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Short communication

Simple and efficient process for large scale preparation of betulonic acid from birch bark extracts

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ABSTRACT

A bioactive triterpenoid betulonic acid is a valuable synthone for preparation of lupane- and oleanane-type derivatives with a wide spectrum of medical applications. A large-scale, cost-effective, and simple preparation of betulonic acid from birch (*Betula pendula*) bark is presented herein. The process includes four steps with the acid overall yield of 51% counting on betulin content in the extract and with the acid purity of 90% or more. The process can be employed for batch production of betulonic acid.

1. Introduction

Betulonic acid (3-oxolup-20(29)-en-28-oic acid) is a bioactive substance exhibiting antiviral, cytotoxic, antiangiogenic activities (Mukherjee et al., 2004; Pavlova et al., 2003; Ryu et al., 1993). It is also widely used as an intermediate in synthesis of various triterpenoid derivatives with anti-inflammatory, antiviral, and antiproliferative properties (Alakurtti et al., 2006; Tolstikova et al., 2006).

Being a secondary metabolite of birches (*Betula* spp.) betulonic acid was found in particular as minor component in bud extracts (Vedernikov and Roshchin, 2012). As for preparative purposes, betulonic acid is produced from betulin, a major component of birch bark extracts. Nowadays the most common synthetic route to betulonic acid is oxidation of betulin with chromium (VI) compounds (Krasutsky, 2006). In this way preparation of betulonic acid combines two multi-step processes: (1) extraction of birch bark followed by concentration and purification of crude betulin; and (2) oxidation of crude or pure betulin into betulonic acid followed by its purification. In most cases, the production is rather laborious and sophisticated, including solvent type change at every subsequent step of these processes. Thus, alcohols and halogenated hydrocarbons are commonly used for bark extraction (Kim et al., 1997; Petrenko et al., 2002), while pure betulin is obtained by crystallization of the extracts from methanolic and chloroformic solutions (Kim et al., 1997). Oxidation of betulin is generally performed with chromium trioxide or potassium dichromate in acetone (Barthel et al., 2008; Melnikova et al., 2012; Kogaj and Kuznetsov, 2008) or acetic acid (Son et al., 1998), whereas lower alcohols are added to eliminate chromium (VI) compounds excess. Water miscible solvents are usually lost with discharged water phases upon sedimentation or extraction of crude betulonic acid. Further, supplementary solvents are used for isolation of the crude acid. Betulonic acid is separated from the reaction mixture either by chromatography (Kim et al., 1997; Barthel et al., 2008) or through precipitation of its salt (Petrenko et al., 2002; Melnikova et al., 2012; Son et al., 1998). In the latter case the sum of oxidation products is dissolved in Et_2O and sodium betulonate is sedimented upon treatment with aq. NaOH solution and further crystallized from methanol. In general, 5–7 solvent types are commonly used changing each other on a technological pathway from birch bark to betulonic acid. The discussed processes are outlined in Fig. 1.

The processes described above are rather complex due to the use of a wide range of organic solvents and multiple concentration-dissolution operations. Time-consuming procedures on isolation and purification of semiproducts also complicate the processes. These technologies could hardly be scaled up for batch production of betulonic acid.

Herein we present a convenient method for large scale production of purified betulonic acid (90%+) from birch (*Betula pendula*) bark using a single solvent, *t*-BuOMe, for bark extraction, oxidation

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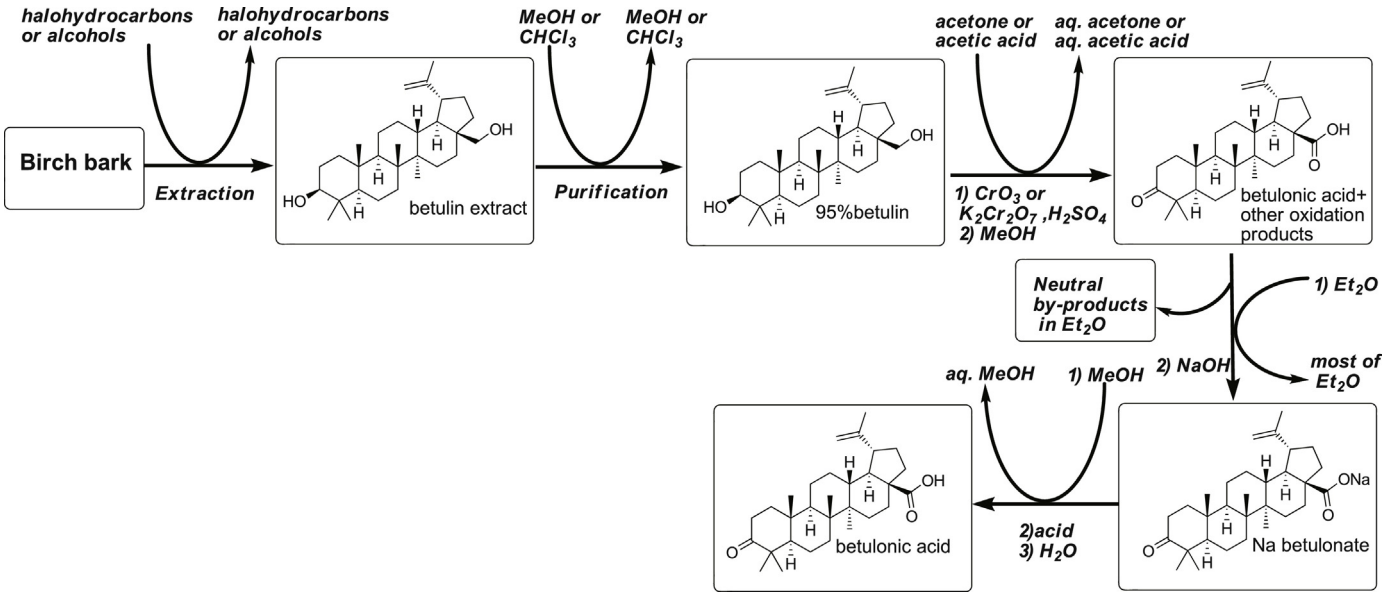

