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Development of the Method of Obtaining Betulin Diacetate and Dipropionate from Birch Bark

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Abstract

Methods of synthesis of betulin diacetate and dipropionate from birch bark are proposed. The methods are based on combined extraction of betulin from birch bark and its acylation with acetic and propionic acids. The structure of the resulting betulin derivatives was confirmed by means of chromatography-mass spectrometry, FT-IR and NMR spectroscopy.

Key words: birch bark, acylation, betulin diacetate, dipropionate, identification

INTRODUCTION

One of the promising directions of birch bark utilization is its extractive treatment with obtaining a number of valuable chemical components among which the most relevant is betulin. Betulin and its derivatives exhibit high biological activity; new pharmacological preparations on its basis are being developed [1–5]. Betulin diacetate exhibits hypolipidemic, choleric and antioxidant action, it serves as the raw material for many organic syntheses, such as the synthesis of betulinic acid, sulphur-containing betulin derivatives, amino derivatives of betulin diacetate *etc.* [1, 6–8].

As a rule, the methods of betulin esters are multistage and based on classical reactions of betulin acylation; betulin should be preliminarily extracted from birch bark [9, 10].

A specific feature of the structure of birch bark is the manifestation of annual rings that are composed of the rows of wide and narrow cells. Wide cells have thin walls and are filled with betulin. While a tree is growing, birch bark gets flaked, and the external row of thin-walled cells is split, so betulin contained in them spills out. The indicated features of the struc-

ture of outer birch bark were used by us to develop new methods of the synthesis of diacyl derivatives of betulin excluding the stage of betulin extraction from birch.

Previously we proposed a new method to synthesize betulin diacetate from birch bark; it allows us to combine the stages of betulin extraction and acetylation [11]. It may be assumed that other betulin diacylates, *e.g.* propionates and butyrates, can be synthesis using a similar method. The goal of the present work was to obtain betulin diacetate and dipropionate from birch bark and to choose the conditions providing the higher yield of the product.

EXPERIMENTAL

The external layer of the bark of birch, *Betula pendula* Roth. was used as the raw material. Birch bark was collected in the vicinity of Krasnoyarsk and ground to particle size of 10–20 mm. Birch bark activated under the conditions of explosive autohydrolysis was also used.

Analysis of the chemical composition of the external layer of birch bark was carried out

TABLE 1

Chemical composition of initial and autohydrolyzed birch bark, % of the mass of absolutely dry birch bark

Compounds	Initial	Autohydrolyzed*
Betulin	44.6	38.8
Suberin	20.2	45.5
Cellulose	1.5	3.3
Lignin	18.1	10.3
Ash	0.3	0.3
Water-soluble	0.4	1.6

*After treatment with vapour at a temperature of 180 °C, pressure 3.4 MPa for 60 s.

using the conventional procedures of wood chemistry and original approaches described in [12–14].

The data on the chemical composition of the samples of initial and autohydrolyzed external part of birch bark are presented in Table 1.

The synthesis of betulin diacetates was carried out by treating the samples of birch bark, ground and fractionated or autohydrolyzed and dried at a temperature of 105 °C to the humidity of less than 2 %, with acetic and propionic acids at their boiling points in a round-bottomed flask equipped with a backflow condenser. After the reaction was complete, the solution was concentrated with a rotary evaporator; the residue was diluted with a five-fold amount of water. The precipitated substance was separated by filtering, washed multiply on the filter with distilled water and dried in the air. The product was recrystallized from ethanol using activated coal of OU-A grade (GOST 4453–74). For the purpose of intensifying acetylation of birch bark in boiling acetic acid and in order to increase the yield of the products of acetylation, birch bark was activated by means of explosive autohydrolysis. This method involves short-time treatment of birch bark with saturated water vapour at increased temperature and pressure, followed by sharp pressure drop. The efficiency of activation of plant raw material by means of explosive autohydrolysis depends on two major factors: hydrolysis of weak bonds in plant polymers under the action of organic acids formed in the decomposition of hemicelluloses, and mechanical loosening of the raw material under pressure drop. This activation may intensify extraction of betulin from birch bark [15–17].

