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Mechanochemical Preparation of the Composites of Betulin Esters with Arabinogalactan and Investigation of Their Physicochemical Properties

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Abstract

Mechanically activated composites of betulin diacetate and dipropionate with water-soluble natural polysaccharide arabinogalactan were obtained. It was established that the composites are characterized by increased concentration of betulin diacyls during their dissolution in water. The formation of molecular complexes of betulin diacetate and dipropionate with arabinogalactan during mechanical activation and during the dissolution of initial and mechanically activated mixtures in water was assumed. The complexes of betulin diacyls with arabinogalactan were also obtained in the form of thin films, readily soluble in water.

Key words: betulin diacetate, betulin dipropionate, arabinogalactan, mechanochemical treatment, dissolution, gel-permeation chromatography

INTRODUCTION

Betulin esters and the simplest carboxylic acids are obtained from the birch bark: betulin diacetate 3β,28-diacetoxy-lup-20(29)-ene (DAB) and betulin dipropionate 3β,28-dipropioxy-lup-20(29)-ene (DPB) (Fig. 1).

It has been established that betulin diacetate possesses hepatoprotective, antioxidant, antitumour and other pharmacological activities. Betulin dipropionate is interesting for pharmacy, too [1–5]. One of the factors holding back the use of betulin diacyls is their poor solubility in water and other solvents.

It is known that mechanical activation of pharmaceutical substances in the presence of auxiliary substances promotes an increase in dissolution rate and solubility of the preparations, thus their biological availability increases [6, 7]. The mechanical activation involves disordering of the crystal structure of pharmaceutical substances and their dispersion in the carrier matrix with the formation of composite materials. Due to this, the rate of dissolution and solubility of substances increases. We demonstrated previously [8] that the mechanical activation of betulin in mixtures with water-soluble polymers, that is polyvinylpyrroli-

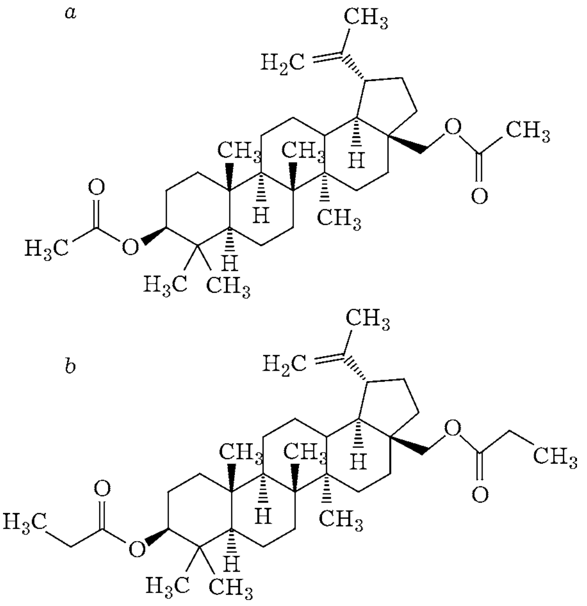

Fig. 1. Structural formulas of betulin diacetate (*a*) and betulin dipropionate (*b*).

done (PVP) and polyethylene glycol (PEG) leads to the formation of composites characterized by betulin structural disordering and its interaction with carriers. During the dissolution of mechanocomposites in water, betulin concentration in a solution turned out to be higher than after the dissolution of initial betulin. However, the mechanical treatment of DAB with PVP and PEG did not cause an increase in its solubility. This may be connected with the fact that DAB does not get disordered during mechanical treatment with polymers and does not form molecular complexes with PVP and PEG [8, 9].

Arabinogalactan (AG) is a water-soluble natural polysaccharide which is extracted from larch wood and is used as a stabilizer, filler, carrier for pharmaceutical substances. The quality and properties of this [polymer are strongly affected by its molecular mass. The preparation of intermolecular complexes of AG with some poorly soluble pharmaceuticals allowed improving their pharmacological characteristics [10–13]. In this connection, it was interesting to use AG for the preparation of mechanically activated composites with betulin diacyls.

