

Article

A Practical Synthesis of Betulonic Acid Using Selective Oxidation of Betulin on Aluminium Solid Support

Nina Melnikova ^{1,*}, Irina Burlova ², Tatiana Kiseleva ¹, Irina Klabukova ¹, Marina Gulenova ³, Aleksey Kislitsin ¹, Viktor Vasin ² and Boris Tanaseichuk ²

¹ Department of Pharmaceutical Chemistry, Nizhny Novgorod State Medical Academy, Minin sq. 10/1, Nizhny Novgorod 603-000, Russia

² Department of Chemistry, N.P. Ogarev Mordovian State University, Mironov St. 3-274, Saransk 430-005, Russia

³ Department of Chemistry, Nizhny Novgorod State University, Kuibishev St. 17-25, Nizhny Novgorod 603-950, Russia

* Author to whom correspondence should be addressed; E-Mail: melnikovaNB@gmail.com; Tel.: +7-902-309-22-98; +7-831-275-24-20.

Received: 10 August 2012; in revised form: 23 September 2012 / Accepted: 24 September 2012 / Published: 9 October 2012

Abstract: The room temperature oxidation of betulin by Cr(VI) compounds in aqueous acetone on solid supports such as alumina, zeolites and silica gel has been studied. The oxidation on alumina support led to a single product—betulonic acid—in quantitative yield. One hundred percent selective oxidation during 30 min of betulin up to betulonic aldehyde was determined when silica gel support was used. The oxidation of betulin using zeolites as a support gives a mixture of betulonic acid and aldehyde in a 2:1 ratio. It is proposed the selective oxidation up to betulonic acid is due to the influence of Al³⁺-ions.

Keywords: oxidation on alumina; betulin; betulonic acid; protection of double bond by Al³⁺-ions

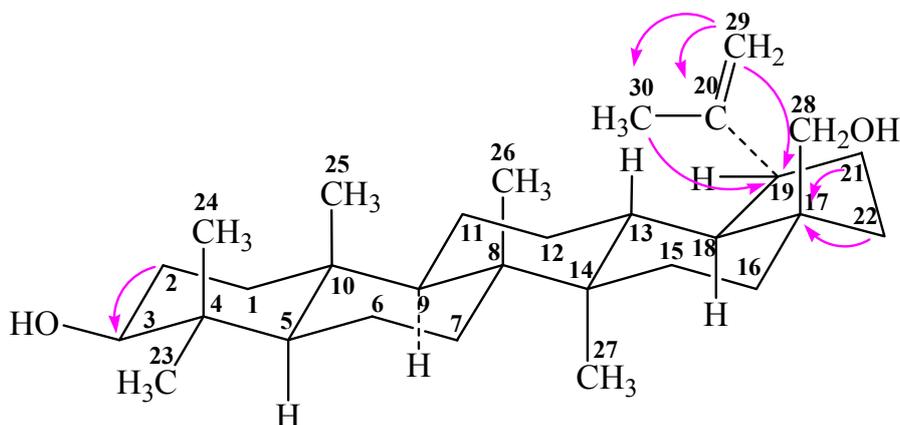
1. Introduction

Betulonic acid [lup-20(29)-en-3-oxo-28-oic] has valuable biological properties such as antiviral, antitumor, anti-inflammatory, antimicrobial, hepatoprotective, as well as immunostimulant activities [1–5].

Until the 2000s interest to betulonic acid was primarily due to its role as the precursor for synthesis of betulinic acid, which is an effective drug against human melanoma. As a rule, betulonic acid was produced using betulin oxidation by Jones' reagent ($\text{CrO}_3/\text{H}_2\text{SO}_4/\text{acetone}$) or similar synthesis routes with yields less more 75%. This synthesis is long, not very profitable and requires challenging purification steps, including column chromatography, multiple recrystallizations and extraction using very large volumes of solvents, making it unsuitable for extensive scale industrial application [1,2,6–10].

The main problems in the oxidation of betulin are due to, firstly, nonselectivity of the process, because the molecule contains three active centers: primary (at C-28) and secondary (at C-3) alcohol groups and an isopropenyl moiety. Secondly, it is difficult to regulate the range of oxidation because products may be aldehydes or ketones as well as acids. Thirdly, the lability of the betulin structure (Figure 1) provides conditions for different rearrangements as follows on the basis NOESY spectra calculation taking into account of Overhauser effect [11,12].

Figure 1. Structure of betulin (1).



Taking into account the features of the betulin structure, historically oxidation selectivity was achieved in synthetic routes with protection of the primary alcohol groups of betulin, for example, by acetylation using acetic anhydride [13]. Then betulin diacetate was hydrolyzed up to betulin monoacetate at the C-3 position. The non-protected C-28 alcohol group is oxidized to a carboxyl group by CrO_3 in acetic acid and betulonic acid is formed after removing the acetate protective groups.

A number of new chromium (VI)-containing compounds with heterocyclic bases, like pyridinium chlorochromate, pyridinium bromochromate, quinolinium chlorochromate, quinolinium fluoro-chromate, quinolinium bromochromate, imidazolium fluorochromate, pyridinium fluorochromate, imidazolium dichromate and quinolinium dichromate have been developed to improve the selectivity of oxidation of organic compounds [14–16].

Quite recently, a two-step route [17] utilizing solid-supported chromium oxide and potassium permanganate has been suggested and even more recently, a TEMPO-mediated electrochemical approach has been devised [7]. Whereas the oxidation of betulin with TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl)/ $\text{NaClO}_2/\text{NaOCl}$ at 35 °C furnished 92% of the betulonic aldehyde (4), the reaction of betulin with 4-acetamido-TEMPO/ $\text{NaClO}_2/\text{NaOCl}$ at 50 °C gave betulonic acid in an 86% isolated yield. Both of these approaches, however, are small-scale preparations and the obtained yields only

moderate. The increased selectivity of betulin oxidation was developed only for betulinic acid or betulinic aldehyde production.

Betulin oxidation to betulonic acid must be similar to the oxidation of steroid hydroxyls. Usually the oxidation of steroid or other natural triterpenes having hydroxyl groups at the C-3 position by chromic acid proceeds selectively in the presence of acetone, but the reaction gives double bond oxidation products if acetone is changed for other solvents. The oxidation of primary alcohol groups by chromic acid in acetone medium (Oppenauer's oxidation) may proceed up to aldehydes or acids like by isopropyl Al-salt catalysis [18]. Concurrent directions of oxidation characteristics didn't seem reasonable for practical synthesis of betulin derivatives, consequently, development of a practical synthesis of betulonic acid with a mild, selective and high-yielding oxidation in the first stage is a very important problem. Accordingly, several methods have been recently reported for the environmentally benign oxidation of primary and secondary alcohols to carbonyl compounds using solid supports [17,19,20].

Recently the possibility of high selective oxidation of betulin by chromium (VI) compounds immobilized on aluminium oxide or silica gel solid support in donor solvents up to betulonic acid as a precursor of betulinic acid was shown [21]. In this paper we study the protective function of surface aluminum ions as the reason behind the high yields seen in the practical synthesis of betulonic acid from betulin at room temperature in a one-pot reaction by using low cost, easily available potassium dichromate ($K_2Cr_2O_7$) which may be regenerated as oxidant in the presence of H_2SO_4 and acetone on a solid support such as alumina.