The morphology of the initial and activated external layer of birch bark was studied with a REM-100U scanning electron microscope in the Krasnoyarsk Collective Use Centre (KCUC) of the KSC, SB RAS. Figure 1 shows electron microscopic images of the initial bark and the sample activated with water vapour. Autohydrolyzed birch bark has loosened structure, which simplifies the transport of reagents in the pores of birch particles and diffusion of reaction products in the solution.

Analysis of the products was carried out using an Agilent 6890 N GC (USA) gas chromatograph with flame ionization detector and a capillary column (HP-5, 30 m × 0.32 mm). The

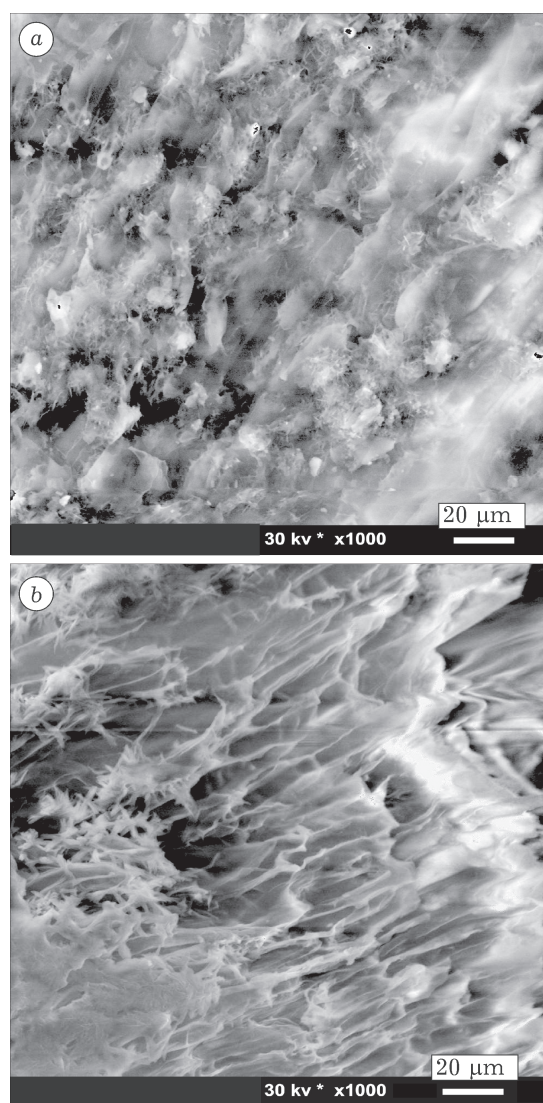


Fig. 1. Electron microscopic images of birch bark: initial (a) and activated with overheated water vapour (b). Magnification 1000.

flow rate of nitrogen carrier gas was 1.5 mL/min. Temperature sample introduction: 250 °C; initial temperature: 180 °C; temperature rise to 320 °C at a rate of 10 °C/min; isothermal mode for 10 min. Identification was carried out on the basis of retention time values for standards.

To identify the products of birch bark acetylation, analysis by means of gas chromatography-mass spectrometry was carried out with a mass spectrometer (GCD Plus, Hewlett Packard, USA) in the KCUC of the KSC, SB RAS. Chromatography conditions: temperature of sample introduction: 250 °C; heating rate: 5.0 °C/min, temperature of transfer line: 250 °C, temperature of ion source: 165 °C, electron beam mode at 70 eV; mode of scanning the fragments from 45 to 450 m/z at 0.5 s/s. A capillary column HP-5S (30 m \times 0.25 mm) was used. The concentrations of components were determined from the areas of gas chromatographic peaks. Qualitative analysis of the products was carried out by comparing the data on retention times and complete mass spectra with the corresponding data from the mass spectral database.

