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INFLUENCE OF MECHANICAL TREATMENT ON THE PROPERTIES OF BETULIN, BETULIN DIACETATE, AND THEIR MIXTURE WITH WATER-SOLUBLE POLYMERS

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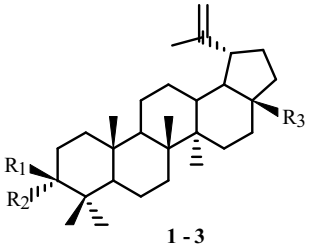
The influence of mechanical treatment on the properties of betulin, betulin diacetate, and their mixture with water-soluble polymers was studied. It was shown that mechanical treatment in a planetary mill-activator disordered the crystalline structure of betulin and betulin diacetate. Composites of betulin with polyvinylpyrrolidone (PVP) and polyethyleneglycol (PEG) that gave an increased betulin concentration upon dissolution in water were prepared. It was shown that H-bonds formed between betulin and PVP upon mechanical activation of their mixtures.

Keywords: betulin, betulin diacetate, mechanical treatment, amorphization, composites, polyvinylpyrrolidone, polyethyleneglycol, solubility.

Betulin [3β,28-dihydroxy-20(29)-lupene] (**1**) is obtained from birch bark [1] and is widely investigated owing to its use as a biologically active compound [2]. Betulin derivatives such as betulin diacetate (**2**) exhibit hypolipidemic and antioxidant activity [3]. One of the main obstacles to the use of betulin and its derivatives is their low biological availability related to poor solubility.



1 - 3

1: R_1 = OH, R_2 = H, R_3 = CH_2OH
2: R_1 = OAc, R_2 = H, R_3 = CH_2OAc
3: R_1 = R_2 = H, R_3 = CH_2OH

One method for improving the solubility is mechanical activation (MA) of drugs in the presence of excipient carriers [4, 5]. MA of drugs leads to dispersion or disorder of the crystal structure up to complete amorphization, which increases the rate of dissolution or solubility of the substances [6]. Addition of a polymeric carrier can stabilize the disordered state of the drug by coating the particles and/or interacting with them to form hydrogen bonds or other bonds [4].

The aim of the present work was to study the influence of MA on betulin and its diacetate, to prepare composites of betulin and its diacetate with water-soluble polymers, and to study their physicochemical properties including their solubility in water.

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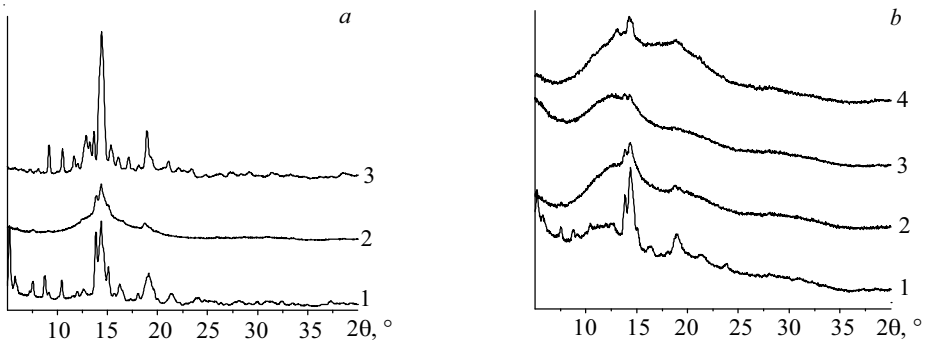

Fig. 1. X-ray diffraction patterns of starting betulin (1), betulin after MA for 30 min (2), and mechanically activated betulin after heating at 130°C for 30 min (3) (a); betulin:PVP 360000 (1:3, by mass) mixtures: physical mixture of starting components (1), physical mixture of mechanically activated components (2), mixture after 30 min of MA (3), and mechanically activated mixture after heating at 130°C for 30 min (4) (b).