2. Results and Discussion

It is well known that Lewis acids may catalyze the oxidation of alcohols up to aldehydes [22]. The most catalytic activity was shown by aluminum salts, being also the most selective catalysts. In the first stage of our study the effect of Al^{3+} on oxidation selectivity and conversion of betulin (**1**) was demonstrated in experiments by using $Al_2(SO_4)_3$ in aqueous acetone at room temperature. We obtained betulonic acid (**2**) as a single product in quantitative yield at a molar ratio $v_{Cr^{6+}}/v_{betulin}$ equal to 3, and it was easily separated out after 1.5–3.0 h by adding water to the supernatant liquid. The crude betulonic acid didn't require challenging purification, if it was used for synthesizing betulonic acid derivatives, for example, betulinic acid. The synthesis of betulinic acid by $NaBH_4$ reduction of betulonic acid without challenging purification in isopropanol or THF medium is described in the Experimental section.

In the case when $Al_2(SO_4)_3$ was absent in the reaction mixture, conversion of betulin was only 40% with the formation of two main products—betulonic acid **2** and betulonic aldehyde **3**—and several undetermined products (Table 1). It may be supposed that they were the results of isopropenyl moiety oxidation as estimated by ^{13}C -NMR-spectra [decrease of the signals with $\delta = 109$ ppm (C-29) and $\delta = 150$ ppm (C-20)]; and by IR-spectroscopy [decreasing intensity of bands at 882 cm^{-1} (methylene terminal double bond) and 1642 cm^{-1} (double bond)].

Table 1. Time distribution of selectivity in betulin oxidation without $\text{Al}_2(\text{SO}_4)_3$ at molar ratio $v_{\text{Cr}^{6+}}/v_{\text{betulin}}$ equal to 3 in acetone at room temperature.

Time, min	Conversion of betulin (1), %	Products in reaction mixture, % (HPLC, IR and NMR-spectra control)			
		Betulinic aldehyde (4)	Betulinic aldehyde (3)	Betulinic acid (2)	Other unknown products
2	5	15–20	55	–	20–25
5	7	15	50–60	–	20–25
10	12	5	60	10	25
20	17	–	60	10	30
65	25	–	50	20	30
80	30	–	40	30	30
160	37	–	35	35	30
300	40	–	20	50	30
400	50	–	–	70	30
150 *	100	–	0–5	95–100	–

*: The experiment with $\text{Al}_2(\text{SO}_4)_3$.

The feature of chromium oxidation in the presence of Al^{3+} , in addition to high selectivity, was the acceleration of the reaction: 100% betulin conversion was achieved in 150 min. This may mean a protective role of Al^{3+} as well as a catalytic effect. The disadvantage of this high selective oxidation in the presence of $\text{Al}_2(\text{SO}_4)_3$ was the formation of a green coloured inorganic precipitate of $\text{Cr}_2(\text{SO}_4)_3$ with toxic properties.

It is a very interesting approach to use a solid support containing Al^{3+} -ions due to the high oxidation selectivity by using Cr^{3+} sorption on a solid support to minimize sediments. The experiments with aluminosilicate (zeolites) with similar dispersity as a granular solid support were carried out to demonstrate the role of Al_2O_3 as the best regulator of selective betulin oxidation. SiO_2 was chosen as solid support with other acid properties than the Lewis acid— Al^{3+} .

Selectivity of oxidation and conversion of betulin (1) on alumina support in aqueous acetone was due to molar ratio $v_{\text{Cr}^{6+}}/v_{\text{betulin}}$, mass ratio $m_{\text{solid support}}/m_{\text{betulin}}$, duration of processes and didn't depend on the Cr^{6+} -containing compounds (Table 2, examples 3–5). The best selectivity of betulin oxidation up to betulinic acid (2) (100%) with high yield (93%–98%) was obtained under the conditions of experiments 3a–c. The decrease of selectivity in betulin oxidation was shown in experiments with zeolites (Table 2, example 6), since the surface concentration of Al^{3+} in zeolite is much less than in alumina.

Table 2. Oxidation of betulin ($0.02 \text{ mol}\cdot\text{L}^{-1}$) with Cr^{6+} -containing compounds on solid support in aqueous acetone at 15–25 °C during 1.5–3 h.

Example	Oxidant				Conversion of betulin 1, %	Ratio 2:3	Selectivity, %
	Cr^{6+}	Solid support	$\frac{v_{\text{Cr}^{6+}}}{v_{\text{betulin}}}$	$\frac{m_{\text{solid support}}}{m_{\text{betulin}}}$			
1	$\text{K}_2\text{Cr}_2\text{O}_7$	$\text{Al}_2(\text{SO}_4)_3$ *	3:1	–	100	100:1	100 (2)
2	$\text{K}_2\text{Cr}_2\text{O}_7$	–	3:1	–	40	1:1	50 **
3a	$\text{K}_2\text{Cr}_2\text{O}_7$	Al_2O_3	3:1	6:1	100	100:1	100 (2)

Table 2. Cont.

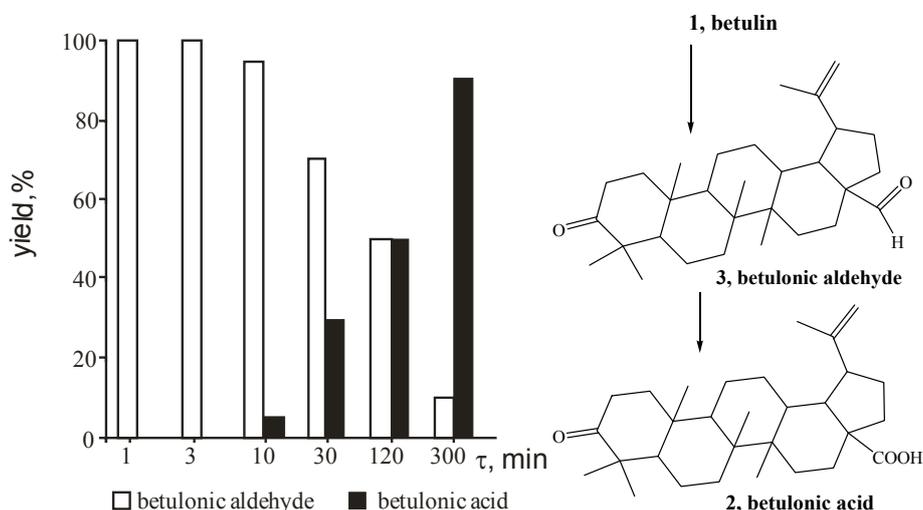
Example	Oxidant				Conversion of betulin 1, %	Ratio 2:3	Selectivity, %
	Cr ⁶⁺	Solid support	$\frac{v_{Cr^{6+}}}{v_{betulin}}$	$\frac{m_{solid\ support}}{m_{betulin}}$			
3b	K ₂ CrO ₄						
3c	CrO ₃						
4	K ₂ Cr ₂ O ₇	Al ₂ O ₃	1.5:1	6:1	100	3:1	75 (2)
5	K ₂ Cr ₂ O ₇	Al ₂ O ₃	1.5:1	3:1	80	1:1	50
6	K ₂ Cr ₂ O ₇	zeolites	3:1	2:1	100	7:3	70 (2)
7 ***	K ₂ Cr ₂ O ₇	SiO ₂	3:1	10:1	100	1:100	100 (3)

* C_{Al₂(SO₄)₃} = 1.6 mmol·L⁻¹; ** selectivity was estimated and the oxidation of isopropenyl moiety wasn't taken into account; *** synthesis during 30 min.

