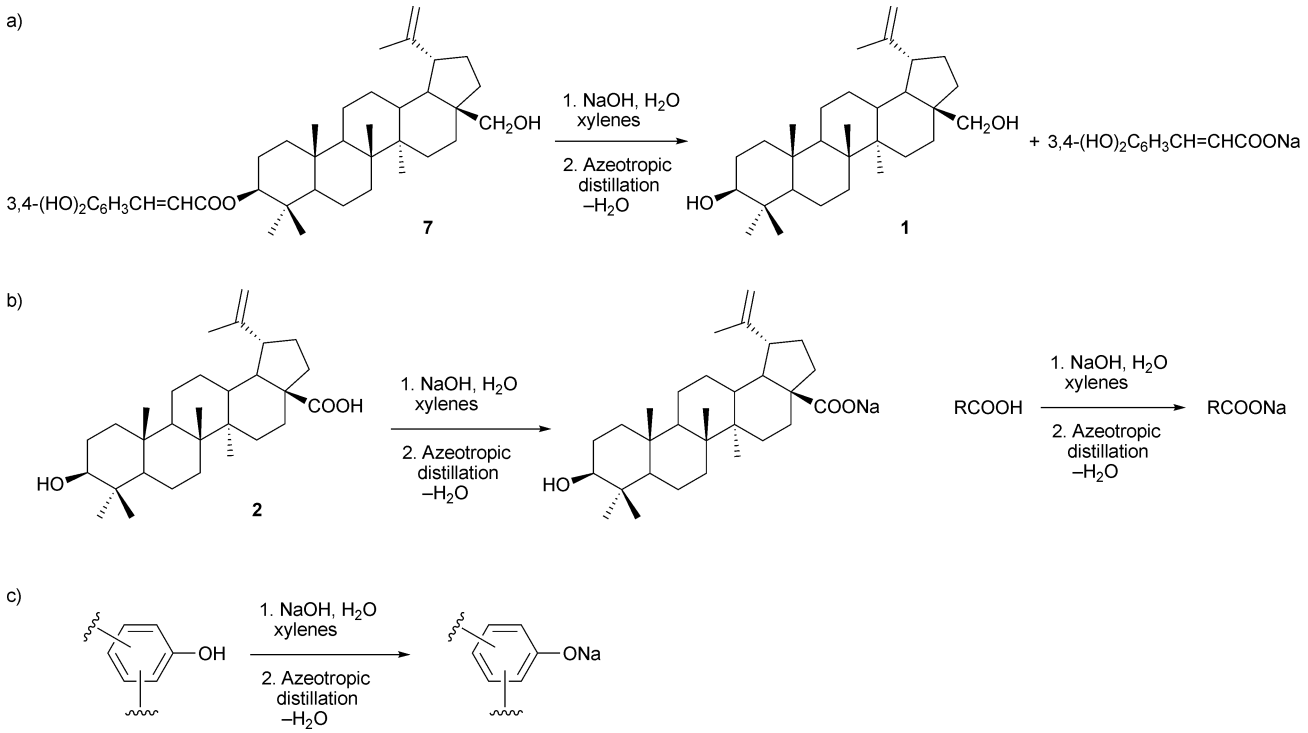
The invention of Krasutsky *et al.*[339] is suitable for obtaining all major birch bark triterpenoids, betulin (**1**), lupeol (**4**), and betulinic acid (**2**), at yields of about 10–12%, about 2.5% and about 2%, respectively. By employing this method, commercial quantities (*i.e.* tons) of the above triterpenoids can be obtained from birch bark. When birch bark extract is boiled with a water-immiscible solvent that is capable of forming an azeotropic mixture with water and with an aqueous base, the following important processes take place (Scheme 8): a) hydrolysis of betulin 3-caffeate (**7**) into betulin and the corresponding caffeic acid salt; b) formation of salts of betulinic acid and other fatty acids (on average, birch bark extracts contain ~5% fatty acids); c) formation of alcoholates of polyphenols and tannins. The discoloration of the neutral fraction (Scheme 8) is a very important feature of this process for producing white crystalline NPs. Thus, pure white betulin (**1**) and lupeol (**4**) can be obtained after removing water through azeotropic distillation and subsequent filtering and crystallisation. Natural betulinic acid (**2**) is obtained after separation and treatment of solids (mixture of salts). Removing water by azeotropic distillation with non-polar solvents (xylenes) is a very important approach. This process results in the ultimate precipitation of all organic fatty acid salts and their separation from the neutral triterpenoid fraction. Other extractive processes using basic aqueous solutions cannot provide such an efficient separation and good yield.

It is worth noting that birch bark is not the only possible large-scale natural source of betulin. It is well-known that the presence of betulin in birch wood pulp causes harmful pitch deposits on papermaking machines.[340] Nikulenkova *et al.*[341] reported that betulin may be isolated in large amounts from the crude sulfate soap fraction from pulp mill manufacturing plants. Hamunen[342,343] proposed a method for betulin isolation from the crude soap of the sulfate process at paper mill manufacturing plants that use birch wood. A betulin content of 5–22% in four species of birch trees can also be considered as a good source of this NP.[344]

The above observations of different methods and approaches to birch bark processing show that the contemporary state of research and development is ready to meet high-scale commercial interests for the manufacture of such NPs as betulin (**1**), lupeol (**4**), betulinic acid (**2**), and birch bark suberinic acids (**39–43**). It is also clear that birch bark extracts may be manufactured on any scale.