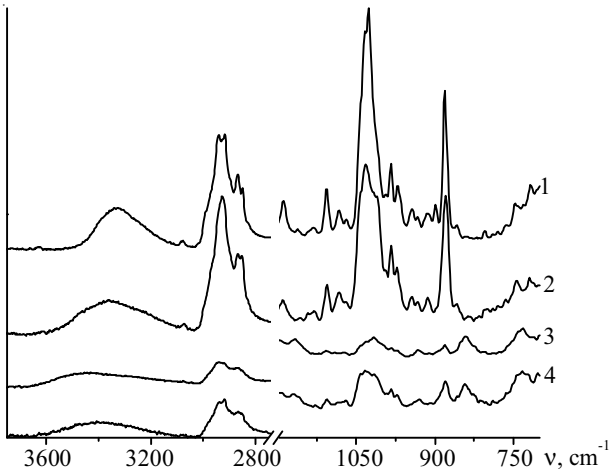

Fig. 2. IR spectra of starting betulin (1), after 30 min of MA (2), betulin:PVP 360000 (1:3, by mass) mixtures: physical mixture of mechanically activated components (3) and mixture after 30 min of MA (4).

Influence of Mechanical Treatment on Betulin and Betulin Diacetate. Chemical changes did not occur upon MA of **1** and **2** according to HPLC studies. The compositions of the resulting samples were identical to those of starting **1** and **2**. Furthermore, the crystal structure of the compounds was highly disordered as a result of the mechanical treatment.

Reflections in the x-ray diffraction patterns of **1** became broad because of the disordering (Fig. 1a, 2). Exothermic effects in the range 80–130°C that corresponded to annealing of the newly formed defects appeared in the DSC curves. New reflections atypical of starting betulin appeared in diffraction patterns of MA samples after they were heated (130°C, 30 min) (Fig. 1a, 3). This suggested that various polymorphic modifications of **1** may have existed and that polymorphic transformations occurred in the mechanically activated samples. The widely varying published melting points of betulin [7, 8] and our results on the properties of the decomposition products of various betulin solvates [9] are consistent with the assumption that **1** exists in different polymorphic modifications.

Betulin diacetate (**2**) behaved analogously. Annealing mechanically activated **2** also not only narrowed lines in the diffraction patterns but also generated new reflections that did not correspond to the starting modification of **2**, the structure of which was published [10].

Effect of Mechanical Treatment on Mixtures of Betulin and Betulin Diacetate with PVP. MA of mixtures of **1** and **2** with PVP homogenized them as a result of dispersing the components and forming composites. The crystal structure of **1** was disordered to the point where it was completely amorphous if the polymer content was increased (Fig. 1b, 3). The presence of the polymer stabilized the amorphous state of **1**. Reflections of **1** did not appear in diffraction patterns upon annealing to 130°C, in contrast with pure **1** (Fig. 1b, 4). The melting peak of **1** in the DSC curves disappeared for the amorphous samples.

TABLE 1. Betulin Concentration upon Dissolution of Mixtures with Polymers, mg/mL ($\beta = 0.95$, $n = 3$)

Composition	PVP 360,000	PVP 12,600	PEG 4,000
Physical betulin–polymer (1:3) mixture	$(1.9 \pm 0.2)\cdot10^{-3}$	$(2.3 \pm 0.5)\cdot10^{-3}$	$(2.1 \pm 0.5)\cdot10^{-3}$
MA betulin–polymer (1:3) mixture	$(11.8 \pm 0.3)\cdot10^{-3}$	$(23.2 \pm 1.0)\cdot10^{-3}$	$(6.4 \pm 0.6)\cdot10^{-3}$
Physical betulin–polymer (1:10) mixture	$(2.8 \pm 0.4)\cdot10^{-3}$	$(2.3 \pm 0.5)\cdot10^{-3}$	$(2.1 \pm 0.4)\cdot10^{-3}$
MA betulin–polymer (1:10) mixture	$(49.7 \pm 0.9)\cdot10^{-3}$	$(29.1 \pm 0.8)\cdot10^{-3}$	$(12.1 \pm 0.9)\cdot10^{-3}$

Betulin concentration in water upon dissolution of starting betulin (37 ± 1°C): $(1.90 \pm 0.55)\cdot10^{-3}$ mg/mL; betulin concentration in water upon dissolution of MA betulin: $(2.80 \pm 0.32)\cdot10^{-3}$ mg/mL.