The goal of the present work was to obtain the composites of betulin diacetate and dipropionate with AG and study their physicochemical properties.

EXPERIMENTAL

DAB, DPB and AG obtained according to the developed original procedures were used [14–16].

The mechanochemical treatment was carried out using SPEX 8000 mill (CertiPrep Corp., the USA) in steel cylinders 40 mL in volume. The treatment mode was: milling bodies – steel balls 6 mm in diameter, ball mass 30 g, acceleration of milling bodies 8–10*g*, mass of the mixture under treatment 1 g, maximal treatment time 30 min. The ratio of DAB (DPB)/AG was 1 : 9 (by mass).

X-ray phase analysis was carried out using a D8 DISCOVER diffractometer with two-coordinate detector (Bruker), CuK_α radiation, $2\theta = 5–40°$. IR spectra were recorded by means of disturbed complete internal reflection within frequency range 4000–500 cm^{-1} using an Excalibur 3100 Fourier-Transform IR spectrometer (Varian) without sample pressing. Electron micrographs were taken with a TM-1000 scanning electron microscope (Hitachi, Japan). Thermoanalytical measurements were carried out with a DSC-204 calorimeter (Netzsch). Standard aluminium crucibles were not sealed but closed with caps. Heating rate was 6 K/min, argon flow rate was 20 mL/min, and weighted portion of sample was 5.0 mg.

The molecular mass distribution of samples was studied by means of gel penetrating chromatography with an Agilent 1200 instrument equipped with refractometer detector 1260 Infinity (30 °C, PL aquagel-OH 40 300×7.5 mm, 0.1 M $LiNO_3$, 1 mL/min). Column calibration was carried out with the help of standard dextran samples (Sigma-Aldrich) with molecular masses 10 600, 20 000, 41 272, 70 000. Chromatograms were normalized per maximum absorption of the refractometer detector.

Studies of the solubility of composites in water were carried out in the solubility tester Varian 705 DS. A weighted portion of DAB-AG or DPB-AG 200 mg in mass composite was placed into the vessel with water and kept for 2 days under permanent mixing at 37 °C. Then the solution was filtered through the filters with pore diameter 0.2 μm. Filtrates were evaporated at reduced pressure and temperature 35–40 °C (IR-IM rotary evaporator, Russia). The dry residue (9–10 mg) was subjected to a triple extraction with chloroform. The chloroform extracts were united and evaporated; the solid residue after evaporation was dissolved in 1 mL of ethanol. DAB and DPB concentrations in ethanolic solutions were determined chromatographically with Milikhrom A-02 chromatograph (Ekonova, Russia) (chromatographing conditions: 35 °C, column 2.0×75 mm, ProntoSil, 120-5C18 AQ, particle size 5.0 µm, mobile phase H_2O (A)–CH_3CN (B), gradient elution 80–100–100 % B, the flow rate of the mobile phase 100 µL/min) at the wavelengths of 200 and 210 nm.

RESULTS AND DISCUSSION

After the mechanical activation of mixtures of betulin diacyls with AG for 5 min, reflections attributed to initial DAB and DPB are still noticeable in the diffraction patterns. However, after the activation for 15 min their intensity decreases substantially, and these reflections disappear almost completely after mechanical treatment for 30 min (Fig. 2). The disappearance of reflections related to betulin diacyls may be the evidence of DAB and DPB distribution in AG matrix with the formation of X-ray amorphous composites.