This result was different in comparison with the experiments carried out without aluminum-ions (Table 2, example 2) or Al₂O₃-produced ones but under other conditions being equal (Table 2, examples 3–5). The reaction time increased up to 3–5 h (80%–100% betulin conversion), if the molar ratio $v_{Cr^{6+}}/v_{betulin}$ was decreased down to 1.5:1 (Table 2, examples 4–5). At the same time the selectivity of betulin oxidation was decreasing (Table 2). The colourless flakes of inorganic nature were formed immediately, and they became green. The flakes adhered to the alumina granules increasing in size, and the supernatant liquid became more homogeneous.

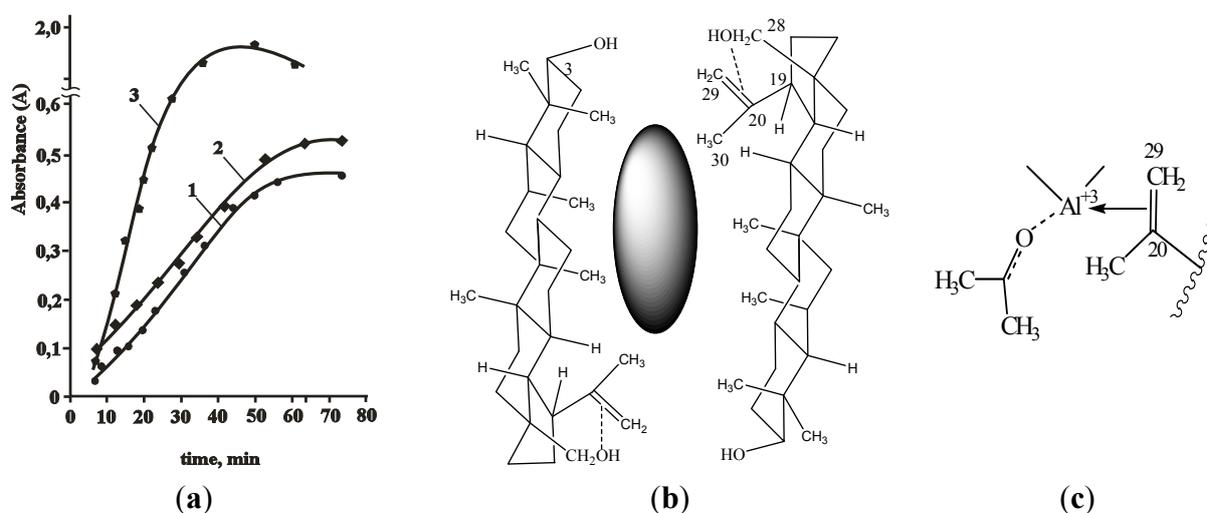
Surprisingly, oxidation of betulin (1) on silica gel for 30 min gave betulonic aldehyde (3) as a single product (Table 2, example 7) with 100% conversion of starting compound. Later on betulonic acid was obtained in the reaction mixture and at 8 hours the molecular ratio of betulonic aldehyde (3) to betulonic acid (2) became 1:1. The betulonic acid formation takes place as betulonic aldehyde oxidation. Assuming that a two-stage mechanism would be realized on alumina support we investigated betulin oxidation under the same conditions but at 0 °C. A two-step mechanism of betulin oxidation on Al₂O₃ was suggested, but the rate of betulonic acid formation was increased many times (Figure 2).

Figure 2. Diagram of yields of betulonic acid (black colour) and betulonic aldehyde (white colour) in time.



The data of Table 2 may be explained by the influence of homogeneity in the reaction zone on the selectivity of betulin oxidation in the presence Al^{3+} -ions on solid surface or in the solution. It was been shown that water solution of $\text{Al}_2(\text{SO}_4)_3$ as well as H_2SO_4 conc. dissolve betulin (**1**) in ultra-sound dispersed suspension in acetone giving a transparent colourless solution. The absorbance band in the 190–220 nm region of the UV spectra of aqueous acetone solutions of betulin in the presence of H_2SO_4 or $\text{Al}_2(\text{SO}_4)_3$ mixture was increasing with time up to 70 min and after that it became a constant value (Figure 3a, curves 1,2). The influence of $\text{Al}_2(\text{SO}_4)_3$ on betulin dissolution was much more pronounced (Figure 3a, curve 3).

Figure 3. Betulin dissolving by chelation of components in the reaction medium.



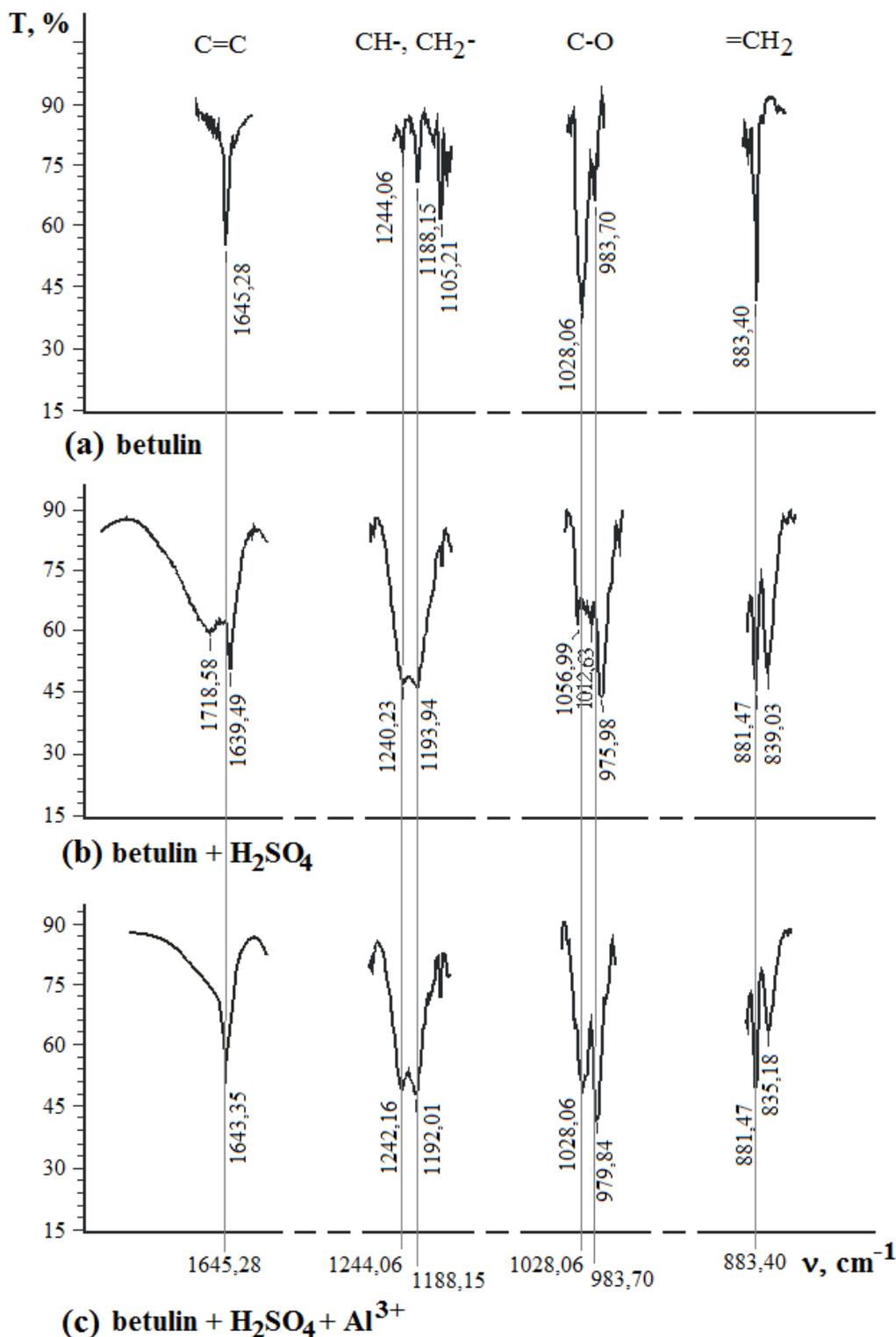
(a) The changes of absorbance (A) at 210 nm of 1% betulin in acetone solution containing H_2SO_4 (curve 1), mixture of H_2SO_4 and $\text{Al}_2(\text{SO}_4)_3$ (curve 2) and $\text{Al}_2(\text{SO}_4)_3$ (curve 3) in time; (b) betulin dimer as “host” to form inclusion complexes; (c) π -complex of Al^{3+} with isopropenyl moiety.