3.3 Chemistry of birch bark NPs

During the reviewed ten year period the chemistry of triterpenoids has been intensively developed, thanks to the efforts in design of new drugs, as well as in the novel approach of the cosmeceuticals[345–348] and neutraceuticals industries.[349–351] In the



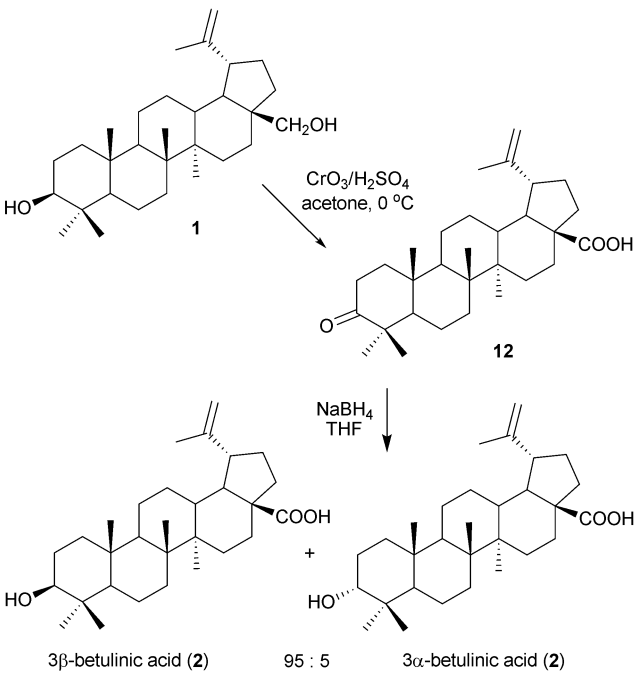
Scheme 8 Chemical processes during birch bark extract treatment with xylenes–NaOH–water and azeotropic distillation.

previous section the high potential for manufacture of birch bark NPs **1**, **2** and **4** is described. In this section it will be shown that on the basis of these three burgeoning NPs, almost all other birch bark triterpenoids and their derivatives can be synthesised.

A lot of research effort has been devoted to different methods of synthesis of betulinic acid (**2**) from betulin (**1**), because the latter is the most commonly available triterpenoid, and betulinic acid (**2**) and its derivatives have high potential for use as anti-cancer drugs and as precursors for anti-HIV drugs. Some independent approaches have recommended straight oxidation of betulin (**1**) into betulonic acid (**12**) with the Jones reagent (CrO_3/H_2SO_4/acetone), and subsequent relatively stereoselective $NaBH_4$/THF reduction to 3α- and 3β-betulinic acids (5 : 95 by weight) (Scheme 9).[352–355]

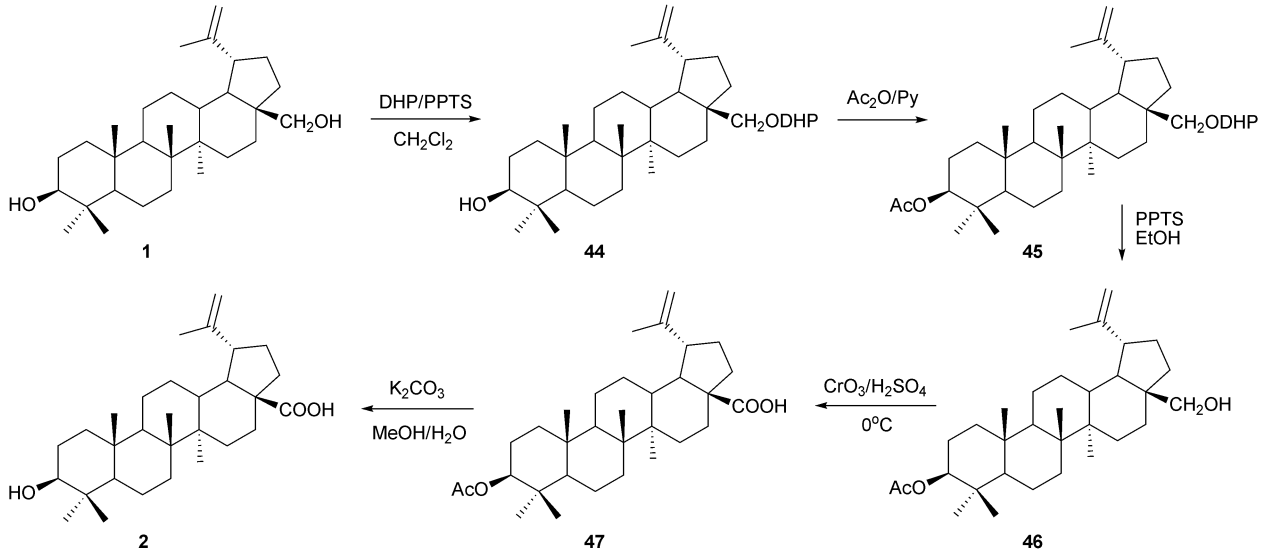
One study[352] (Scheme 10) claimed a five-step process involving selectively protecting the group at the C28-position of betulin (**1**) (to give DHP ether **44**), protecting the group at the C3-position (to give acetyl ester **45**), removing the C28-protection (to give ester **46**), carrying out Jones oxidation, and hydrolysing the resulting betulinic acid 3-acetate (**47**) to 3β-betulinic acid (**2**), identical to the natural compound. The overall yield was 55%.

Levdanskii *et al.*[356] invented an improved process for the preparation of betulinic acid by oxidation of betulin to betulonic acid with CrO_3/AcOH and subsequent reduction with sodium borohydride, without the isolation of free betulonic acid. The overall yield of betulinic acid from betulin was 65% using this process. Kogai *et al.*[357] invented a process for the preparation of betulinic acid (**2**) by the oxidation of betulin with CrO_3/AcOH and subsequent sodium borohydride reduction of sodium betulonate in water. Pichette *et al.*[358] reported the mild selective oxidation of betulin (**2**) into betulinic aldehyde (**3**) on specially designed solid-phase chromic oxide adsorbed on silica gel. Betulinic aldehyde (**3**) can then be almost quantitatively oxidised to betulinic acid



Scheme 9 Two-step synthesis of 3α- and 3β-betulinic acids (**2**).

with potassium permanganate. Roshchin *et al.*[359] invented another modification of betulin (**1**) oxidation into betulonic acid (**12**) by pyridine dichromate complex with DMF–acetic anhydride (2.5 : 3.0). Betulonic acid (**12**) was then reduced to a mixture of 3α- and 3β-betulinic acid (**2**). The ratio of isomers (5 : 95) was identical to ratios previously reported.[352–354] All of these methods[351–358] of oxidation using common chromium oxide reagents can be



Scheme 10 Five-step synthesis of betulinic acid (**2**) from betulin (**1**).

regarded as an improvement on the first publication on this subject by Ruzicka *et al.*[360]