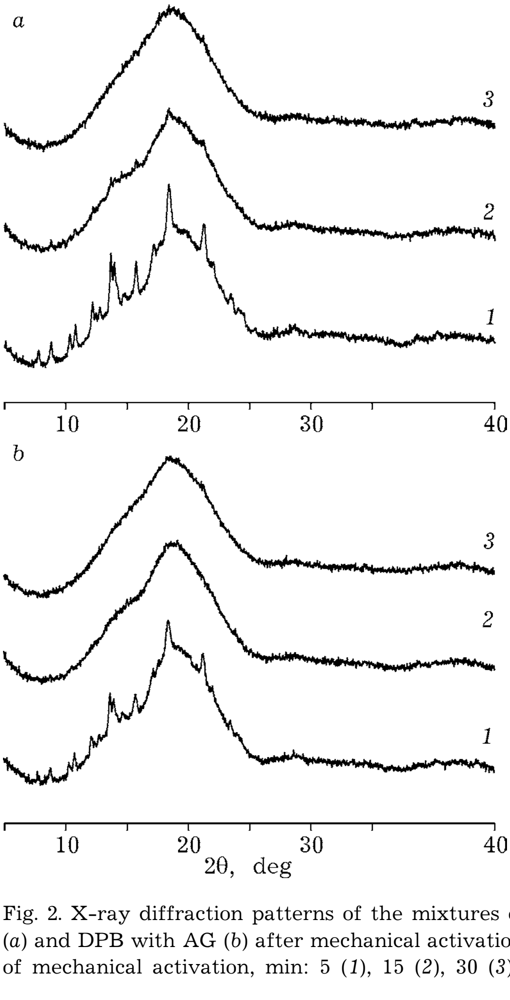

Fig. 2. X-ray diffraction patterns of the mixtures of DAB (*a*) and DPB with AG (*b*) after mechanical activation. Time of mechanical activation, min: 5 (*1*), 15 (*2*), 30 (*3*).

The same evidence is provided by the data of differential scanning calorimetry. Only the traces of DPB melting peak are observed on the DSC curve of the mechanically activated mixture of DPB with AG (Fig. 3). A large endothermal peak that starts almost at room temperature and finishes at ~150 °C corresponds to water removal from AG. A weak peak of DPB melting with the maximum at around 160 °C is shown in Fig. 3 on a magnified scale. The presence of this peak suggests that not the whole matter may have transformed into the amorphous state, or its part was crystallized during heating. Nevertheless, the absence of the true peak of DPB melting on the DSC curve provides evidence of the formation of rather strong DPB–AG composite during mechanical treatment. This may be the distribution of betulin diacyl molecules (or an ensemble of molecules) in the polymer, as it was proposed for some other low molecular mass pharmaceutical substances in [11, 12], or DAB and DPB dispersing to the nanometer-sized state with the formation of a uniform mechanical mixture with AG. The stability of this composite to heating may be due to the interaction between components during mechanical activation.

Electron microscopic images of the samples of initial betulin diacyls and mechanically activated (MA) DAB–AG and DPB–AG compos-

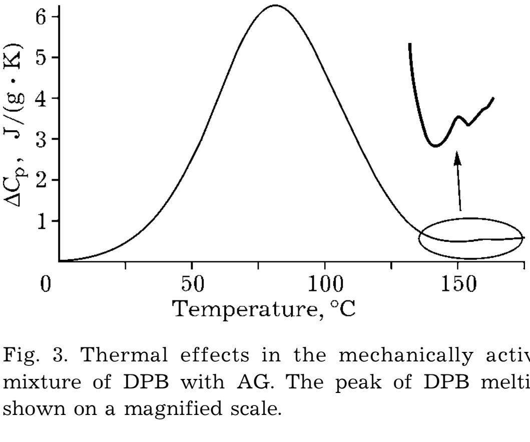

Fig. 3. Thermal effects in the mechanically activated mixture of DPB with AG. The peak of DPB melting is shown on a magnified scale.