It is common knowledge that selectivity is increased when homogeneity is achieved in the reaction zone [23]. Variation of the dispersion state of the reaction zone on a solid support may be due to the sorption of some components by the solid support, formation of intermediate products and implication of betulin in complexes with Al^{3+} having better solubility than complexes only with H_2SO_4 .

Such dissolution is typical for inclusion complexes of betulin derivatives with solvents such as benzene, toluene, isopropanol and several metal ions [24], while clathrates are formed by betulin dimers as “head-to-tail” structures. The interaction of H_2SO_4 with steroid compounds due to their solution and such types of reactions are usually used for identification of steroid rings.

It has been estimated that some red solid products were formed after betulin oxidation by conc. H_2SO_4 (3 mass %). The IR region ($900\text{--}800\text{ cm}^{-1}$) relating to the double bond in the isopropenyl moiety of betulin was changed: one narrow band of 883 cm^{-1} was transformed into two bands at 881 cm^{-1} and 832 cm^{-1} and at the same time, the primary alcohol band (1028 cm^{-1}) disappeared, but the bands of the epoxy group (1242 cm^{-1} and 976 cm^{-1}) and of allobetulin (1056 cm^{-1}) were established. Changes of the $1740\text{--}1640\text{ cm}^{-1}$ region (C=O, C=C) were noted too (Figure 4).

Figure 4. IR spectra of solid products evolved from 1% betulin solution in acetone.



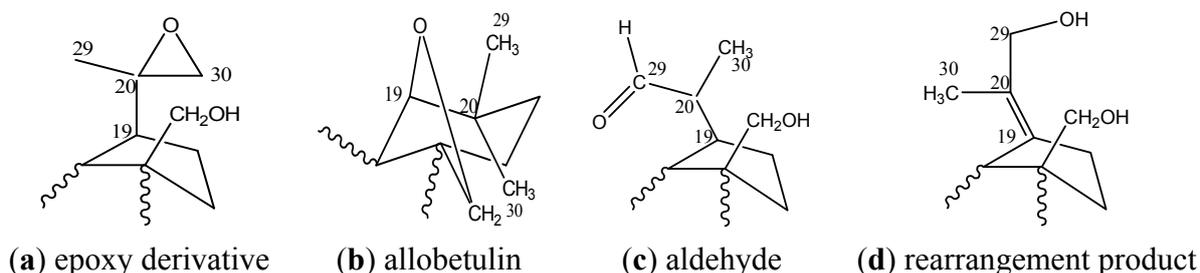
(a) starting betulin; (b) in the presence of H₂SO_{4 conc.}; (c) from reaction mixture containing aqueous solution of Al₂(SO₄)₃ and H₂SO_{4 conc.}.

We suggest that changes to betulin by the action of H₂SO₄ occurred upon the oxidation of the isopropenyl moiety as well as the isomerization of the primary alcohol group. The most probable fragments of betulin derivatives are presented in Figure 5. This proposal has been corroborated with

$^1\text{H-NMR}$ data. The intensity of olefin protons signals in isopropenyl moiety in $^1\text{H-NMR}$ spectra ($\delta = 4.71$ ppm and $\delta = 4.59$ ppm) was decreased two-fold. The intensity of signals of C-20 ($\delta = 150.24$ ppm) and C-29 ($\delta = 109.46$ ppm) in the $^{13}\text{C-NMR}$ spectra was decreased too, but $\delta = 86.79$ ppm, $\delta = 70.33$ ppm and $\delta = 63.52$ ppm signals were observed.

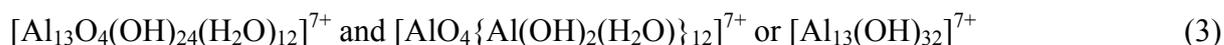
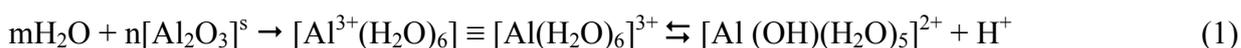
In the presence of Al^{3+} -ions (0.01%) the betulin oxidation up to allobetulin or other oxidation products was decreased (Figure 4). Aluminum as an element with a d-configuration with Lewis acid properties that may protect the isopropenyl moiety by formation of a π -complex (Figure 3c) in the presence of H_2SO_4 as well as Cr^{6+} - H_2SO_4 system.

Figure 5. Proposed fragments of oxidation products of the isopropenyl moiety.



The most important advantage of betulin oxidation when Al^{3+} -ions were generated from Al_2O_3 was the ability to collect Cr^{3+} compounds as flakes by sorption or by other interactions on the solid surface. This result allows one to remove Cr^{3+} -containing granules from reaction mixtures very easily, in contrast to the SiO_2 support which can't absorb Cr^{3+} .

