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MECHANICAL COMPOSITES BASED ON BIOLOGICALLY ACTIVE COMPOUNDS FROM LARCH WOOD

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Mechanical composites were prepared by mechanical chemical processing of a mixture of biologically active preparations of arabinogalactan (AG) and dihydroquercetin (DQ) isolated from larch wood. Their properties were studied using HPLC, ^{13}C NMR, and IR spectroscopy. It was found that AG and DQ did not react chemically under the studied conditions. According to x-ray phase and thermal analyses, mechanical processing destroyed the DQ crystalline structure and dispersed it into the AG matrix. The resulting mechanical composites had significantly higher (up to 38 times) solubility in water compared with starting DQ and an unprocessed AG/DQ mixture. It was shown that DQ reduces the extent of destruction of polysaccharide macromolecules during mechanical processing.

Keywords: larch wood (*Larix* sp.), arabinogalactan, dihydroquercetin, mechanical chemical processing, nanocomposites, intermolecular complexes.

Larch (*Larix* sp.) wood is a source of a wide range of biologically active compounds (BAC), of which a water-soluble polysaccharide arabinogalactan (AG) and the flavonoid dihydroquercetin (DQ) are the most significant.

Arabinogalactan from larch wood has low toxicity (does not exhibit acute toxicity at a dose of 5 g/kg and chronic toxicity at a dose of 500 mg/kg per day) [1], exhibits immunomodulating and prebiotic properties, and interacts strongly with membranes.

The biological activity of the bioflavonoid DQ is well studied. It is sold as the drug preparation Diquertin, which has a broad spectrum of therapeutic activity [2]. Furthermore, several highly effective drugs and biologically active food additives are based on DQ [3]. Its antiviral activity was recently observed [4].

DQ is poorly soluble in water and is a short-lived preparation. Its elimination half-life is less than 1.25 h [5]. Therefore, conjugation of DQ with AG seemed promising for creating preparations with increased bioavailability and prolonged action.

The goal of our work was to prepare mechanochemically from DQ and AG intermolecular complexes, nanocomposites, and to study their properties.

Use of AG as the matrix and carrier of the BAC ensured the bioavailability and duration of action and in certain instances reduced the toxicity while increasing the therapeutic activity of the BAC [6, 7]. Targeted delivery into cells or organs of x-ray contrast and therapeutic agents using AG was carried out through receptor-mediated endocytosis [8].

Mechanochemical methods enabling the desired products to be prepared without solvents in one processing step had significant advantages for creating such composites over traditional liquid-phase processes [9]. Known drugs had reduced toxicity and significantly higher (up to 50 times) solubility and, therefore, bioavailability after mechanochemical processing with AG. This enabled the treatment dose of these drugs to be reduced by an order of magnitude while retaining the therapeutic properties [7, 9].

A mild regime was selected for mechanochemical processing of the samples. As shown by us previously [10], it had a smaller effect on the molecular and mass properties of the polysaccharide.

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TABLE 1. Effect of Mechanical Processing Conditions on Molecular Weight Properties of AG and AG/DQ Composites

Sample	Processing time, h	M_w	M_n	M_w/M_n
AG-1	–	18552	12525	1.48
AG-1	2	19543	7674	2.55
AG-1	14	23865	8667	2.75
AG-1–DQ 10:1	2	14226	9007	1.58
AG-1–DQ 20:1	14	14716	8831	1.67
AG-2	–	18746	11167	1.68
AG-2–DQ 20:1	2	16583	8163	2.03

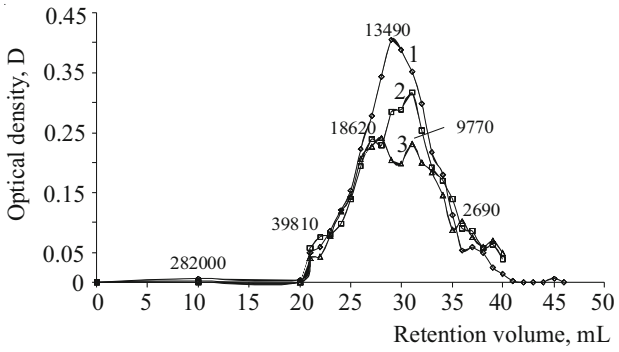

Fig. 1. Gel chromatograms of AG/DQ mechanical composites: starting AG-1 (1), AG/DQ (10:1), 2 h (2), AG/DQ (20:1), 14 h (3).