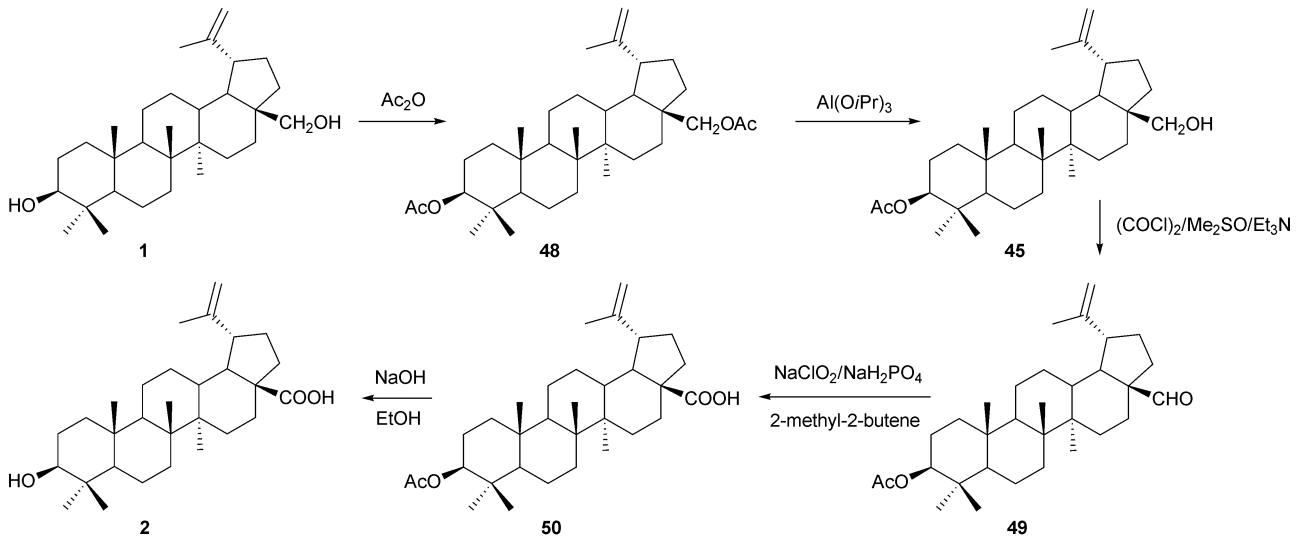
Another approach, by Krasutsky *et al.*,[361–363] claims a five-step betulinic acid (**2**) synthesis (Scheme 11), involving diacetylation of betulin (**1**) to betulin 3,28-diacetate (**48**), followed by selective alcoholysis with $Al(OiPr)_3$ in *i*PrOH to give betulin 3-acetate (**45**), Swern oxidation to betulinic aldehyde 3-acetate (**49**), oxidation with sodium or potassium chlorite to give betulinic acid 3-acetate (**50**) and final hydrolysis of ester **50** to provide betulinic acid (**2**).

A recent patent[364] claimed a different sequence of operations to provide betulinic acid 3-acetate (**50**) (Scheme 12): regioselective silylation of betulin (**2**) with $tBuMe_2SiCl$ to give ether **51**; acetylation with Ac_2O into the ether-ester **52**; desilylation with TBAF to give betulin 3-acetate (**46**); Oppenauer oxidation with $Al(OtBu)_3$ with 1,4-quinone to give betulinic aldehyde 3-acetate (**49**); and oxidation of **49** with $NaClO_2/NaH_2PO_4$ and 2-methyl-2-butene to give **50**.

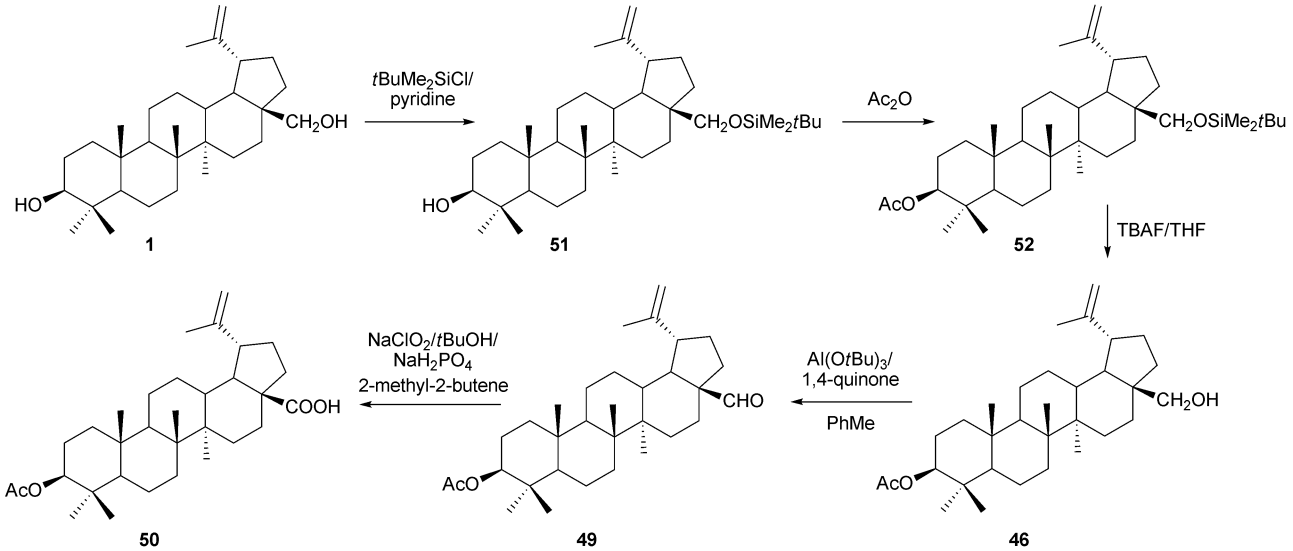
All three methods shown in Schemes 10–12 use different regioselective reactions with betulin (**1**) as the key steps: Scheme 10 the regioselective formation of DHP-ether **44**, Scheme 11 the regioselective alcoholysis of 3,28-diacetate **48**, and Scheme 12 a regioselective C28-silylation process.