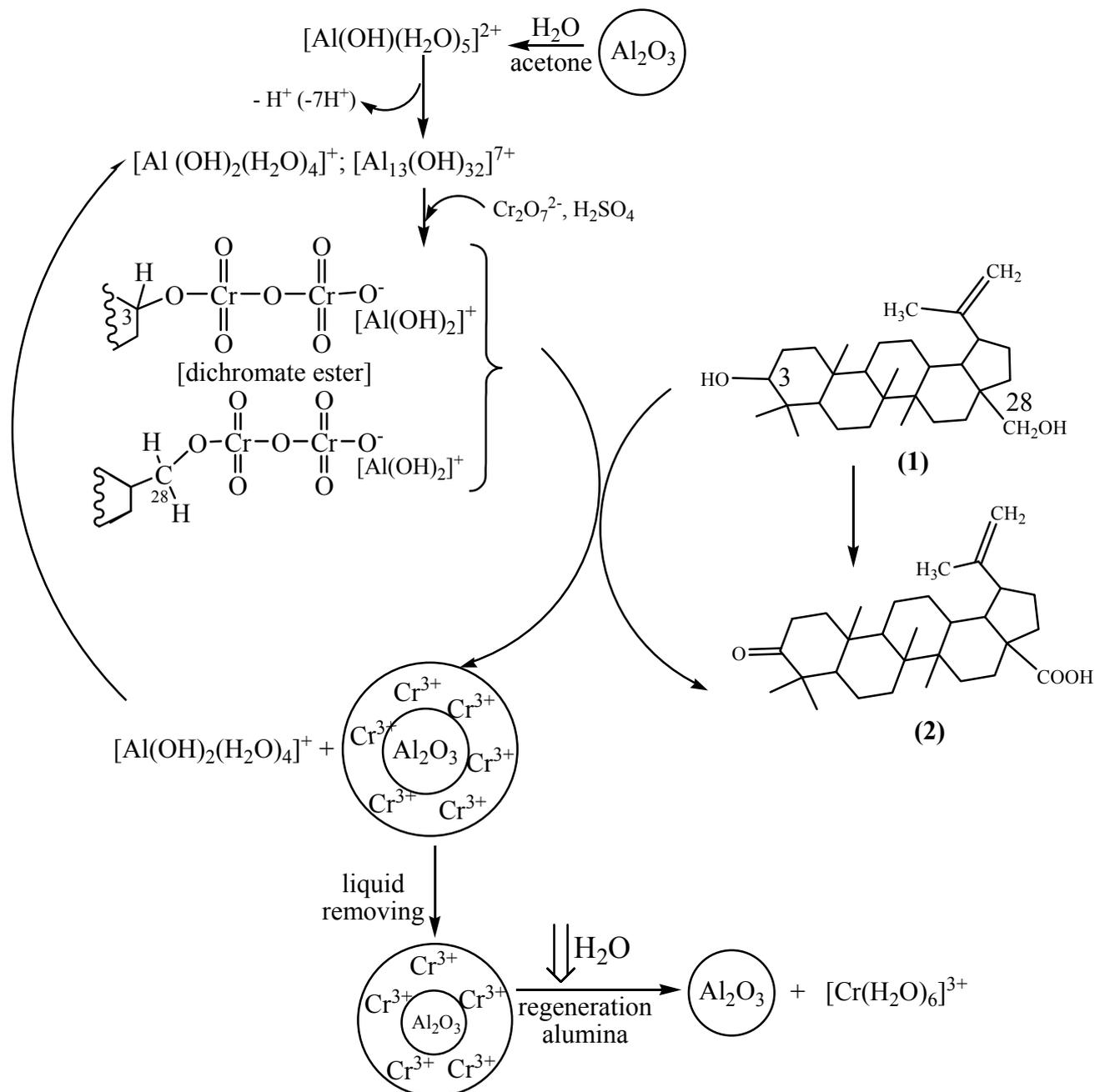
Aluminium oxide surface layers in aqueous acetone medium are transformed under: (1) surface dissociation, (2) deprotonation and (3) dimerization via hydroxy-bridge joint into different kinds of ions and multinuclear cations respectively [25]:



If such large amounts of sulfuric acid as in our experiments were used, it is most probable that reaction product type (2)— $[\text{Al}(\text{OH})_2(\text{H}_2\text{O})_4]^+$ —occurs. The features of high reactivity of wet aluminium oxide surface with $[\text{Al}(\text{OH})_2]^+$ or $[\text{Al}(\text{OH})_2(\text{H}_2\text{O})_4]^+$ in aqueous acetone medium containing $\text{K}_2\text{Cr}_2\text{O}_7$ - H_2SO_4 are due to: (i) anionic species of chromium derivatives with betulin; (ii) formation of inclusion complexes of reduced Cr^{3+} -ions; (iii) solid solution due to replacement of Cr^{3+} - Al_2O_3 [25,26].

The improved homogeneity in the reaction zone may be explained by stabilization of chromium esters with betulin, formed according to a Westheimer mechanism [27] which is due to aluminium cations (Scheme 1).

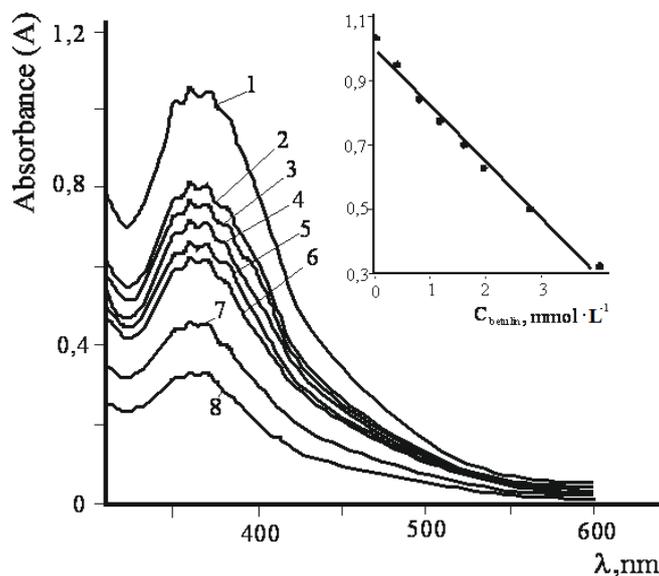
Scheme 1. General formulation of synthesis of betulonic acid using oxidation of betulin on alumina in aqueous acetone by $K_2Cr_2O_7-H_2SO_4$.



The oxidation of betulin by $K_2Cr_2O_7-H_2SO_4$ in aqueous acetone in the presence of Al^{3+} was studied in more a detail by UV spectrophotometry. A UV absorption characteristic of Cr^{6+} in different media occurs at $\lambda_{max} = 350-360$ nm. The absorbance (A) at a fixed wavelength follows the Beer-Lambert Law. Dichromate-ion may exist as the following forms: $Cr_2O_7^{2-}$, $HCrO_4^-$, $HCr_2O_7^-$. Lately $HCr_2O_7^-$ has been considered as the main particle in $K_2Cr_2O_7-H_2SO_4$ system [25,26].

The reaction mixtures were at the constant concentration of oxidant ($K_2Cr_2O_7-H_2SO_4$) and $Al_2(SO_4)_3$ but with variable concentration of betulin. From Figure 6, the peak intensity at 360 nm decreased linearly as the concentration of betulin increased.

Figure 6. UV-visible spectra of reaction mixtures; $C_{K_2Cr_2O_7} = \text{const} = 6.05 \text{ mmol}\cdot\text{L}^{-1}$; $C_{Al_2(SO_4)_3} = \text{const} = 0.27 \text{ mmol}\cdot\text{L}^{-1}$. Initial concentration of betulin corresponds to N curves ($\text{mmol}\cdot\text{L}^{-1}$) 1–0; 2–0.40; 3–0.80; 4–1.21; 5–1.61; 6–2.01; 7–2.82; 8–4.03. The inset figure is magnification for the absorbance A: $A = f(c_{\text{betulin}})$.

