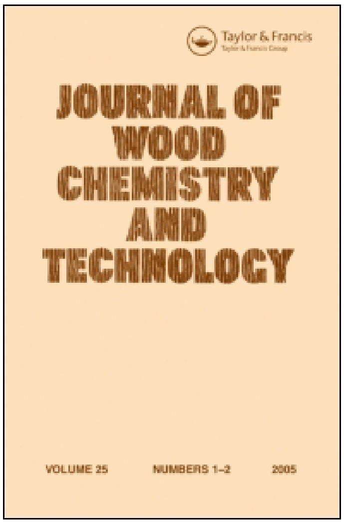

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EXTRACTION OF BETULIN BY VACUUM PYROLYSIS OF BIRCH BARK

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ABSTRACT

Separation of betulin from the outer bark of *Betula papyrifera* birch tree by sublimation in a batch vacuum pyrolysis reactor was studied in the temperature range of 250–300°C and under a total pressure of 0.7 kPa. Yield of 9.5% by weight of betulin on an anhydrous bark basis was obtained. The total yield of extractives was in the range of 28–32%. The purification of betulin was achieved by solvent extraction followed by crystallization. In comparison, soxhlet extraction using dichloromethane yielded 11.5% of betulin. The structural analysis of the purified betulin has been made by GC/MS and ^{1}H NMR.

Key Words: Betulin; Birch; Bark; Extraction; Sublimation; Vacuum; Pyrolysis; Analysis

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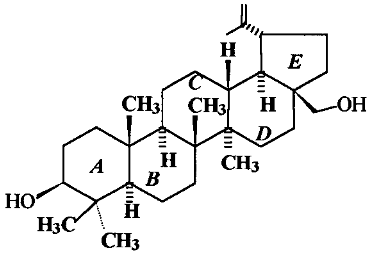
INTRODUCTION

Betula papyrifera is also called white birch. The generic name derives from *betu*, the Latin name of birch. It means shiny or glitter, referring to its white colour. It's specific name is *papyrifera* from the Greek *papuros* (or *papyros*), meaning thin bark like paper which peels off easily from the internal part of bark. There are 30–50 species of birch tree that are primarily distributed in Asia, America and Europe. Birch is distributed almost everywhere in Canada, but is less abundant in the areas with the leafy trees. It can reach 15–20 m in height, and its trunk is 30–60 cm in diameter. The most abundant North American birch species are: the yellow birch (*Betula alleghaniensis*), the sweet birch (*Betula lenta*) and the white birch (*Betula papyrifera*). They are mainly distributed in the North-Eastern and of the Great Lakes areas with a slight variation from one species to another. The yellow birch is hard and dense (689 kg/m^3). Sweet birch has a density of 737 kg/m^3. White birch is soft and tender with a density of 609 kg/m^3.

For the forest industry, birch constitutes a species with an economic value. In Quebec for example, birch is used in the construction industry and as a domestic solid fuel, but birch bark content is regarded as a residue. Birch bark is made up of two layers; the alive internal layer or phloerene and the dead external layer or the rhytidome. The external part of bark is mainly made up of fatty and resinic acids, suberin, and triterpenes which represent 3–4% of the total bark.[1,2] Bark accounts for 10–15% of the total weight of the wood.[2,3] The two physiological and chemical roles of birch bark are the transport of the nutriments towards the rest of the plant and the defence of the tree against the phytophagous insects as well micro-organisms.[4]

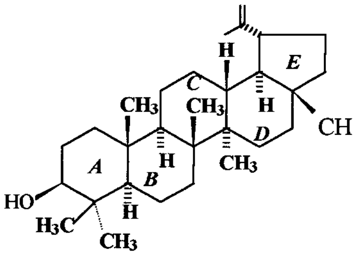
Betulin or lup-20-(29)-ene-3,28-diol, also called betulinol, is an abundant natural product of the external part of bark of several species of *Betula* birch. The triterpenes are by far the most significant compounds of birch bark. According to their structure, the triterpenes are subdivided into three classes; lanostanes, lupanes and oleananes. The C_{30} triterpenoides by definition are composed of six isoprenes (C_5H_8) units. Several compounds of natural origin of more than 30 carbon atoms, come from the alkylation of triterpene. However, the majority of the compounds have less than 30 carbon atoms which are derived from the catabolic degradation processes of triterpenes.

Betulin concentration has been reported to reach about 12% by weight of the anhydrous external bark of North American *Betula papyrifera*.[5] A higher concentration of about 30 wt.% has been reported in Scandinavian birch bark.[6]



Betulin

Lup-20(29)-ene-3,28-diol



Lupeol

(3β)−Lup-20(29)-en-3-ol

Applications

Sheth et al.[7] reported that betulin has the potential to be used in the manufacture of disinfectant and insecticide. Jääskeläinen[8] believes that betulin has anti-tumour properties. Betulin was found to possess hair care effects when added in shampoo formulation.[9] The utilization of betulin has been investigated to some extent, but so far not many applications have been demonstrated. Recently, Russian researchers found antioxidant properties to betulin when used in alcoholic beverages.[10] Furthermore, betulin has shown sun-protective and antimelanome activity in sun creams.[10] Considering the availability of betulin from natural renewable sources, and its reactivity with other organic compounds, it is expected that new areas of application will be found in the future. Extensive investigations are needed however to exploit the potential of this interesting compound.