Figure 2 shows the ion chromatograms of the products of birch bark acetylation with acetic acid;

Fig. 3 shows the mass spectra of lupeol, betulin **I** and betulin diacetate **II**. It was established that the major components of products extracted from birch bark are betulin **I** and betulin diacetate **II**. In addition, lupeol and lupeol acetate are present in small amounts. Identification of betulin **I** by comparing with the database of mass spectra was carried out with the good probability. The mother ion coinciding with the molecular mass of the compound is m/z 442. The identifying fragment which is formed after the detachment of the alcohol group in position 28 is the fragment with m/z 411. In addition, the fragments with m/z 69, 81, 95, 107, 189, 207 were identified, which points to the fact that this compound belongs to the lupan series. Another identified compound is lupeol (m/z 426). According to the data obtained, the product of birch bark acetylation recrystallized from ethyl alcohol contains betulin diacetate **II** 95.3 % and lupeol acetate 4.7 %.

Betulin diacetate **II** (3 β ,28-di-O-acetyl-lup-20(29)-lupene) extracted and recrystallized from ethyl alcohol is coloured light-cream, its purity is 95 %, its melting point is 222 °C.

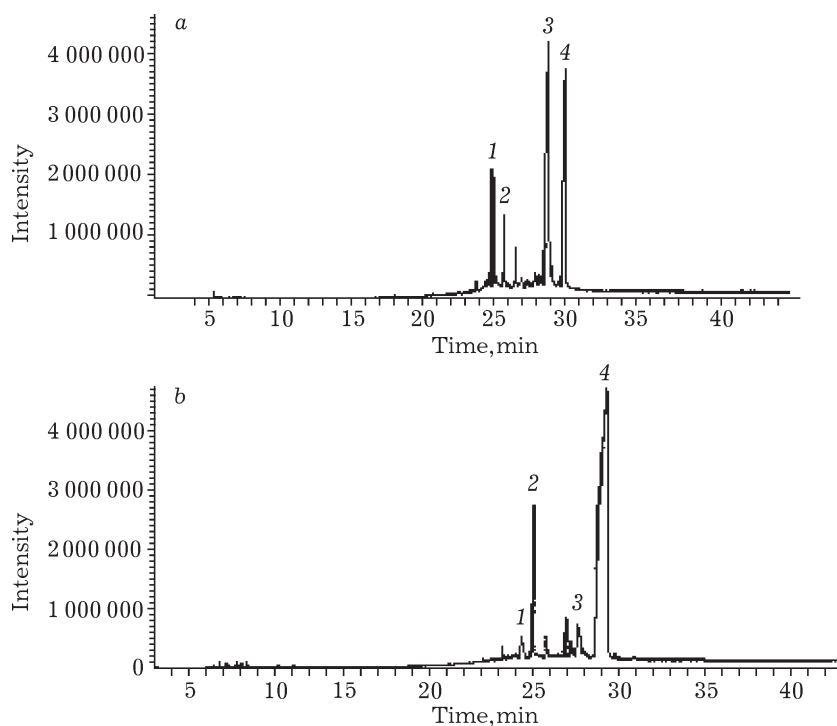


Fig. 2. Ion chromatograms of the products of acetylation of birch bark, fraction 10–20 mm, with acetic acid, after treatment for 0.5 (a) and 18 h (b): 1 – lupeol, 2 – lupeol acetate, 3 – betulin, 4 – betulin diacetate.