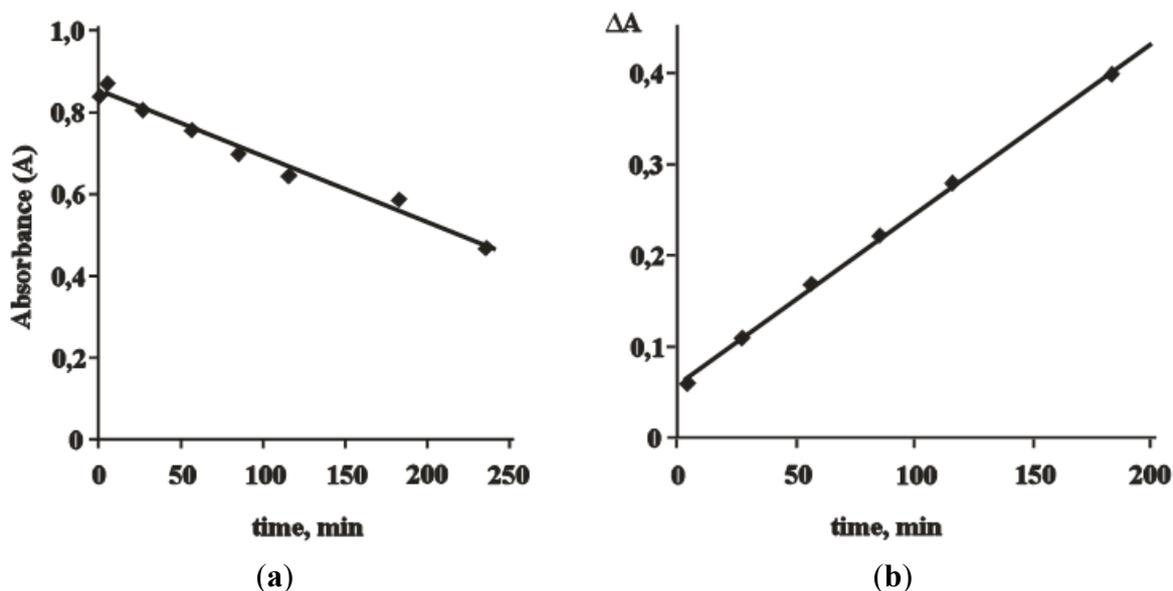


The decrease of the absorption at $\lambda_{\text{max}} = 360 \text{ nm}$ means the concentration of Cr^{6+} is decreasing and may be explained by the interaction of betulin (**1**) with $\text{Cr}^{6+} - \text{H}_2\text{SO}_4$ – oxidant resulting in formation chromate ester according to the Westheimer mechanism for the oxidation of alcohols [27]. The Westheimer mechanism involves the rapid initial formation of a chromate ester followed by the low rate determining, decomposition of the ester by removal of the α -proton by base B. In aqueous acetone the base was considered to be water. Some reports consider chromium oxidation in acid medium as a process of conversion to the protonated bimetallic chromium (VI) species, resultant in the formation of the monochromate ester which, under the decomposition in the rate-determining step, to give the product [25,27]. Monitoring the absorption at 360 nm in time $A = f(\tau)$ in the initial reaction mixtures was studied. The sampling was carried out from supernatant liquid using reaction mixture during synthesis of betulonic acid (**2**). Dilution of supernatant liquid by $\text{CH}_3\text{CN} - \text{DMSO}$ in a volume ratio of 10:1 conserved the homogeneity of the analyzed sample.

The absorbance (**A**) at 360 nm was decreased 2-fold during 1.5–2 h (Figure 7), while 100% conversion of betulin (**1**) was achieved during this time. The analysis of the crude from supernatant liquid corresponded only to the betulonic acid (**2**). This result was due to the participation of only monochromate ester in betulin oxidation although dichromate ester was formed initially.

The value of ΔA depends proportionally on the concentration of Cr^{6+} -containing complexes or ester with betulin. The linearity of absorbance against time plots means that the reaction was found to be of zero order with respect to Cr^{6+} -containing compound mathematically and, most probably, had catalytic character. Actually, it is very difficult to estimate kinetic parameters in such multistage reactions passing through parallel and sequential reactions (ester or complex formation, oxidation).

Figure 7. Dynamic range of the absorption at 360 nm (a) $A = f(\tau)$ during synthesis for supernatant liquid of reaction mixture under condition: $C_{\text{betulin}} = 2.01 \text{ mmol}\cdot\text{L}^{-1}$; $v_{\text{Cr}^{6+}}/v_{\text{betulin}} = 3$; $m_{\text{betulin}}/m_{\text{Al}_2(\text{SO}_4)_3} = 10$. 20-times dilution of sample was carried out by CH_3CN – DMSO in volume ratio as 10:1. (b) $\Delta A = f(\tau)$, where $\Delta A = A_{\text{in}} - A_{\text{car}}$, A_{in} – initial absorption; A_{car} – the absorption in a time of synthesis.



3. Experimental

3.1. General

IR spectra were run from on a Shimadzu IR-Prestige-21 instrument (KBr tablets). The ^1H - and ^{13}C -NMR spectra were recorded on a Bruker Advance DPX-200 or a Bruker DRX SF-500 spectrometers in $\text{DMSO}-d_6$ solution using TMS as an internal standard. The UV-Vis spectra were obtained on a Analytik Jena Specord S-100 instrument. Melting points were measured using the capillary tube method on an Electrothermal 9200 apparatus. HPLC analysis were done using a Shimadzu LC-10 Avp equipped with a Discovery C_{18} column ($250 \times 4.6 \text{ mm}$, $5\mu\text{m}$) and UV-detector. The eluent was acetonitrile—water 80:20 (v/v) as isocratic mobile phase at a flow rate of $1 \text{ mL}\cdot\text{min}^{-1}$ at $25 \text{ }^\circ\text{C}$, the injection volume was $20 \mu\text{L}$, the detection was accomplished at 210 nm , and the analysis time was 15 min . Residual traces of Al (III) and Cr (VI) were determined by a Shimadzu AA 7000 Atomic Absorption spectrophotometer using standard solutions. Electron-impact mass spectra (EI-MS) were obtained on a JMS-HX 110 mass spectrometer.

3.2. Materials and Reagents

Deionized water (pH 5.6, χ 18mSm) was used, solvents (acetone, isopropanol, methanol) were purified by known methods. As a solid support we used silica gel 60 (15–49, 40–63 and 63–200 μm , Merck), Alumina - $\gamma\text{-Al}_2\text{O}_3$ (Aldrich), zeolite 13X, NaA (270, 325 and 400 mesh, Merck). “Wet” solid support was formed by impregnation of oxidant (Cr^{6+} -compound– H_2SO_4).

Betulin (**1**, C₃₀H₅₀O₂) was prepared according to the literature method [28], m.p. 260 °C (lit. 254–256 °C [28]); purity 99.5%, IR, ν , cm⁻¹: 3470 st (OH), 1640 st (C=C); ¹H-NMR δ , ppm: 4.67 m (1H, =CH₂), 4.57 m (1H, =CH₂), 3.78 br. s (1H, 28-CH₂OH), 3.31 m (1H, 28-CH₂OH), 3.17 m (1H, 3-CHOH), 2.36 m (1H, 19-CH), 1.66 s (3H, CH₃), 1.23 s (3H, CH₃), 0.96 s (3H, CH₃), 0.94 s (3H, CH₃), 0.80 s (3H, CH₃), 0.74 s (3H, CH₃). ¹³C-NMR, δ , ppm: 76.71 (C-3), 109.46 (C-29), 150.24 (C-20), 57.87 (C-28). EI-MS m/z (%): 442 (M⁺, 40), 411 (60), 203 (95), 189 (100), 95 (85).