Mitrofanov *et al.*[365] reported selective oxidation of betulin (**1**) into betulinic acid (**2**) by micro-organisms in chloroform. It was shown that the dormant cells of *Mycobacterium* perform this reaction with a 36% yield.

The reviewed period has brought many interesting synthetic approaches to the chemistry of birch bark triterpenoids through intensive efforts in drug design and research into structure–activity relationships. A number of methods for betulin and betulinic

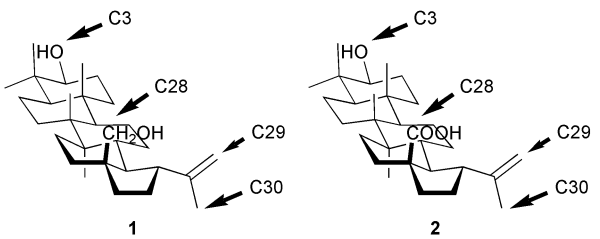


Scheme 11 Five-step synthesis of betulinic acid (**2**).



Scheme 12 Five-step synthesis of betulinic acid 3-acetate (**50**).

acid derivatisation at the C3-, C28-, C29- and C30-positions (Scheme 13) have been developed and reported in publications and patents.

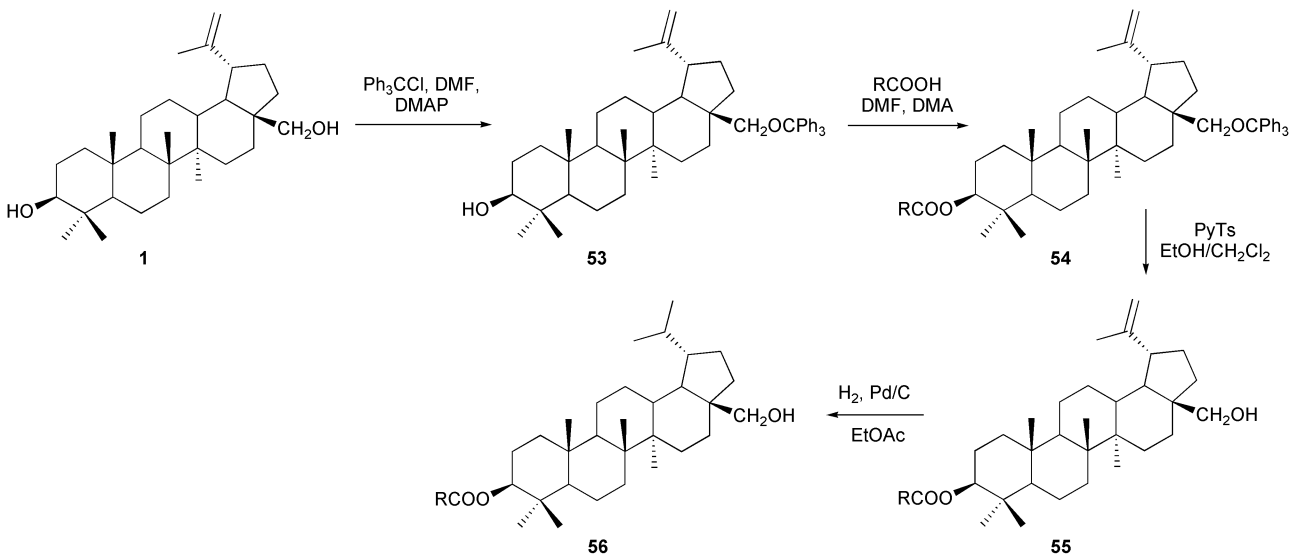


Scheme 13 Four methods for modification of betulin and betulinic acid.

Schemes 10–12 manifest the different methods of C3-protection of betulin (**1**) for betulinic acid synthesis (**2**).[351–353,359–362] Kim *et al.*[352] have reported a modification of the C28-position of betulin (**1**) through the selective formation of tetrahydropyranyl ether **44**. Selective C28-silylation of betulin (**1**) has been claimed by patent.[364] Tietze *et al.*[366] reported the selective C28-acetylation of betulin into betulin 28-acetate and its oxidation to betulone 3-acetate. In this work, betulone 3-acetate was a precursor to the synthesis of [^{13}C]- and [D]-betulin for biological transformations.

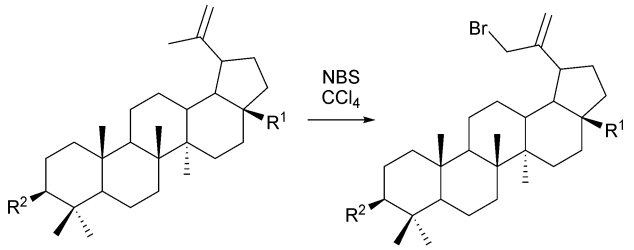
A method for the synthesis of betulin and dihydrobetulin 3-acylated anti-HIV derivatives used a rather different approach (Scheme 14) involving selective C28-tritylation of betulin (**1**) with Ph_3CCl/DMAP in DMF.[54,56] After esterification with RCOOH in pyridine/DMAP, betulin 28-*O*-trityl ether (**53**) gave 3-*O*-acylated betulin derivatives **54**. The following detritylation with catalytic pyridinium tosylate in EtOH–CH_2Cl_2 results in esters **55**, and hydrogenation with catalytic Pd/C yields dihydrobetulin 3-acylated products **56**.

Activation of the C30-position (Scheme 13) in betulin or other lupane triterpenoids can be achieved through bromination with



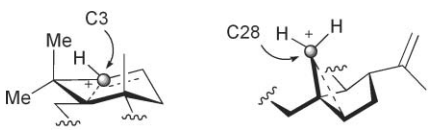
Scheme 14 Synthesis of betulin and dihydrobetulin 3-acyl derivatives.

NBS in CCl_4 (Scheme 15).[52,224,367] This reaction is actually directed at the C29-unsaturated carbon atom, with consequent double bond isomerisation leading to functionalisation with bromine at the C30-position. Nucleophilic substitution of this active C30-brominated compound with Y^- then leads to the corresponding C30-Y triterpenoid derivatives. These processes are quite selective, and have been reported in numerous publications.[52,224,367]



Scheme 15 Activation of the C30-position in lupane triterpenoids through bromination with NBS.