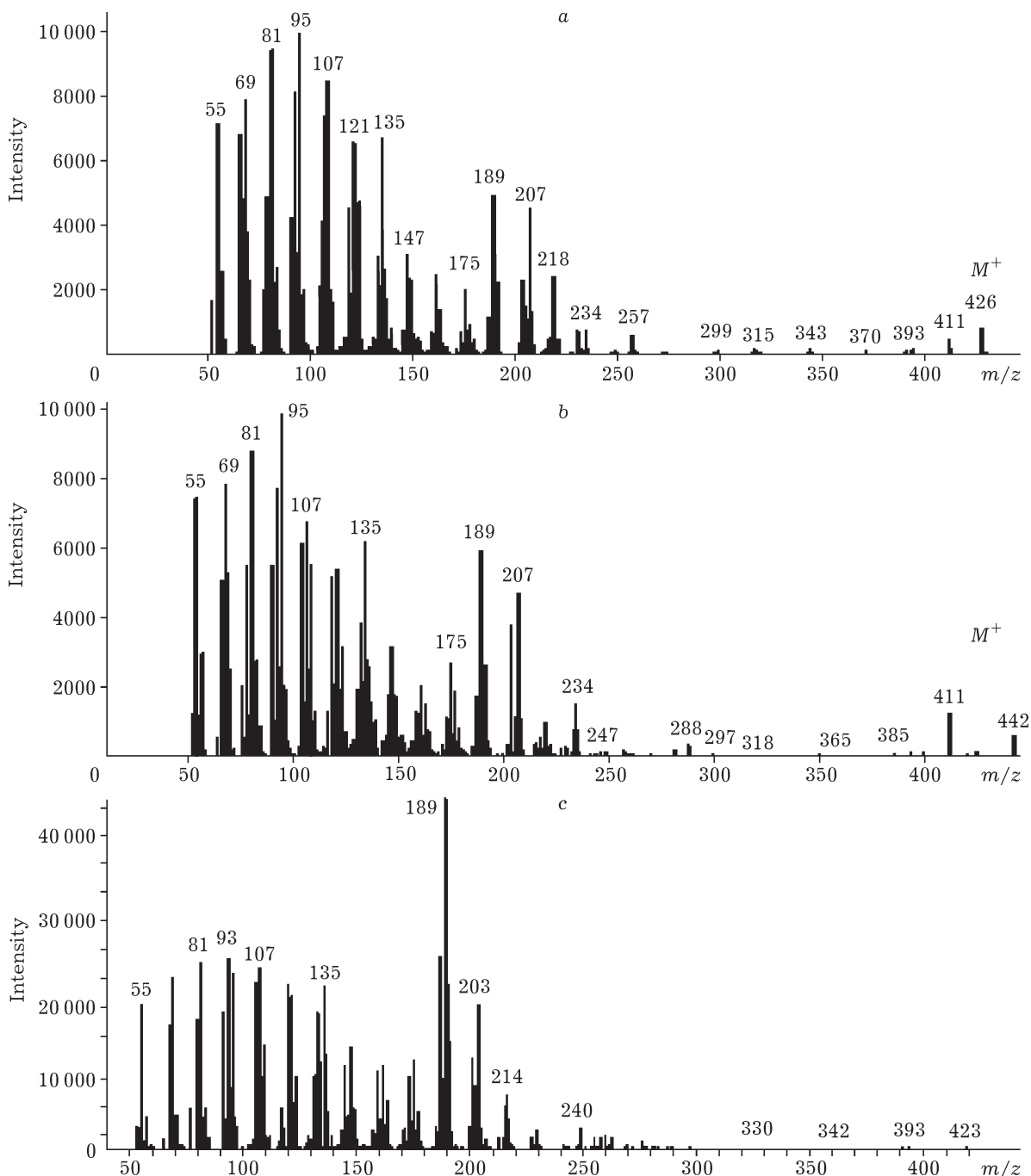


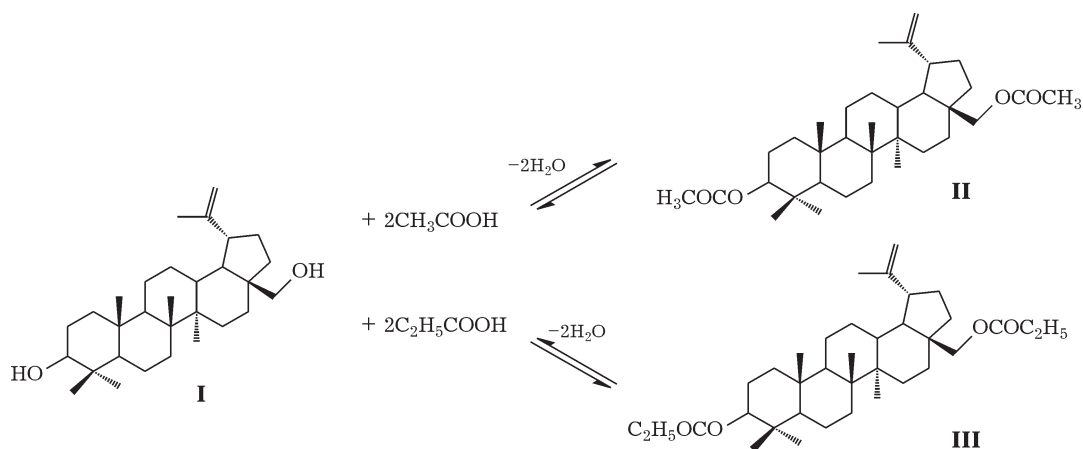
Fig. 3. Mass spectra of lupeol (a), betulin (b), and betulin diacetate (c).