3.3. Oxidation of Betulin (**1**) to Betulonic Acid (**2**) by K₂Cr₂O₇-H₂SO₄ on Alumina

Granules of Al₂O₃ (9.2 g, A_{sp} = 500 m²/g) was treated with potassium dichromate solution (3 g, 10 mmol), H₂O (20 mL) and conc. H₂SO₄ (5 mL) in sequence. Betulin (**1**, 1.5 g, 3.4 mmol) was dispersed by ultra-sound in acetone (138 mL) to give a white suspension. Oxidant with “wet” alumina was added to the suspension of betulin in a reaction flask, then the reaction mixture was stirred for 1.5 h (HPLC-control). After the residue of inorganic compounds was removed, water (500 mL) was added to the liquid phase. Crude betulonic acid (**2**) precipitated (as white flakes) and after filtration **2** was washed multiple times with hot water. The precipitate (1.48 g, 97% yield) was recrystallized from methanol. The crystals were isolated and dried in a vacuum oven to afford pure (>95% by HPLC) **2**, m.p. 250–252 °C (methanol) (lit. 245–248 °C [6]). IR, ν , cm⁻¹: 1705 st (C=O), 1641 st (C=C); 883 st (=CH₂); ¹H-NMR δ , ppm: 4.68 s (1H, 29-H), 4.55 s (1H, 29-H), 2.23 m (1H, 19-H), 1.65 s (3H, 30-CH₃), 1.02–1.95 (3H, complex, CH₂, CH), 1.02 s (3H, 26-CH₃), 1.00 s (3H, 25-CH₃), 0.98 s (3H, 27-CH₃), 0.86 s (3H, 23-CH₃), 0.85 s (3H, 24-CH₃). ¹³C-NMR δ , ppm: 216.52 (C-3), 109.67 (C-29), 150.33 (C-20), 177.26 (C-28). EI-MS m/z (%): 454 (M⁺, 58), 248 (64), 219 (42), 205 (76), 189 (88), 136 (100), 121 (90). The precipitate of betulonic acid after recrystallisation (methanol) didn't include any residual traces of Cr and Al.

3.4. Oxidation of Betulin by K₂Cr₂O₇-H₂SO₄ on Silica Gel

Granules of SiO₂ (10 g, A_{sp} = 800 m²/g) were treated with potassium dichromate solution (1.5 g, 5 mmol), H₂O (40 mL) and conc. H₂SO₄ (2.5 mL) in sequence. Betulin (**1**, 1 g, 2.3 mmol) was dispersed by ultra-sound in acetone (40 mL) to give a white suspension. Oxidant with “wet” SiO₂ was added to suspension of betulin in a reaction flask, then the reaction mixture was stirred during 10 min (HPLC-control). After that the SiO₂ was removed and water (500 mL) was added to the liquid phase. The white flake precipitate was filtered, washed with hot water and was dried. The finished product was identified as betulonic aldehyde (**3**), m.p. 163–165 °C (lit. 165–166 °C [29]). IR, ν , cm⁻¹: 1730–1728 st (C=O), 1641 st (C=C); 883 st (=CH₂); ¹H-NMR δ , ppm: 9.67 s (1H, 28-CHO), 4.68 s (1H, 29-H), 4.55 s (1H, 29-H), 2.99 dd (1H, 3 α -H), 2.23 m (1H, 19-H), 1.65 s (3H, 30-CH₃), 1.02–1.95 (3H, complex, CH₂, CH), 1.02 s (3H, 26-CH₃), 1.00 s (3H, 25-CH₃), 0.98 s (3H, 27-CH₃), 0.86 s (3H, c, 23-CH₃), 0.85 s (3H, c, 24-CH₃); ¹³C-NMR δ , ppm: 216.52 (C-3), 109.67 (C-29), 150.33 (C-20), 206.55 (C-28). EI-MS m/z (%): 438 (11.5), [M-CHO]⁺ 409 (20.0), 273 (3.2), 219 (20.2), 205 (38.4), 189 (39.6), 133 (35.6), 105 (55.8), 81 (65), 55 (100).

3.5. Synthesis of Betulinic acid Using Crude Betulonic Acid (2)

The crude betulonic acid (2) was subjected to NaBH₄ (THF or isopropanol) reduction (10 molar ratio to betulonic acid) at room temperature to yield a mixture (5:95) of 3 α - and 3 β -betulonic acid (1.4 g), m.p. 295–300 °C (methanol), after recrystallization m.p. 310–311 °C (lit. 290–293 °C [30]). IR, ν , cm⁻¹: 3450 st (OH), 1683 st (C=O), 1641 st (C=C); 883 st (=CH₂); 1031, 1042 st (α , β -3-C-OH). ¹H-NMR δ , ppm: 4.68 s (1H, 29-H), 4.55 s (1H, 29-H), 3.24 t (1 H, 3 β -H), 2.99 dd (1H, 3 α -H), 2.23 m (1H, 19-H), 1.65 s (3H, 30-CH₃), 1.02–1.95 (3H, complex, CH₂, CH), 1.02 s (3H, 26-CH₃), 1.00 s (3H, 25-CH₃), 0.98 s (3H, 27-CH₃), 0.86 s (3H, 23-CH₃), 0.85 s (3H, 24-CH₃). ¹³C-NMR δ , ppm: 76.83 (C-3), 109.67 (C-29), 150.33 (C-20), 177.26 (C-28). EI-MS m/z (%): 457 (M⁺+H, 7), 307 (25), 154 (100), 136 (60). The precipitate of betulonic acid after recrystallization (methanol) didn't include anyresidual traces of Cr and Al.

4. Conclusions

We have developed the effective methods for selective oxidation of betulin up to carbonyl derivatives. K₂Cr₂O₇–H₂SO₄ oxidizes betulin to betulonic acid in aqueous acetone at 15–25 °C with 93%–98% yields in the presence of Al³⁺-ions. The same procedure using silica gel gives a single product—betulonic aldehyde—after 30 min.

The high selectivity of oxidation is determined by the protection of the interaction of betulin with Si⁴⁺ on silica gel surface or by π -complex formation of Al³⁺-ions as Lewis acid with terminal the double bond of the isopropenyl moiety. An alternative reason for the high selectivity may be complexes formed by betulin with chromium intermediate products, for example chromium ester, stabilized by [Al(OH)₂]⁺-ions. The main advantage of this procedure is the possibility to remove toxic Cr³⁺ from reaction mixtures by sorption on alumina and provide a simple and environmentally friendly reaction, resulting in a less a hazardous method for betulin oxidation.