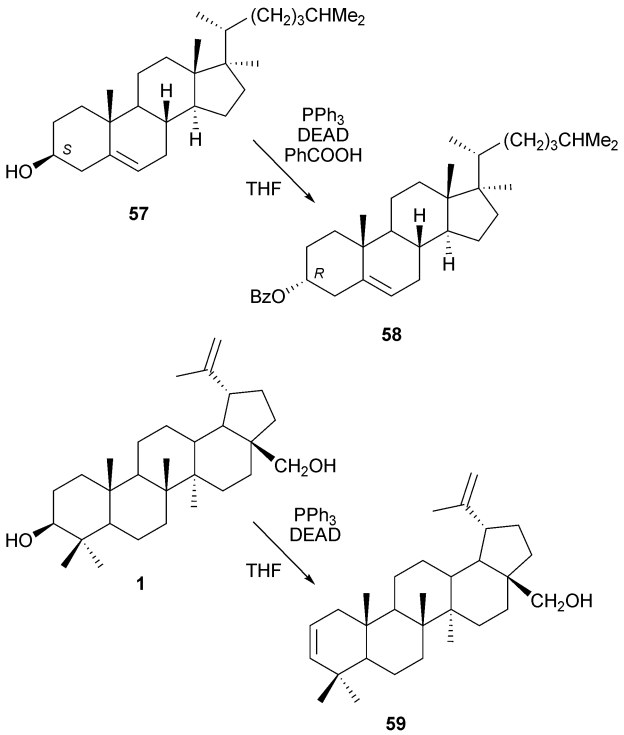
The chemical specifics of lupane triterpenoids appear in their resistance to nucleophilic substitution near the C3- and C28-atoms. Such behaviour is quite understandable from a theoretical point of view, because these positions are especially prone to carbocationic Wagner–Meerwein rearrangement, similar to those in neopentyl or norbornyl systems (Scheme 16).[368,369] For example, the reaction of lupane triterpenoids with $POCl_3$/pyridine did not yield the corresponding chlorides, but a complicated mixture of Wagner–Meerwein rearrangement products.[370] The "neopentyl"-type carbocationic fragments of the betulin structure involved are shown in Scheme 16.



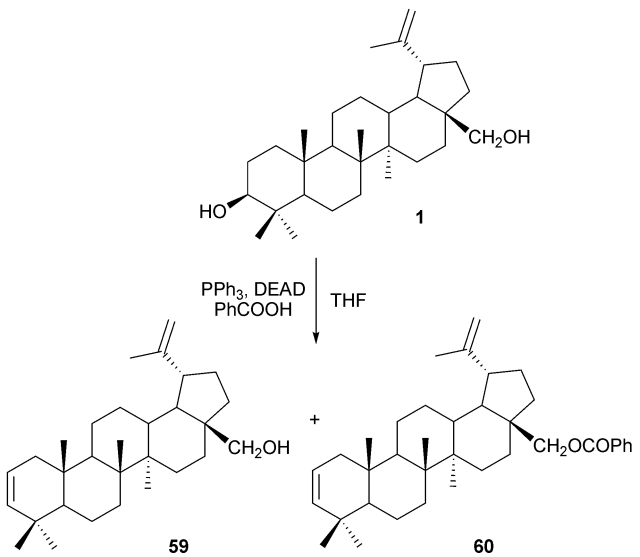
Scheme 16 Fragments of the "neopentyl"-type carbocations at C3 (A-ring, prone to elimination and rearrangement) and C28 (E-ring, prone to rearrangement through enlargement) of betulin (**1**). The dashed lines indicate the main direction of the elimination or rearrangement process.

Attempts to accomplish S_N2 nucleophilic substitution near the C3- and C28-atoms have not been very succesful so far. Even such a well-known S_N2-process as the Mitsunobu reaction,[371] which inverts the configuration at the C3-atom of the unhindered sterol **57**, providing **58**,[372] led to olefinisation at the C3-atom of betulin (**1**), giving 3-deoxy-2,3-dihydrobetulin (**59**)[52] (Scheme 17).

Symon *et al.*[225] reported that attempts at bimolecular substitution in lupeol 3-tosylate resulted only in $\Delta^{2,3}$-elimination products, with none of the expected products of S_N2 substitution. Attempted Mitsunobu reaction of betulin (**1**) in dry THF with benzoic acid led to the formation of 3-deoxy-2,3-dehydrobetulin (**59**) (44%) and 3-deoxyhydrobetulin 28-benzoate (**60**) (16%) (Scheme 18). The formation of **60** proves the possibility of an S_N2 process at the C28-position, but such reactions have not yet been reported for the C3 positions of triterpenoids. These results demonstrate conditions where the process of carbocation olefinisation at the



Scheme 17 Mitsunobu reaction for (−)-3β-cholesterol (**57**) and betulin (**1**).

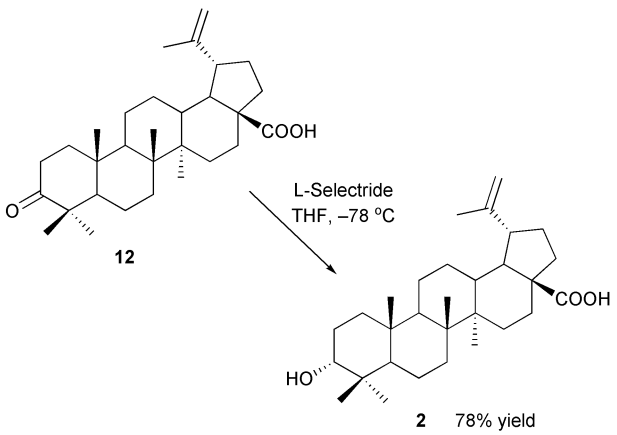


Scheme 18 Mitsunobu reaction for betulin (**1**) with DEAD.