The elemental analysis of recrystallized products was carried out with the help of element analyzer FLASHTM 1112. According to the data of elemental analysis of betulin diacetate **II**, found, %: C 77.9, H 10.2, O 11.9. Calculated, %: C 77.6, H 10.3, O 12.1.

Betulin dipropionate **III** ($3\beta,28$ -di-*O*-propionyl-lup-20(29)-lupene) extracted and recryst-

tallized from ethyl alcohol is light yellow, its purity is 93 %, its melting point is 150–152 °C. According to the data of elemental analysis of betulin dipropionate **III**, found, %: C 80.0, H 10.8, O 12.0. Calculated, %: C 77.9, H 10.5, O 11.6.

IR spectroscopic examination of betulin diacylates was carried out with a Bruker Vector 22 FT-IR spectrometer. Purified products were



I – betulin, **II** – betulin diacetate, **III** – betulin dipropionate

Scheme 1.

pressed with KBr into a special matrix. The spectral information was processed using the OPUS/Y software (version 2.2).

The following characteristic absorption bands (a. b.) are present in the IR spectrum of betulin diacetate **II** (ν , cm⁻¹): 3070.90 (=C-H), 1739.93 (C=O), 1649.86 (C=C), 1246.14, 1085.78 (C-O-C). The IR spectrum of betulin dipropionate **III** contains characteristic a. b. (ν , cm⁻¹): 3067.02 (=C-H), 1735.79 (C=O), 1641.88 (C=C), 1276.09, 1083.88 (C-O-C).

The ¹H NMR spectra were recorded at the KCUC of the KSC with a Bruker DPX-200 spectrometer at the frequency of 200 MHz (= ¹H), solvent: C₆D₆.

The ¹H NMR spectrum of betulin diacetate **II** coincides with the data reported in [7].

The ¹H NMR spectrum of betulin dipropionate **III** (C₆H₆), δ , ppm (*J*, Hz): 4.83 (1H, m, =CH₂); 4.74 (1H, m, =CH₂); 4.72 (dd1H, H₃); 4.53 (1H, d, H₂₈); 4.0 (1H, d, C₂₈H); 2.126 (2H, q, CH₂, COCH₂CH₃); 2.124 (2H, q, CH₂, COCH₂CH₃); 1.034 (3H, CH₃, COCH₂CH₃); 1.015 (3H, CH₃, COCH₂CH₃); 1.676 (3H, CH₃, CH₂=CHCH₃); 0.945, 0.938, 0.907, 0.900, 0.737 (15H, 5 CH₃).

The yield of the products of acetylation of non-activated birch bark with acetic acid (fraction 10–20 mm) was 20 to 45 mass % of absolutely dry birch bark (a.d.b.b.) after acetylation for 0.5 to 10 h. The maximal content of betulin diacetate **II** in non-recrystallized product reach-

es 84 mass %. After acetylation of the same fraction of non-activated birch bark with propionic acid for the same time, the yield of products was 29 to 51 mass % of a.d.b.b. The maximal concentration of betulin dipropionate **III** in non-recrystallized product is about 90 mass %.

RESULTS AND DISCUSSION

Effect of the conditions of birch bark acylation on the yield of extractable products

During the treatment of birch bark with acetic and propionic acids, along with betulin extraction into solution, its acylation with the formation of diacetate (**II**) or dipropionate (**III**) of betulin occurs (Scheme 1).