Betulinic acid is obtained by oxidation of betulin. It has a stronger biological activity and exhibits a potential therapeutic activity against the AIDS virus when compared with betulin.[11,12]

Physicochemical Properties

Betulin is a white crystal with a melting point of between 254 and 256°C. Under 0.01 kPa of pressure, it sublimates at 240°C. It is slightly soluble in water, petroleum ether and in carbon disulphide. One part of this product is soluble in 149 parts of alcohol, 251 parts of ether, 113 parts of chloroform and 417 parts of benzene. It is freely soluble in acetic acid. Both hydroxyl functional groups on A and E rings of betulin offer substantial possibilities of structural modifications by several chemical reactions.

Extraction Methods

Conventional soxhlet extraction of betulin from birch bark has been reported in the literature using solvents such as toluene, chloroform and alcohol. The extraction lasts at least 12 h, and is followed by one or more crystallization or elution steps on a silica-gel column, yielding 95% pure betulin.[13]

The qualitative analysis of the fraction obtained by extraction with solvents has revealed the presence of compounds other than betulin. Among triterpenes, lupeol is the major impurity in this fraction.[6]

EXPERIMENTAL

Material

The birch wood bark sample (*Betula papyrifera*) investigated in this work was obtained from live birch wood trees from Quebec city, Canada. The feedstock was shredded to fibrous particles with the longest dimension ranging between 5 and 20 mm and dried in an oven at 102–103°C until a constant weight was obtained.

Pyrolysis

Pyrolysis was performed with approximately 480 g sample in a 15-L bench-scale reactor. The temperature of the reactor was raised at a rate of 20°C/min to a maximum temperature of 250–300°C in a series of experiments by means of a heating element installed at the bottom of the reactor (Run #G71-73). A vacuum pump was used to maintain a total pressure of less than 0.7 kPa in the reactor. Three dry ice in limonene condensers were used as cooling medium to trap the pyrolysis vapours at −72°C. When the final temperature was reached, the temperature was maintained for 1 h. Then the reactor was cooled down to room temperature and kept under nitrogen. The pyrolysis product recovered was collected, sealed and stored in the refrigerator at 4°C for further chemical analysis. A detailed description of the pyrolysis set-up used in this work can be found elsewhere.[14]

Solvent Extraction

Approximately 46 g of shredded bark was extracted with 250 mL of dichloromethane in a soxhlet apparatus during 43 h. Samples of extracts after 1, 4, 8, 10, 23 and 43 h were collected and analyzed.

Analysis

The GC/MSD analysis was performed on a HP-5890 gas chromatograph with split injection at 290°C. The HP5-MS fused silica capillary column from Hewlett Packard was 30 m long × 0.25 mm i.d. with 0.25 μm film thickness. Helium was the carrier gas with a flow rate of about 1 mL min^{-1}. The GC initial oven temperature was 50°C for 2 min, then programmed to increase to 210°C at 10°C min^{-1} and then to 290°C at 30°C min^{-1}. The oven temperature was held at 290°C for 20 min. The end of the column was introduced directly into the ion source of a HP-5970 series quadrupole mass selective detector. The transfer line was set at 270°C and the mass spectrometer ion source was at 250°C with 70 eV ionization potential.

A volume of 1 μL of sample was injected into the GC using a HP-7673 automatic sampler. Data acquisition was done with a PC base G1034C Chemstation software and a NBS library database. The mass range of $m/z = 30$–500 Dalton was scanned every second.

RESULTS AND DISCUSSION

Approximately 28–32% by weight of the initial anhydrous sample was recovered as a betulin-rich pyrolysis product under a pyrolysis temperature range of 250–300°C. Quantitative analysis revealed a total betulin content of $9.5 \pm 0.5\%$ by wt. on an anhydrous bark basis for all the experiments. The balance was a mixture of the residual humidity, bark extractives and a very low quantity of the thermal degradation products of the initial bark sample. The quantity of degradation products increased as the reactor final temperature was increased from 250 to 300°C while no significant increase in yield of betulin was observed. Three pyrolysis tests were carried out at 300°C. The total betulin yield was slightly higher as compared with the pyrolysis tests at 250 and 275°C. The betulin condensation process was different from that of the other condensed products. A betulin sample with approximately 90% purity was recovered from the reactor inside wall. Betulin was condensed practically all over the internal reactor surface and the condensers. No data is available concerning the temperature gradient above the sample inside the reactor and the reactor inside wall. Further work in envisaged for an efficient control over the reactor heating system and vacuum to eliminate any cold spot over the reactor inside surface.