Because the properties of the AG from larch depended on the isolation and purification method [11], two samples of AG (AG-1 and AG-2) that were purified by different methods were studied.

Gel-permeation chromatography (GPC) of samples of starting AG typically had a unimodal molecular-weight distribution (MWD). Their average molecular weight (MW) was 13,490 Da (AG-1) and 17,780 Da (AG-2). Gel chromatograms of mechanochemically treated AG-1 and AG-2 had several characteristic maxima corresponding to MW in the range 282,000–800 Da. This was consistent with the increased degree of polydispersion (ratios of average-mass M_w and average number M_n molecular weight) of the mechanically treated samples (Table 1).

Mechanical treatment of AG caused partial destruction of its macromolecules and recombination of the resulting fragments [10]. Therefore, the amount of high-molecular-weight fractions of processed AG-1 samples increased with the duration of processing whereas the amount of low-molecular-fractions decreased compared with the starting sample.

AG in mechanical composites with DQ had a narrower MWD than AG treated under analogous conditions (Table 1) because of the reduction in the amount of high-molecular-weight and low-molecular-weight fractions. Gel chromatograms of the resulting composites differed insignificantly from those of the untreated samples (Fig. 1).

IR spectra of mechanically treated and starting AG samples were identical and indicated that destruction of its macromolecules during mechanochemical processing was not accompanied by oxidative processes. This was confirmed by a lack in ^{13}C NMR spectra of AG samples of resonances at 160–220 ppm. DQ under the studied mechanical processing conditions probably stabilized the polysaccharide macromolecules.

HPLC analyses of alcohol extracts of mechanically processed mixtures indicated that chemical reactions involving DQ did not occur under the used processing conditions.

X-ray phase analysis suggested that solid AG and DQ phases were disordered during mechanical processing. Reflections decreased and disappeared (Fig. 2). Solid DQ lost its crystallinity. Its molecules were probably dispersed into the AG matrix. An analogous loss of DQ crystallinity was observed on the DSC thermograms (Fig. 3).

Mechanical processing of AG and DQ samples together did not change the frequencies and intensities of absorption bands in their IR spectra as compared with spectra of the starting compounds. We observed a similar effect during processing of AG together with other BAC [12]. Analogous results were obtained during mechanochemical processing of mixtures of organic acids with AG [13].

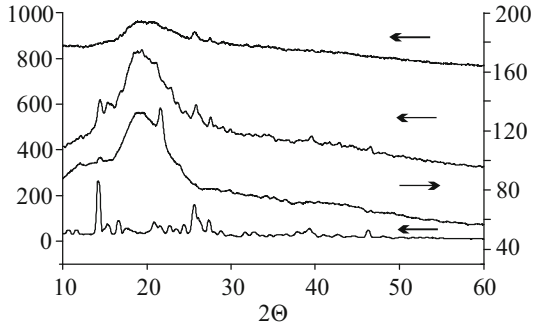

Fig. 2.

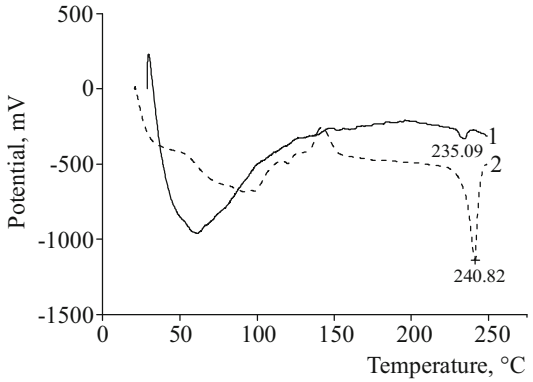

Fig. 3.