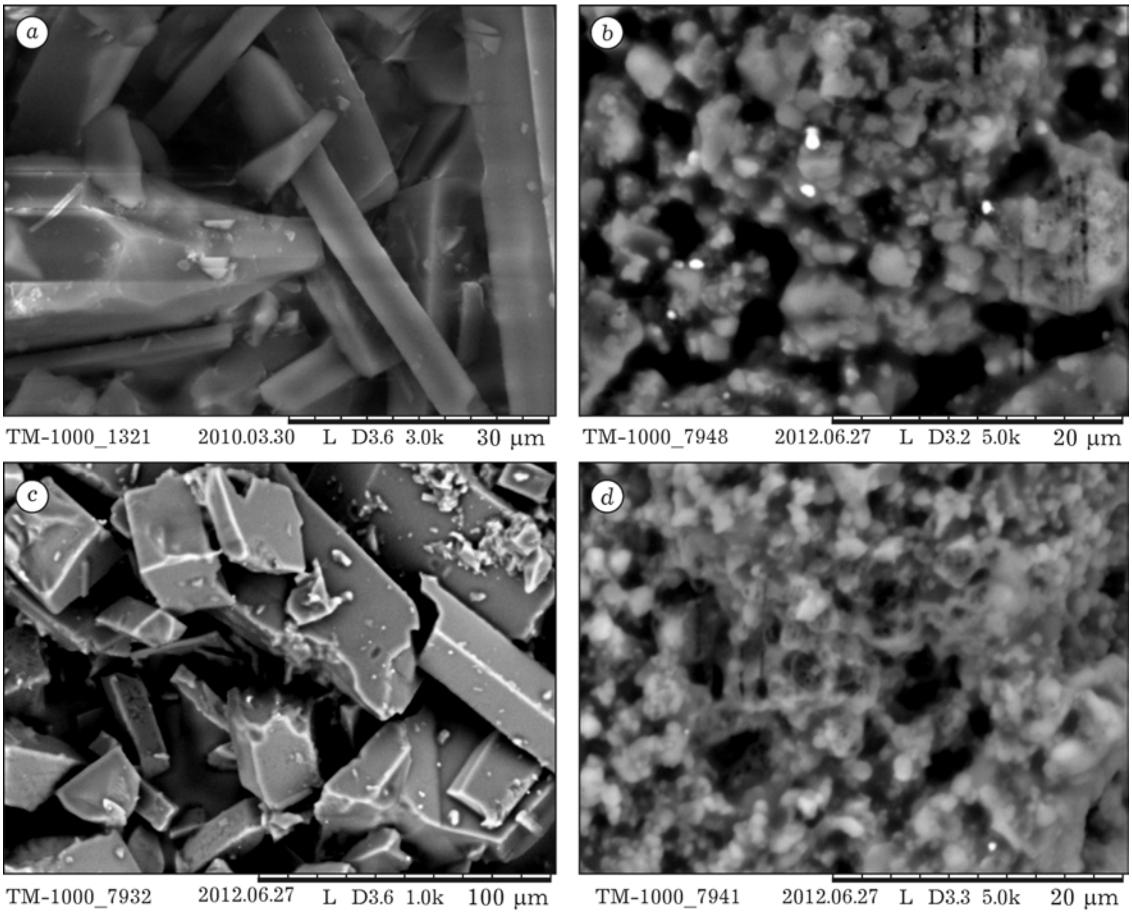

Fig. 4. Electron microscope images: *a* – DAB; *b* – mechanically activated DAB–AG composite; *c* – DPB; *d* – mechanically activated DPB–AG composite.

ites are presented in Fig. 4. Initial crystals of betulin diacyls have needle-like shape with the size 10 to 100 μm. Mechanically treated mixture of betulin diacyls with AG is uniform and composed of the aggregates of particles 5–30 μm in size.

In the IR spectra of the mixtures of DAB and DPB with AG after the mechanical activation (Fig. 5), broadening and a decrease in the intensities of bands are observed in the region of stretching vibrations ν(O–H) (3200–3500 cm^{-1}), ν(C=O) (1720 cm^{-1}), ν(C–O) (1150 cm^{-1}) is observed, which may be caused by amorphization of betulin diacyls. It is difficult to reveal any shifts of diacyl and AG bands, so it seemed impossible to reveal the presence of hydrogen bonds between the components. It may be assumed that the bonds between betulin diacyls and AG in mechanically activated mixtures are due to van der Waals interactions.