IR spectra of mixtures of **1** with PVP changed after MA in the region of OH stretching vibrations (3200–3500 cm^{-1}). The band maximum shifted to low frequencies by about 60 cm^{-1} compared with the band in the spectrum of a physical mixture of the components that underwent MA separately. The shape of the band in the region 1020 cm^{-1}, corresponding to ν(C–O) of **1**, changed. The band shifted by 5–10 cm^{-1} and the intensity at the higher frequencies was redistributed compared with the physical mixture of the mechanically activated components and mechanically activated **1**. This suggested that the C–O bond length in **1** in the composite decreased. This indicated that **1** interacted with PVP upon MA to form H-bonds between the OH groups of **1** and the C=O groups of PVP. The amorphous state of activated **1** was stabilized by the formation of the molecular complex (Fig. 2).

MA of **2** also caused disordering. However, it was easily annealed. IR spectra of mechanically activated mixtures of **2** with PVP did not change relative to a physical mixture of the mechanically activated components. This suggested that interactions did not occur upon MA. This may have been due to the lack of functional groups in **2** that could participate in the formation of H-bonds with PVP.

Effect of Mechanical Treatment on a Mixture of 1 and 2 with PEG. MA of **1** and **2** with PEG did not form H-bonds between the components. This was indicated by the lack of changes in the IR spectra. Reflections broadened and their intensities weakened in x-ray diffraction patterns of mixtures of **1** with PEG after MA. However, diffraction patterns of mechanically activated mixtures of **2** with PEG did not change substantially. This may have been related to dissolution of **2** in the PG melt and subsequent crystallization of it from the melt. Examples are known where drugs dissolved in PEG upon heating [11]. It was assumed [12] that indomethacin dissolved in a PEG melt upon mechanical treatment without forming intermolecular bonds according to IR spectroscopy data. Considering the low melting point of PEG (61–63°C), a melt probably formed when the particles came into contact during collisions and were impacted by the balls during MA. Recrystallization of **2** without substantial formation of the amorphous state could be expected to occur as a result of contact melting and subsequent solidification of the compound during mechanical treatment. The presence of the amorphous state in mechanically activated mixtures of **1** with PEG was apparently due to the fact that **1** was practically insoluble in PEG at these temperatures.

Release of 1 from Mixtures with Polymers into Solution. Dissolution in water of composites of **1** with polymers that were produced using MA showed that the content of **1** in solution was significantly increased compared with dissolution of starting **1** and mechanically activated **1** and physical mixtures of **1** with PVP and PEG (Table 1). The increased solubility may have been related to the amorphous state of **1**, the solubilizing action of the polymers, and the formation of intermolecular complexes of **1** with PVP. The effect of PEG, which did not form H-bonds with **1**, may have been related to the formation of a composite as a result of the distribution of the drug in the polymer that facilitated its subsequent dissolution [11]. **1** may have been dissolved through a micellar mechanism, so-called colloidal dissolution.

Addition to the treated mixtures of small quantities of EtOH had an even greater effect. The concentrations of **1** from dissolution of mixtures with polymers that were mechanically activated in the presence of EtOH (mg/mL, β = 0.95, n = 3) are given below.

Composition	*C, mg/mL*
MA **1**:PEG 4,000 mixture 1:10 + 10% EtOH	$(14.8 \pm 0.9)\cdot10^{-3}$
MA **1**:PEG 4,000 mixture 1:10 + 5% EtOH	$(17.1 \pm 1.9)\cdot10^{-3}$
MA **1**:PVP 12,600 mixture 1:10 + 5% EtOH	$(52.4 \pm 2.0)\cdot10^{-3}$

The positive effect of adding EtOH to the reaction mixture on the solubility of **1** depended on its amount. Thus, adding 5% EtOH gave a greater effect than adding 10%. The effect of adding "catalytic" amounts of liquids during mechanical treatment of mixtures of molecular crystals on the acceleration of syntheses is well known [13, 14]. In our instance, adding much smaller volumes of solvent than before [13] had a positive effect on the drug solubility. The effect of small amounts of solvent on the synthesis mechanism of mixed crystals during mechanical treatment of reaction mixtures was discussed [14]. Mechanical treatment may have formed supersaturated solutions from which the molecular complex crystallized. In our instance, the action of EtOH may have been related also to a plastifying effect on the polymer carrier that had a positive influence on the formation of the complexes with **1** owing to a shift of the polymer glassification point and the increased mobility of its molecules.