C3-position of lupane structure prevailed over the process of carbocation rearrangement. The carbocation at C28 (Scheme 16, E-ring fragment) has the option for E-ring enlargement and subsequent olefinisation.[370,373,374]

Since birch bark triterpenoids are 3β-isomers, research into their conversion into their 3α-isomers is also worth observation. 3α-Epimers of triterpenoids (such as *epi*-lupeol or *epi*-betulinic acid) are less common in nature,[375–377] and therefore their synthesis from available birch bark triterpenoids is quite interesting. As mentioned above, the C3-position is sterically restricted with

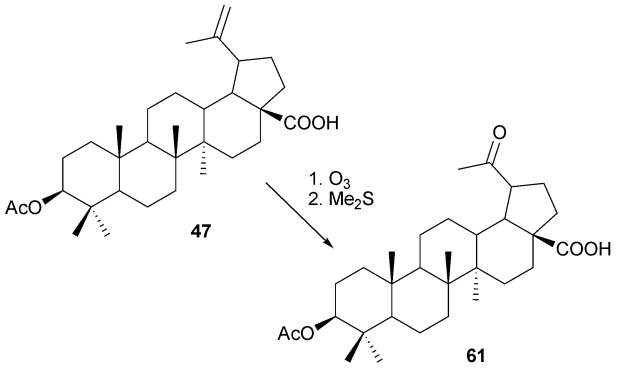
respect to S_N2 reactions. This problem was bypassed by the design of various stereoselective methods for the reduction of triterpen-3-one derivatives. Das[378] provided the first report on the stereochemistry of the Meerwein–Ponndorf–Verley reduction (with $Al(OiPr)_3$) of betulonic acid into 3β-betulinic acid (80%) and 3α-betulinic acid (20%). It was later shown that reduction of betulonic acid with $NaBH_4$/THF yielded a mixture of 3β- and 3α-betulinic acid (95 : 5 by weight).[352–355] This level of selectivity may be used for the synthesis of 3β-betulinic acid from betulonic acid (**44**) (Scheme 9). The best selectivity for the 3α-isomers synthesis (78%) was achieved by Sun *et al.*[52] by reducing betulonic acid with L-Selectride in THF at −78 °C (Scheme 19). A similar study[225] reported lower selectivities for the reduction of betulonic acid (**12**) with L-Selectride and with Raney nickel (3α/3β ratio = 60 : 40).



Scheme 19 Synthesis of 3α-betulinic acid (**2**) from betulonic acid (**12**).

Synthesis of 20-oxo-30-norlupane derivatives (involving modification at C29, Scheme 20) can be accomplished by transformation of the corresponding lupane derivatives with ozone.[379] For example, synthesis of platanic acid 3-acetate (**61**) from betulinic acid 3-acetate (**47**) can be accomplished in 66% yield (Scheme 20).[224]

Ashavina *et al.*[380] have reported the stereoselective epoxidation of 20(29)-lupene triterpenoids with dimethyldioxirane. Another interesting modification, at C29 of betulin 3,28-diacetate (**48**), was

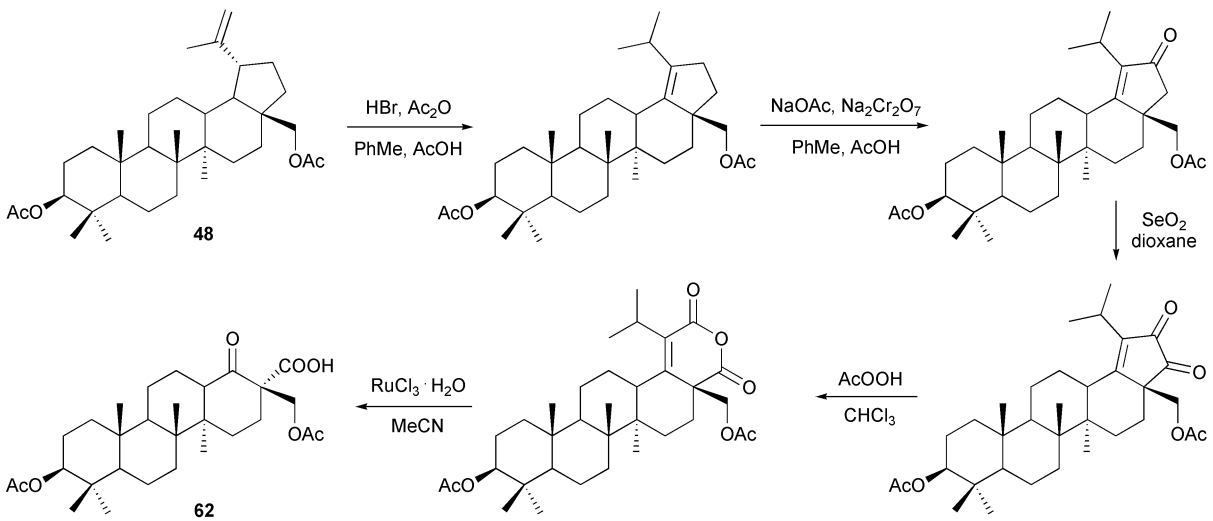


Scheme 20 Synthesis of platanic acid 3-acetate (**61**).