As mentioned previously, initial DAB and DPB are practically insoluble in water. Table 1 presents the data on DPB concentration in water after the dissolution of initial mixtures with AG and the mixtures mechanically activated for 15 min. One can see that after the mechanical activation of DPB with AG the concentration of DPB in aqueous solution increases almost by a factor of 4 in comparison with the physical mixture and reaches 10.8 μg/mL. An increase

TABLE 1

DPB concentration in water after the dissolution of initial and mechanically activated mixtures of DPB with AG

Mixtures	Dissolution time, h	Concentration, μg/mL
Initial	24	2.8
Mechanically activated	1	2.9
	2	3.8
	3	4.4
	21	5.3
	24	10.8

in the time of mechanical activation to 30 min did not promote an increase in the solubility of the composite. The data on the dissolution of DAB–AG composite also provide evidence of an increase in DAB concentration in solution to 9.4 μg/mL.

The changes of molecular mass distribution caused by mechanical treatment were studied. It was established that molecular mass (*M*) for some AG molecules decreases approximately by a factor of 2 – from ~16000 to ~8000, as it was observed in [13] for mechanical treatment in other milling devices. Indeed, gel chromatogram of AG (Fig. 6, *a*, curve *1*) has the major maximum with $\log M = 4.2$. Mechanical activation for 10 min leads to its splitting as a consequence of the formation of a new maximum with $\log M = 3.9$; after activation for 30 min, a complete shift of the maximum from $\log M = 4.2$ to $\log M = 3.9$ occurs (see Fig. 6, *a*, curve *3*). The ratio of peak areas $\lg M = 3.9/\log M = 4.2$ (in coordinates: intensity–retention time) after activation for 10 and 30 min is 1.2 and 6.3, which corresponds to the degree of AG transformation into the low molecular state ~55 and 87 %, respectively.

The dissolution of mechanically activated AG samples in water and subsequent evaporation of the solvent lead to partial recovery of molecular mass (see Fig. 6, *b*, curve *1*). In the case of AG mixtures with DAB, chromatograms of mechanically activated samples (see Fig. 6, *b*,

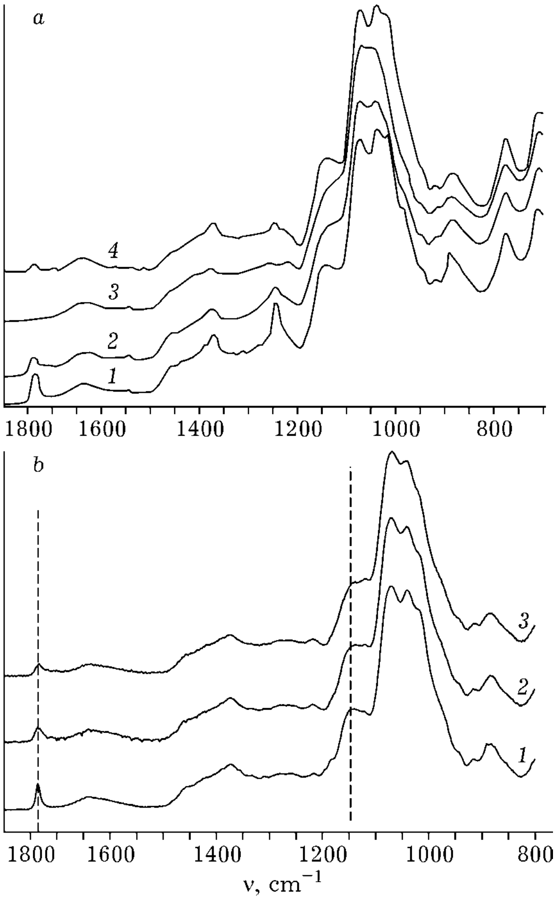

Fig. 5. IR spectra: *a* – initial mixture of DAB with AG (*1*) and after mechanical activation for 15 min (*2*), mechanically activated AG (*3*), film obtained by evaporation of the solution of a mixture of DAB with AG (*4*); *b* – mixture of DPB with AG after mechanical activation for 5 (*1*), 15 (*2*) and 30 min (*3*).