The optimal conditions of birch bark acylation were chosen by varying the duty value (the ratio of liquid to solid) from 10 to 30 and the duration of acid treatment from 0.5 to 18 h. Figure 4 shows the results of the investigation into the effect of duty value on the yield of the products of birch bark acylation with acetic and propionic acids. For duty value equal to 10, the products of birch bark acylation with acetic and propionic acids were obtained with the yields of 30 and 42 % of the mass of a.d.b.b., respectively. The yield of birch bark acylation products increases with an increase in the duty value to 20 and 30 reaching 42 and 48 % of

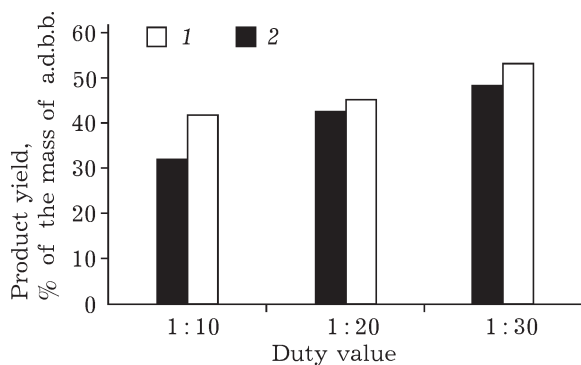


Fig. 4. Effect of the duty value on the yield of products of birch bark acylation with acetic (1) and propionic acid (2).

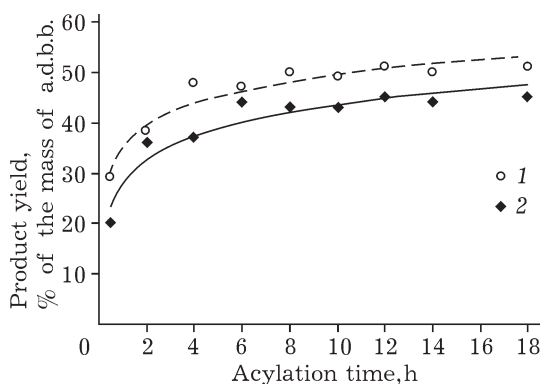


Fig. 5. Effect of the time of birch bark acylation with acetic and propionic acids on the yield of products, for duty value equal to 20: 1 – betulin diacetate, 2 – betulin dipionate.

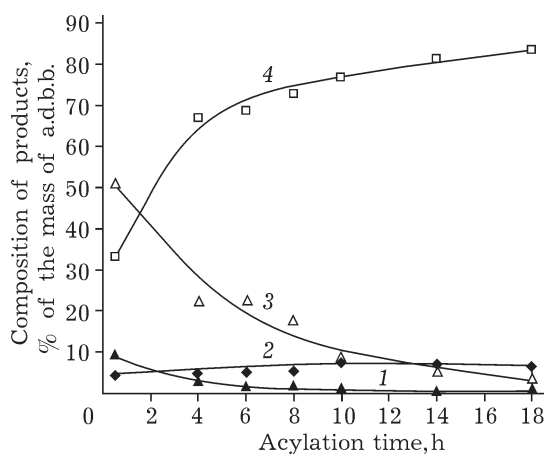


Fig. 6. Effect of the duration of acetylation of birch bark with acetic acid on the composition of the products: 1 – lupeol, 2 – lupeol acetate, 3 – betulin, 4 – betulin acetate.

the mass of a.d.b.b. for propionic acid, 45 and 53 % of the mass of a.d.b.b. for acetic acid, respectively.

The effect of the duration of birch bark acylation with acetic and propionic acids on the yield of products is shown in Fig. 5. With an increase in acylation time from 30 min to 8 h, the yield of the products of acylation with acetic acid increases from 20 to 43 % of the mass of a.d.b.b., while with propionic acid the yield increases from 29 to 50 % of the mass of a.d.b.b. The yield remains unchanged with further increase in treatment time (to more than 8 h).