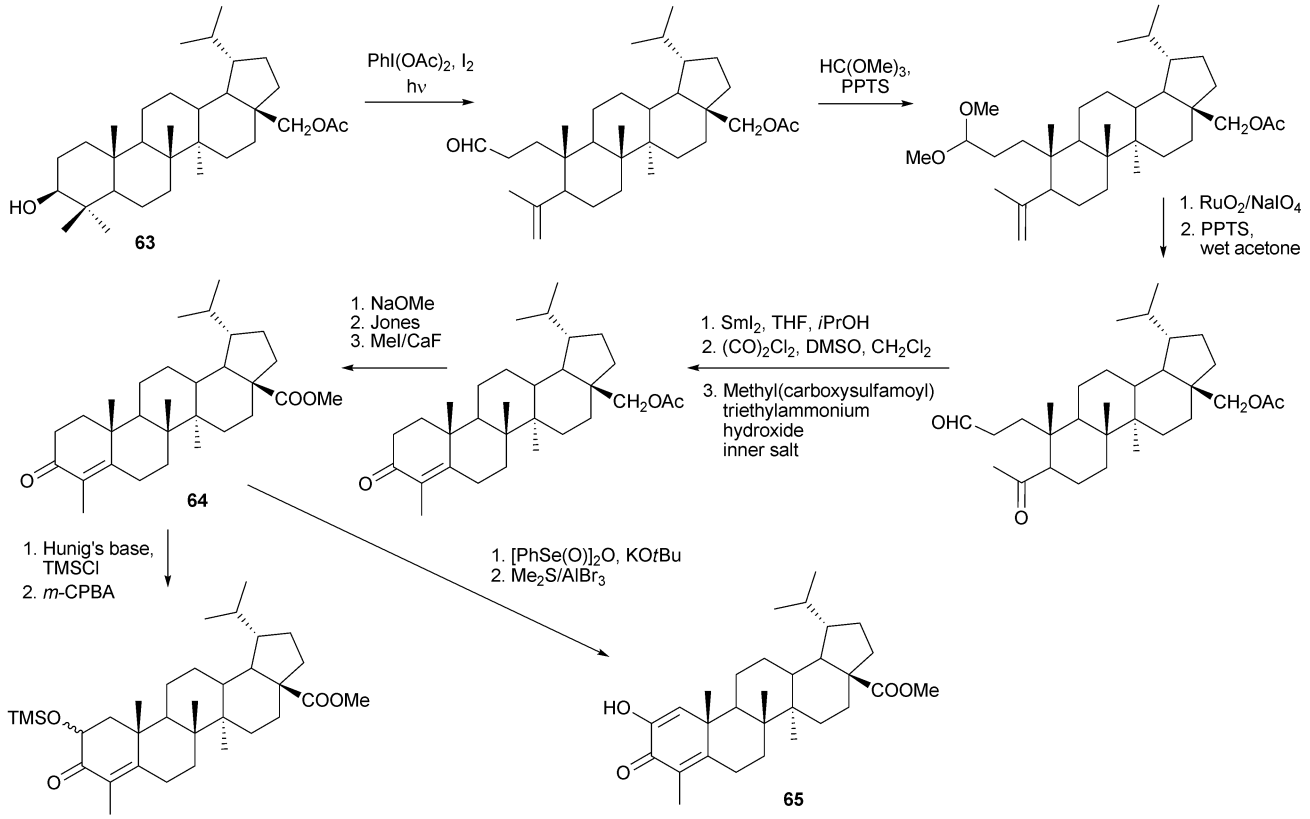
based on a 1975 study by Suokas *et al.*[381] It was found that specific acidic conditions (HBr/AcOH/Ac_2O/PhMe) lead not to allobetulin formation, as with HCl/EtOH/$CHCl_3$,[382] but to the migration of the double bond from C20 to C18. This reaction was the starting point for a five-step transformation (Scheme 21) of betulin 3,28-diacetate (**48**) to 3β,28-diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oic acid (**62**), which is a promising compound for the treatment of proliferative disorders such as cancer and leukaemias.[383] Interestingly, compound **62** forms a fairly stable solvate with methanol (1 : 1.5). Similar solvates of betulinic acid (**2**) with ethanol (1 : 1) have been described in a patent application.[384]

Sarek *et al.*[197,198,385] have developed syntheses for numerous 18-lupene, 18,19-secolupane, des-E lupane, and other oxidised triterpenoids as potential anti-tumour and anti-cancer chemotherapeutics.

The development of different methods of A-ring modification and cleavage has also been a matter of research and synthesis of new bioactives. Urban *et al.*[191,199] have reported the synthesis of A-seco derivatives of betulinic acid with relevant cytotoxic activity. Deng *et al.*[386] have described a new route to the synthesis of 24-nortriterpene derivatives with a modified A-ring (2-hydroxy-$\Delta^{1,4}$-cyclohexadiene-3-one) starting from of betulin (**1**) and betulinic acid (**2**). The principal steps of these transformations were a Suarez cleavage[387] of the A-ring in dihydrobetulin 28-acetate (**63**) and an



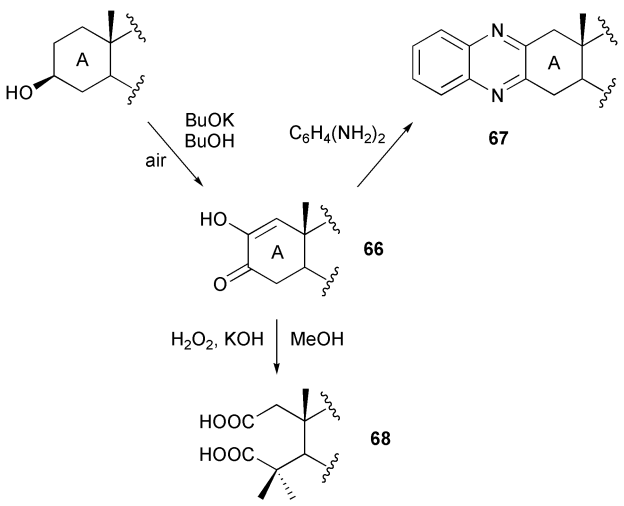
Scheme 21 Synthesis of 3β,28-diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oic acid (**62**).



Scheme 22 Synthesis of methyl 2-hydroxy-3-oxo-24-norlup-1,4-dien-28-oate (**65**).

SmI_2-mediated pinacol coupling to re-close the A-ring following removal of the C24 carbon by oxidative cleavage (Scheme 22).[387]

It is possible that compound **64** could be oxidised to **65** directly with air in BuOH/BuOK. Such an A-ring oxidation (Scheme 23) for lupeol was first reported by Ganguly *et al.*,[388] followed by a method for oleanolic acid by Chattopadhyay *et al.*,[389] for betulinic acid by Evers *et al.*,[224] for lupane and ursane triterpenoids by Korovin *et al.*,[390] and for numerous betulinic acid derivatives by Urban *et al.*[191,199] This simple process led