Analogous experiments with **2** did not increase its solubility. This may have been due to the fact that **2** was not disordered and did not form molecular complexes with the polymers during mechanical treatment.

Thus, mechanical activation produced composites of **1** with the water-soluble polymers PVP and PEG. H-bonds formed between **1** and PVP. The resulting samples were characterized by an increased concentration of **1** upon dissolution in H_2O. Experiments with **2** confirmed the hypothesis that the reason for the formation of the soluble composites was the generation of disordered drug states and the formation of molecular complexes with the polymers that were capable of stabilizing these states.

EXPERIMENTAL

Betulin (**1**) and betulin diacetate (**2**) were isolated from birch bark at the Institute of Chemistry and Chemical Engineering, SB, RAS (Krasnoyarsk) [15]. GC/MS (Agilent) using a 5973N EI/PCI (70 eV) (Varian), DB-1 column, 280°C column temperature, and N_2 carrier gas showed that starting **1** contained two principal components, ~95% **1** (MW = 442) and up to 5% lupeol (**3**) (MW = 426). The components were identified by comparison of their mass spectra with the NIST-05 database. **1** was recrystallized from EtOH. The resulting solvate was decomposed at 180°C for 30 min [9], after which the content of the impurity of **3** was less than 1.5–2.5% according to HPLC. According to HPLC, **2** contained **1** as the major impurity (about 3%) and was used without further purification. The possibility of chemical transformations of **1** and **2** after MA was studied using GC/MS and HPLC (methods described below). Samples were studied after 30 min of MA as ~0.5% solutions in EtOH.

PVP (MW = 12,600 and 360,000, Sigma-Aldrich) and PEG (MW = 4,000, Sigma-Aldrich) were used without preliminary purification and drying.

MA of the starting components and mixtures was performed in an AGO-2 planetary-centrifugal ball mill (IKhTTM, Russia) [16] with water-cooled drums. The volume of the steel drums was 40 mL; ball diameter, 6 mm; ratio of sample mass to ball mass, 1:30. The loading on a ball was 20 g. The treatment times were 15 and 30 min. The **1**(**2**):polymer ratios were 1:3 and 1:10 (by mass). Physical mixtures were prepared for reference simply by mixing the components in the same ratios.

Thermoanalytical measurements were made on a DSC-204 calorimeter (Netzsch) at heating rate 6°C/min and sample mass 5–10 mg. X-ray diffraction analysis was carried out on a D8 Discover diffractometer with a GADDS area detector (Bruker) using Cu Kα-radiation. IR spectra were recorded from unpressed samples using total internal reflection (TIR) in the range 4000–600 cm^{-1} on a Digilab Excalibur 3100 Fourier IR spectrometer (Varian).

Dissolution was carried out for 24 h at 37 ± 1°C using a 705 DS solubility tester (Varian Inc., Netherlands) and 65–67 mg (for 1:3 ratio) or ~200 mg (for 1:10 ratio) of sample placed in H_2O (250 mL). Collected samples were centrifuged for 15–30 min at 8000 rpm and filtered (Millipore, d = 0.2 μm). The filtrate (40–80 mL) was extracted (3×) with an equal volume of hexane. The resulting extracts were dried over Na_2SO_4 and evaporated. The residue was dissolved in EtOH. The concentration of **1** in EtOH was less than its solubility under normal conditions [17]. The concentration of **1** in the resulting EtOH solution was determined in a Milikhrom A-02 chromatograph (Ekonova, Russia). The chromatography conditions were an N2301 column (2.0 × 75 mm), ProntoSIL 120-5C18AQ sorbent, particle size 5.0 μm, mobile phase $LiClO_4$ (A, 0.1 M):CH_3CN (B), 80–100–100% B gradient, aliquot volume 2–5 μL, flow rate 100 μL/min, column temperature 35°C. The detection wavelengths were 200, 210, and 220 nm. The standards were EtOH solutions of **1** and **2**. The results were recalculated for the initial filtrate volume.

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