The quantity of thermal degradation products increased as the degradation temperature increased. Recently a step-wise pyrolysis study of a mixture of 46% birch bark and 54% birch sapwood sample was performed at 20–200, 200–275, 275–350, 350–450 and 450–550°C by the authors to investigate the production of various classes of degradation products under various pyrolysis temperatures.[15] At low temperature in the range of 200–275°C, some degradation of the lignin polymeric structure occurs as evidenced by the recovery of 0.45% by wt. of phenols (anhydrous feed basis).[15]

Soxhlet extraction of the same birch sample produced 18.5 wt.% of soluble materials, which contained 11% betulin. The graphic presentation of the extraction is shown in Figure 1. Major extractives are removed after 8 h. Betulin content of the extract was purified and analyzed. GC/MS and NMR analysis of betulin obtained by soxhlet and pyrolysis revealed no difference, which indicated that the betulin structure remains intact during pyrolysis. The pyrolysis product was washed with 50 mL of pentane–ethanol (90 : 10),

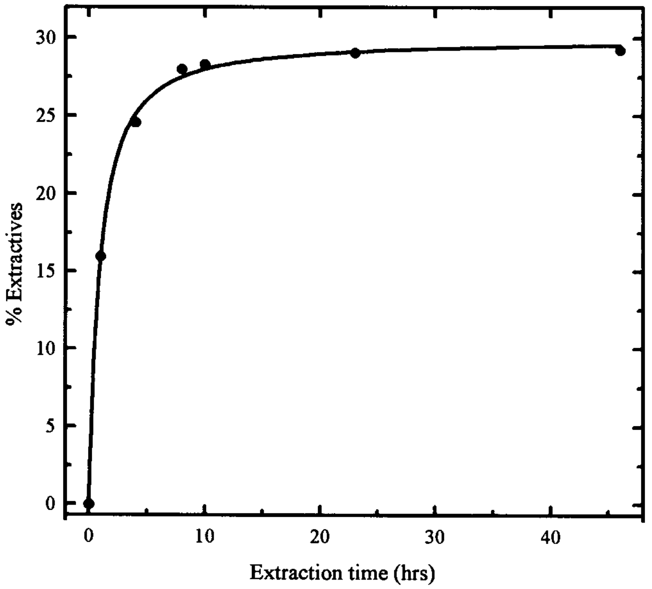


Figure 1. Yield of birch bark extractables vs. time.

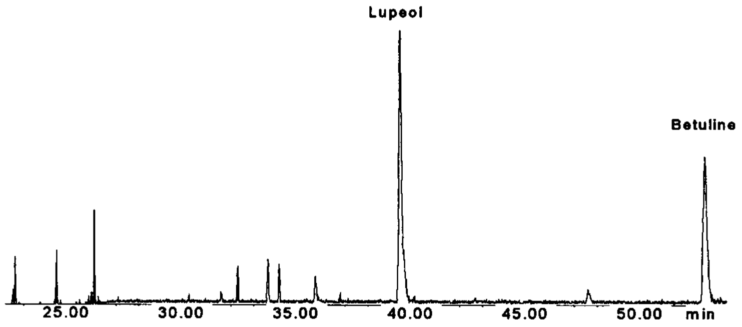


Figure 2. Total ion chromatogram of betulin fraction from pyrolysis.

followed by 3 washings of 50 mL with cold ethanol. A betulin sample with about 89.3% purity was obtained. The qualitative GC/MS analysis results are shown in Figure 2. Lupeol is the principal compound present together with betulin in the mixture. Fatty acids of C_{16}-C_{24} (octadecane-1.16-dioic; 8-hydroxyoctadec-9-enoic; 9,10-epoxy-18-hydroxyoctadecanoic; 9,10,18-trihydroxyoctadecanoic, docosane-1.22-dioic and 2-hydroxydocosanoic) and terpenes (lupenone, sitosterol, β-amyrine, erythrodiol, methylbetulininate) were the main compounds identified among the pyrolysis products. Those compounds are birch bark natural components which were removed by the thermal evaporation. The commercial natural betulin sample is available from JSC 'SPEKTR T.T.& T.', Moscow, Russia at 85–95% purity for approximately 4900 US$/kg (December 2001).

CONCLUSION

Approximately 80% of the betulin present in birch bark was extracted by means of a vacuum pyrolysis reactor operated at 250, 275 and 300°C under a total pressure of less than 0.7 kPa pressure. ^{1}H NMR and mass spectra of betulin extracted and purified by soxhlet extraction and vacuum pyrolysis were similar and revealed no thermal modification of the betulin natural structure. A high pyrolysis temperature at 300°C increased the thermal degradation products without a significant increase of the betulin yield. A temperature range of 250–275°C is optimal for the extraction of betulin from birch bark. Pure betulin was partially recovered inside the reactor wall. The rest was mixed with the other birch extractives and degradation products inside the condensers. Low pressure and absence of cold spots inside the reactor will enable the recovery of betulin at high yield and of high purity.

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