Fig. 2. Diffraction patterns (bottom to top) of crystalline DQ, AG, AG/DQ 10:1 mixture, mechanically processed AG/DQ 10:1 mixture.

Fig. 3. DSC thermograms of mechanically processed DQ/AG (1:10) (1) and DQ (2).

The mechanical composites, AG–DQ intermolecular complexes that were formed as a result of processing of AG and DQ samples together (20:1 and 10:1), were significantly more soluble (up to 38 times) than starting DQ and an unprocessed AG/DQ mixture (the analysis was complicated by the high viscosity of the solution) (see below):

Composition (mass ratio), duration of mechanical processing	*DQ solubility, g/L*	*Composition (mass ratio), duration of mechanical processing*	*DQ solubility, g/L*
DQ	0.7	AG–DQ 20:1, 2 h	>5.8
AG–DQ 20:1, unprocessed	2.0	AG–DQ 20:1, 14 h	26.8.
AG–DQ 10:1, 2 h	7.0		

The results are promising for creating effective drugs, including injectable forms, with increased bioavailability and prolonged action.

EXPERIMENTAL

Attenuated total internal reflectance IR spectra were obtained on a Varian 3100 FT IR spectrophotometer in the range 4,000–600 cm^{-1}.

^{13}C NMR spectra were recorded in D_2O on a Bruker DPX 400 spectrometer at operating frequency 100 MHz with deuteroacetone internal standard.

Thermal analysis was performed using a DSC-550 instrument (Instrument Specialists Inc.). The temperature was programmed for 20–400°C at heating rate 10°C/min.

X-ray powder patterns were obtained on a DRON-3 diffractometer (Cu cathode, $\lambda = 1.54$ Å) at room temperature. The uncertainty of the measured angles was 0.005°.

We used two samples of arabinogalactan (AG-1 and AG-2) that were taken from a pilot-plant stand and different batches of larch wood [14]. Sample AG-2 was used without purification (moisture 3.9%, residual DQ content 0.97%). AG-1 was purified additionally by reprecipitation from aqueous ethanol (10%). The resulting precipitate was dried at 105°C (moisture 0.1%, residual DQ content 0.15%).

Drug substance DQ was obtained from an industrial stand of OOO INPF Wood Chemistry [15]. The content of active ingredient in the sample was 92.1%.

Mechanochemical treatment used a BM-1 ball mill with a drum lined with fluoroplastic and steel balls as the grinders (ShKh-15 grade steel, diameter 15 mm, load 675 g, acceleration of grinders 1 g, free fall, drum volume 300 mL). The total load of the components of the treated mixture was 20 g. The duration of the mechanical treatment was from 2 to 14 h.

Molecular weight (MW) determinations of samples were made by gel-permeation chromatography over a column (1.0×65 cm) of Sephadex G100 that was calibrated using dextrans with MW 70,000, 41,272, 20,000, 10,500, and D-galactose.

The solvent and eluent was NaCl solution (1 M). Fractions (1 mL) were collected at elution rate 15–17 mL/h. The AG content in the fractions was determined by the phenol–H_2SO_4 method [16].

Solubility of mechanically treated AG/DQ mixtures (0.8 g) and weighed portions of DQ equivalent to its content in these mixtures was established by dissolution in water (5 mL) with stirring on a magnetic stirrer (600 rpm) for 6 and 24 h at 24°C. In all instances DQ existed in solution in equilibrium with undissolved precipitate. AG dissolved completely into the solution.

The DQ concentration in solution was determined by HPLC in a Millichrom-2 microcolumn chromatograph equipped with a UV detector and using a Silasorb C_{18} reversed-phase column. The eluent was CH_3CN:CH_3CO_2H (2%, 3:7) at flow rate 100 μL/min, sample volume 2 μL, and detection at 290 nm.

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