The GC-MS data on the effect of the time of birch bark treatment (fraction 2–5 mm) with acetic acid on the composition of acetylation products are presented in Fig. 6. After acylation for 6 h, the product contains about 70 mass % betulin diacetate **II**. With an increase in acetylation time from 0.5 to 18 h, the concentration of betulin diacetate **II** in the product increases from 33 to 84 mass %, while the concentration of betulin **I** decreases from 51 to 4 mass %. We also observe a slight decrease in the concentration of lupeol and an increase in the concentration of lupeol acetate in the product.

Effect of birch bark activation with water vapour on the yield of acylation products

A known method to enhance the reactivity of solid plant raw material is its short-term activation with overheated water vapour under the conditions of explosive autohydrolysis. This kind of activation with water vapour was used to intensify birch bark acylation with acetic and propionic acids. Preliminary treatment of birch bark with water vapour was carried out at a temperature of 180 °C, pressure 3.4 MPa; treatment time was varied from 60 to 300 s.

The data on the effect of the time of activation with water vapour and the time of birch bark acylation with acetic and propionic acids on the yield of products are presented in Tables 2 and 3.

Experimental results showed that the preliminary activation of birch bark under the conditions of explosive autohydrolysis allows one to decrease the duration of acylation process by a factor of 12. After activation and acylation of birch bark for the shortest time interval (60 s and 30 min, respectively), the yield of the products of acetylation with acetic acid

TABLE 2

Effect of the time of birch bark activation with water vapour and the time of acetylation with acetic acid on the yield of products

Acetylation time, h	Activation time, s					
	0	60	120	180	240	300
	Yield of products, % of the mass of a.d.b.b.					
0.5	20	47	51	54	53	54
2	36	52	54	52	52	51
4	39	50	54	53	54	56
6	44	49	52	60	58	58
8	43	59	57	60	60	57

TABLE 3

Effect of the time of birch bark activation with water vapour and the time of acylation with propionic acid on the yield of products

Acylation time, h	Activation time, s					
	0	60	120	180	240	300
	Yield of products, % of the mass of a.d.b.b.					
0.5	29	43	45	47	47	44
2.0	38	45	50	49	51	48
4.0	48	47	51	49	54	49
8.0	50	47	53	50	54	50

increases up to 47 % of the mass of a.d.b.b., while for the products of acylation with propionic acid the yield increases to 43 % of the mass of a.d.b.b. After recrystallization from ethyl alcohol, the purity of resulting betulin diacylates is 93–95 %.

Tests of the samples of betulin diacetate **II** and betulin dipropionate **III** for toxicity, carried out with white mice at the certified testing centre of Krasnoyarsk (Veterinary Laboratory of the Krasnoyarsk Territory) showed that betulin diacetate **II** and betulin dipropionate **III** at the dose of 5000 and 4000 mg/kg, respectively, are not toxic and can be used for pharmacological tests.

CONCLUSION

A new single-stage method of the synthesis of betulin diacetate (**II**) and dipropionate (**III**) directly from the upper layer of birch bark excluding the stage of betulin extraction is pro-

posed. This method is based on the combination of betulin extraction from birch bark and its esterification with either acetic or propionic acid.

Identification of the products was confirmed using physicochemical methods.

The conditions of birch bark acylation with acetic and propionic acids were chosen to provide the maximal yield of betulin diacetate (**II**) and dipropionate (**III**). It was shown that the highest yield of the products of acylation from non-activated birch bark with acetic and propionic acids (about 50 % of the mass of a.d.b.b.) is observed with the duty value of 20 and acylation time longer than 8 h; the maximal yield of betulin diacylates in the products is achieved (84 and 90 mass %, respectively).

It was established that preliminary activation of birch bark under the conditions of explosive autohydrolysis for 60 to 300 s allows one to decrease the duration of acylation with propionic and acetic acids to 0.5 h and to increase the yield of products to 47–54 % of the mass of a.d.b.b., respectively. The purity of betulin diacylates after recrystallization from ethyl alcohol is 93–95 %.

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