Fig. 1. Generalized scheme of betulonic acid production from birch bark in accordance with the published data (Kim et al., 1997; Petrenko et al., 2002; Barthel et al., 2008; Melnikova et al., 2012; Kogaj and Kuznetsov, 2008; Son et al., 1998).

of betulin-containing extracts, and isolation of the acid. In general the developed process scheme is outlined in Fig. 2.

2. Materials and methods

2.1. Materials and chemicals

Outer bark of birch (*Betula pendula*) was purchased on the local market in Novosibirsk, Russia.

t-BuOMe of technical grade was distilled prior to use. Other reagents and solvents were obtained from commercial sources and used without further purification. Reaction was monitored by TLC on PTSC-AF-A plates (Sorbpolimer, Krasnodar, Russia) which were developed by spraying with 10% $FeCl_3$ ethanolic solution followed by heating. HPLC analysis was performed on Milichrom-A-02 chromatograph (Econova, Novosibirsk, Russia) equipped with a column (2× 75 mm, ProntoSIL-120-5-C18) and a UV detector (detection at 206 nm); an eluent was methanol/0.1 M trifluoroacetic acid [9/1 → 10/0 (v/v)] at flow rate 200 μL/min; methyl ursolate (99%+, Carl ROTH, Karlsruhe, Germany) was used as an internal standard.

2.2. Extraction of birch bark

Air-dried ground (3.2 kg, moisture content ≤10%, 3–5 mm) outer bark of birch (*Betula pendula*) in calico sacks was twice extracted with *t*-BuOMe at 50–55 °C for 4 h. The first miscella after draining and filtration (35 L) was directed to the oxidation process. The second miscella was employed in the next extraction cycle of a new portion of the birch bark. According to analytical data for the first miscella, the dry extract yield was ~27% (the content of betulin in the dry extract ~73%). The yield of betulin counted on the birch bark amount was ~20%.

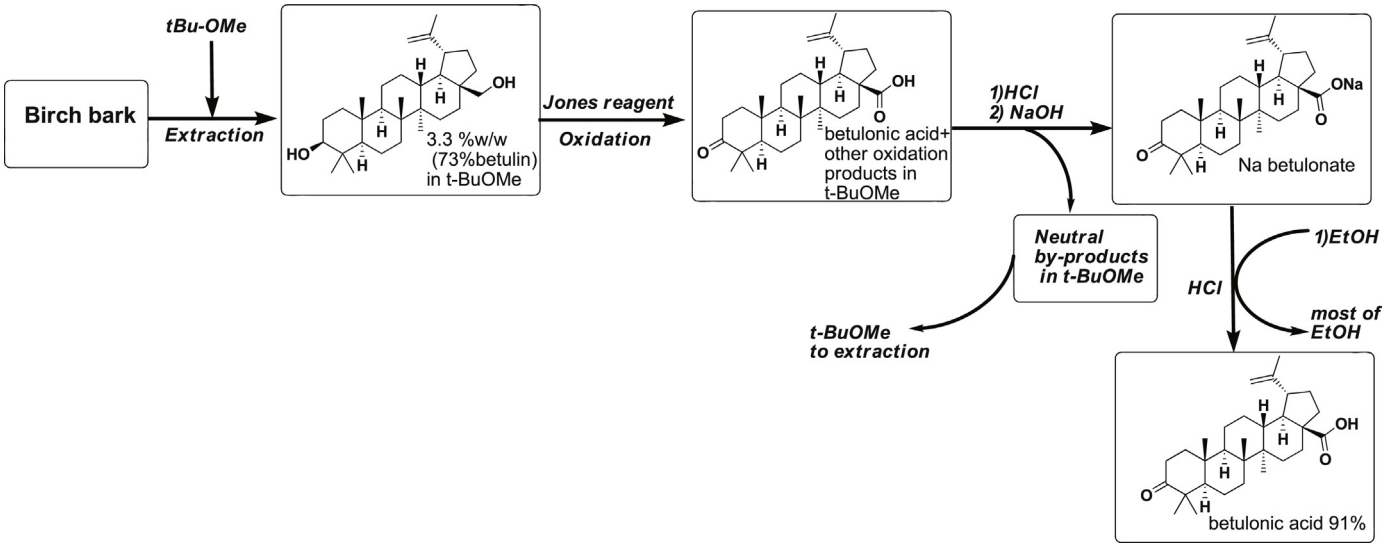

Fig. 2. Preparation of betulonic acid from birch (*Betula pendula*) bark extract.