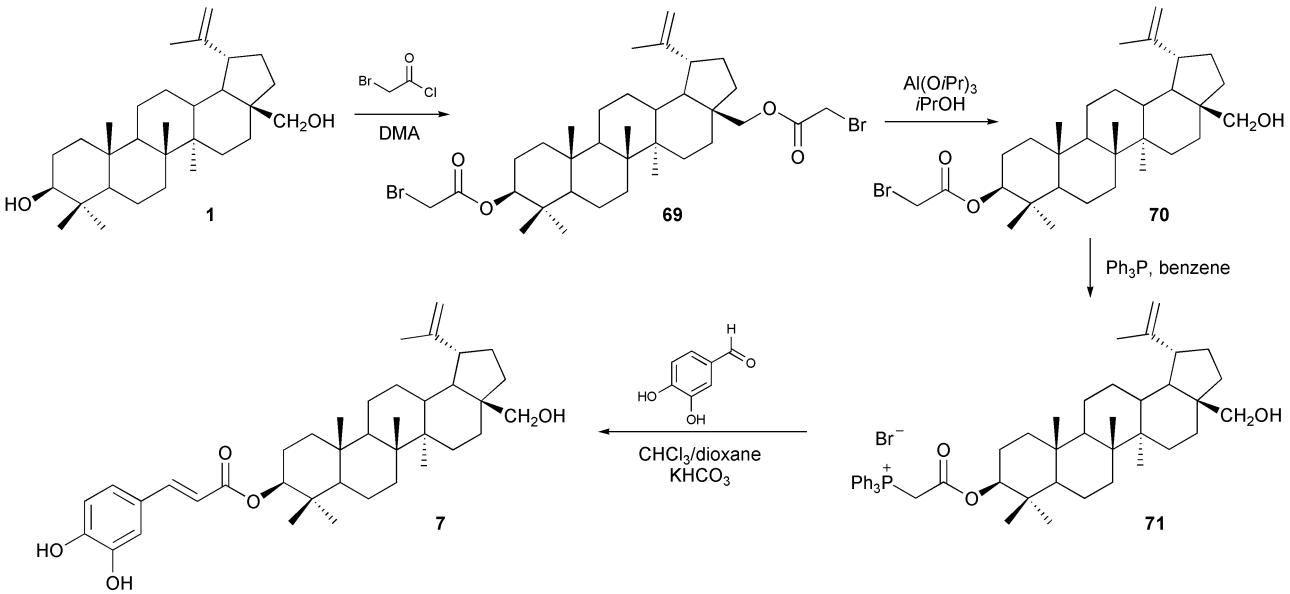
Scheme 23 A-Ring oxidation and cleavage of triterpenes, and synthesis of quinoxalines.

to the corresponding keto-enol derivatives **66** in 90–97% yield. The reaction of these keto-enols **66** with 1,2-diaminobenzene yielded the corresponding quinoxalines **67** in 85–95% yield (Scheme 23).[390] A-ring cleavage of the keto-enols **66** into acids **68** may be accomplished in one step with H_2O_2 and KOH in MeOH (Scheme 23).[199]

Fluorination of the E-ring of betulin with diethylaminosulfur trifluoride (DAST) was reported by Biedermann *et al.*,[391] but these fluorinated betulinines failed to demonstrate significant *in vitro* anti-cancer activity.

As previously mentioned (Section 2.1), different (triterpenoid and non-triterpenoid) caffeates are interesting NPs because of their anti-cancer, imunomodulatory and UV-protection activity.[20,101–104] Therefore, synthesis of these NPs from triterpenoids is of special practical interest. It has been shown[20] that a previously described procedure for the synthesis of caffeates[392] from caffeic acid, and of the corresponding alcohols by using thionyl chloride, do not work well for triterpenoids. This result triggered efforts on the development of a new method for synthesis of triterpenoid caffeates, as shown with Scheme 24.[20] The selective alcoholysis of betulin 3,28-dibromoacetate (**69**) led to betulin 3-bromoacetate (**70**), which was then converted to its triphenylphosphonium salt **71**. Reaction of **71** with 3,4-dihydroxybenzaldehyde yielded betulin 3-caffeate (**7**). This approach may be used to synthesise any natural triterpenoid caffeate.

Several recent and miscellaneous synthetic approaches to the modification of birch bark triterpenoids should also be mentioned. Tolmacheva *et al.*[393] obtained new 30-thio- and



Scheme 24 Synthesis of betulin 3-caffeate (**7**).

30-sulfinylbenzimidazole derivatives of betulin, which exhibited anti-inflammatory activity comparable to that of sodium diclofenac. New 3-amino derivatives of betulin and betulinic acid have been synthesised by Uzenkova *et al.*[394] and Flekhter *et al.*[395] Cyclopropane derivatives of betulin were synthesised by the attachment of dichlorocarbenes or dibromocarbenes to the double bond of betulin diacetate, followed by the deprotection of the hydroxyl groups.[186] You *et al.*[396] reported the synthesis of 3-aminoacetyl derivatives with increased water solubility and potential cytotoxic activity. Petrenko *et al.*[397] have synthesised new C28 amino acid derivatives of betulonic acid as potential bioactives, and Miskiniene *et al.*[398] have described the synthesis of nitroaromatic derivatives of betulin [betulin-(28)-5′-(aziridin-1-yl)-2′,4′-dinitrobenzoate and betulin-(28)-5′-nitro-2′-furoate] as redox cycling reagents.

Addendum

It is worth mentioning the most recent research and developement efforts that have been published during the writing of this review. These new reviews have been about the chemistry and bioactivity of triterpenes and their derivatives.[399–402] Gauthier[403] and Mao-Cai[404] have reported the synthesis of cytotoxic and anti-tumor triterpene glycosides and saponins. Several new triterpenoids and their derivatives with anti-HIV activity have also been reported.[405–408] The possible medicinal use of lupeol derivatives has also been described,[409–411] and there have been studies on the anti-proliferative activity of triterpenoids.[412,413]

4 Summary

The previous ten years of research and development have enabled us to reach a point when birch bark triterpenes or their derivatives will very soon appear in the marketplace. The development of technology for processing birch bark is also ready to meet the demands of industry and relevant markets. The quantity of natural products of the bark from commercially managed birch trees (*B. papyrifera*, *B. pendula*, *B. pubescens* and *B. neoalaskana*) are large enough to satisfy any high-volume need for birch extracts, betulin, betulinic acid or lupeol. These NPs can be also considered precursors for a broad range of synthetic lupane and oleanane derivatives. Birch bark suberinic acids, which can in principle be isolated individually, should also be worthy of attention, but the volume of suberinic acids manufactured will depend very much on the success of the commercialisation of birch bark extract and birch bark triterpenoids.

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