References

1. Tolstikova, T.G.; Sorokina, I.V.; Tolstikov, G.A.; Tolstikov, A.G.; Flechter, O.B. Biological Activity and Pharmacological Prospects of Lupane Terpenoids: I. Natural Lupane Derivatives. *Russ. J. Bioorg. Chem.* **2006**, *32*, 37–49.
2. Kislitsin, A.N. Extractives of Birch Bark: Isolation, Composition, Properties and Application. *Wood Chem.* **1994**, *3*, 3–28.
3. Aiken, C.; Chen, C.H. Betulinic Acid Derivatives as HIV-1 Antivirals. *Trends Mol. Med.* **2005**, *11*, 31–36.
4. Motteran, L.; Pilone, M.S.; Molla, G.; Ghisla, S.; Pollegioni, L. Cholesterol Oxidase from *Brevibacterium sterolicum*. The Relationship between Covalent Flavinylation and Redox Properties. *J. Biolog. Chem.* **2001**, *276*, 18024–18030.
5. Bastos, D.Z.L.; Pimentel, I.C.; de Jesus, D.A.; de Oliveira, B.H. Biotransformation of Betulinic and Betulonic Acids by Fungi. *Phytochemistry* **2007**, *68*, 834–839.

6. Le, B.S.; Kaplun, A.P.; Shpilevskii, A.A.; Andiya-Pravdivyi, Y.E.; Alekseeva, S.G.; Grigor'ev, V.B.; Shvets, V.I. The Synthesis of Betulinic Acid from Betulin and Its Solubilization with Liposomes. *Russ. J. Bioorg. Chem.* **1998**, *24*, 10, 787–793.
7. Csuk, R.; Schmuck, K.; Schäfer, R. A Practical Synthesis of Betulinic Acid. *Tetrahedron Lett.* **2006**, *47*, 8769–8770.
8. Kim, D.S.H.L.; Chen, Z.; Van Tuyen, N.; Pezzuto, J.M.; Qiu, S.; Lu, Z.-Z. A Concise Semi-synthetic Approach to Betulinic Acid from Betulin. *Synth. Commun.* **1997**, *27*, 1607–1612.
9. Levdanskij, V.A.; Polezhaeva, N.A.; Kuznetsov, B.N. Method for Manufacturing Betulinic Acid. RU 2269541, 10 February 2006; *Chem. Abs.* **2006**, 126094.
10. Kogai, T.I.; Kuznetsov, B.N. Method for Manufacturing Betulinic Acid from Betulonic Acid. RU 2271364, 10 March 2006; *Chem. Abs.* **2006**, 218258.
11. Lee, C.-K. A New Norlupene from the Leaves of *Malaleuca Leucadendron*. *J. Nat. Prod.* **1998**, *61*, 375–376.
12. Chen, I.-H.; Du, Y.-C.; Lu, M.-C.; Lin, A.-S.; Hsieh, P.-W.; Wu, C.-C.; Chen, S.-L.; Yen, H.-F.; Chang, F.-R.; Wu, Y.-C. Lupane-type Triterpenoids from *Microtropis fokienensis* and *Perrottetia arisanensis* and the Apoptotic Effect of 28-Hydroxy-3-oxo-lup-20(29)-en-30-ol. *J. Nat. Prod.* **2008**, *71*, 1352–1357.
13. Ruzicka, L.; Lambertson, A.H.; Christie Ruzicka, C.W. Synthetic Approach to Betulinic Acid. *Helv. Chim. Acta* **1938**, *21*, 1706–1717.
14. Mansoor, S.S.; Shafi, S.S. Studies on the Kinetics of Benzyltrimethylammonium Fluorochromate Oxidation of Substituted Benzaldehydes in Aqueous Acetic Acid Medium. *Int. J. ChemTech Res.* **2009**, *1*, 1206–1212.
15. Chaubey, G.S.; Susngi, A.; Das, S.; Mahanti, M.K. Kinetic Features of the Oxidation of Aliphatic Dialdehydes by Quinolinium Dichromate. *Kinet. Catal.* **2002**, *43*, 789–793.
16. Medien, H.A.A. Kinetics of Oxidation of Benzaldehydes by Quinolinium Dichromate. *Z. Naturforsch.* **2003**, *58b*, 1201–1205.
17. Pichette, A.; Liu, H.; Roy, C.; Tanguay, S.; Simard, F.; Lavoie, S. Selective Oxidation of Betulin for the Preparation of Betulinic Acid, an Antitumoral Compound. *Synth. Commun.* **2004**, *34*, 3925–3937.
18. Jacobi, A.; Schroeder, J. (to Boehringer Ingelheim Pharma GmbH & Co. KG) Procedure for the Production of 3-Hydroxy Protected Betulinic Acid. Ger Pat 102004012951, 6 October 2005.
19. Shirini, F.; Zolfigol, M.A.; Khaleghi, M. Oxidation of Alcohols Using $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$ in the Presence of Silica Chloride/Wet SiO_2 in Solution and under Solvent Free Conditions. *Bull. Korean Chem. Soc.* **2003**, *24*, 1021–1022.
20. Lekhal, A.; Glasser, B.J.; Khinast, J.G. Impact of Drying on the Catalyst Profile in Supported Impregnation Catalysts. *Chem. Eng. Sci.* **2001**, *56*, 4473–4487.
21. Melnikova, N.B.; Burlova, I.V.; Kislitsin, A.N.; Klabukova, I.N. Method for Manufacturing Betulinic Acid. RU 242826, 10 September 2011.
22. Mohammadpoor-Baltork, I.; Sadeghi, M.M.; Adibi, A.H. Efficient, Solvent-Free Oxidation of Organic Compounds with Potassium Dichromate in the Presence of Lewis Acids. *Molecules* **2001**, *6*, 900–908.

23. Reutov, O.A.; Kurtz, A.L.; Butin, K.P. *Organic Chemistry*, 3rd ed.; Binom: Moscow, Russia, 2005; pp. 123–125.
24. Cao, D.; Zhao, G.; Yan, W. Solubilities of Betulin in Forteen Organic Solvents at Different Temperatures. *J. Chem. Eng. Data* **2007**, *52*, 1366–1368.
25. Greenwood, N.N.; Ernschaw, A. *Chemistry of Elements*; Binom: Moscow, Russia, 2008; Volume 1, p. 601.
26. Larkworthy, L.F.; Nolan, K.B.; O'Brien, P. Chromium. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R.D., McCleverty, J.A., Eds.; Pergamon Press: Oxford, UK, 1987; Volume 3, p. 699.
27. Lee, D.G.; Downey, W.L.; Maass, R.M. Kinetics of the Chromic Acid Oxidation of Alcohols in Aqueous Acetone Solutions. *Can. J. Chem.* **1968**, *46*, 441–449.
28. Unusov, M.S.; Komissarova, N.G.; Belenkova, N.G. Method for Manufacturing Betulin and Lupeol. RU 2270201, 20 February 2006; *Chem. Abs.* **2006**, 137751.
29. Monaco, P.; Previtera, L. Isoprenoids from the Leaves of *Quercus suber*. *J. Nat. Prod.* **1984**, *47*, 673–676.
30. Fujioka, T.; Kashiwada, Y. Anti-Aids Agents. Betulinic Acid and Platonic Acid as Anti-HIV Principles from *Syzygium claviflorum*, and the Anti-HIV Activity of Structurally Related Triterpenoids. *J. Nat. Prod.* **1994**, *57*, 243–247.

Sample Availability: Samples of the compound **1** and **2** are available from the authors.

© 2012 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).