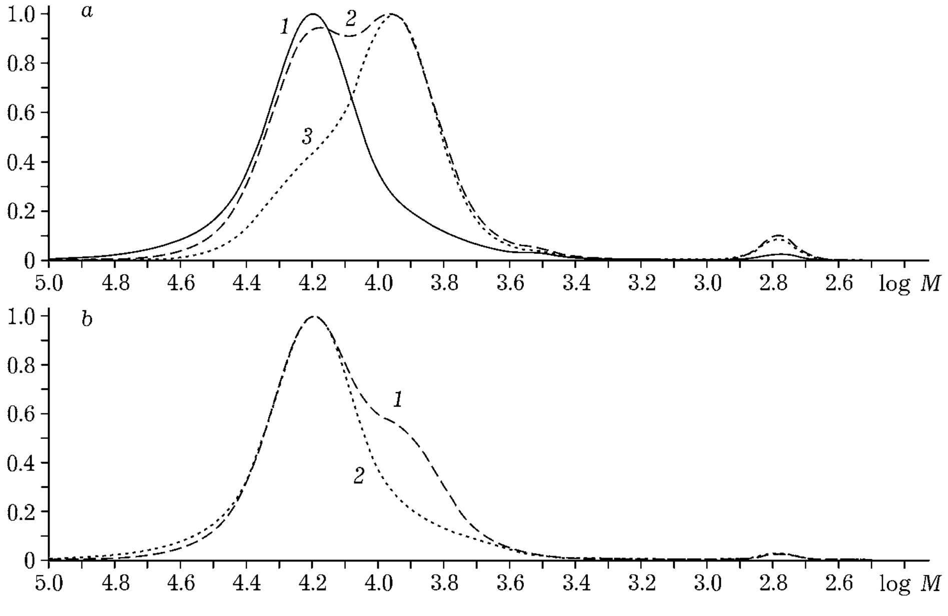

Fig. 6. Gel chromatograms of AG and its mixtures with DAB: *a* – initial AG (*1*), after MA for 10 (*2*) and 30 min (*3*); *b* – AG (*1*) and a mixture of DAB with AG (*2*) after MA for 10 min, dissolution in water and subsequent evaporation of the solvent.

curve *2*) after their dissolution in water and evaporation of the solvent are identical with the chromatograms of initial AG. The reason may be the formation of a complex of DAB with several AG macromolecules in solution. Indeed, it was discovered that DAB is not extracted by hexane from the aqueous solution of a mixture of DAB with AG. This suggests that a strong intermolecular complex is likely to be formed in the aqueous solution.

Similarly to DAB, DPB is not extracted by hexane from the solutions of DAB–AG mixtures. As a result of evaporation of the aqueous solutions of mechanically activated mixtures of DAB and DPB with AG, the complexes were obtained in the form of transparent flexible yellowish films completely soluble in water. The IR spectra of these films contain the bands of physical mixtures of betulin diacyls with AG (see Fig. 5, *a*), therefore, the bonds between diacyls and AG in the films are realized mainly due to van der Waals forces. Taking into account the fact that the films are dissolved in substantially smaller water volume than that consumed for their preparation, investigation of the pharmacological activity of these materials may be of interest for their further use in medicine.

CONCLUSION

It was established that DAB and DPB after their mechanical activation with AG spread over the matrix of the carrier with the formation of amorphous composites. It is assumed that the components interact with each other forming molecular complexes. It was found that the concentrations of DAB and DPB in water after the dissolution of mechanocomposites were higher than in the case of initial substances.

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