

A simple preparation of betulinic acid from sycamore bark

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Abstract Betulinic acid is a biologically active compound, which has been well-known for anti-cancerous properties especially for skin. Recently, it has been drawn attention as a compound for combating AIDS. It can be chemically derived from betulin or by solvent extraction from bark. However, bark extracts are complex mixtures that always contain compounds with a wide spectrum of polarity. In this study, we have found a simple method to get high purity betulinic acid from Sycamore outer bark using hot water pre-treatment followed by hydrophilic organic solvent extraction with a yield of 5–6% (w/w).

Keywords Sycamore bark · Betulinic acid · Hot water pre-treatment · Solvent extraction · Hydrophilic organic solvent

Introduction

Plant extracts are usually complex mixtures of various polar compounds and their study often includes a purification step, such as solid-phase extraction, to isolate interesting compounds prior to analytical investigation. Betulinic acid is a naturally occurring pentacyclic triterpene that exhibits a variety of biological activities including potent antitumor properties. Its anti-cancer and anti-HIV activities have been investigated by many scientists.

Betulinic acid has several botanical sources, but can also be chemically derived from betulin [1–3], a substance found in abundance in the outer bark of white birch trees. Betulinic acid has been found to selectively kill human melanoma cells while leaving healthy cells alive. For the past four decades, the incidence of melanoma has been increasing at a higher rate than any other type of cancer. Betulinic acid has also been found to retard the progression of HIV 1 infection, which eventually leads to AIDS, by preventing the formation of syncytia (cellular aggregates) [4]. In addition, betulinic acid has antibacterial properties and inhibits the growth of both *Staphylococcus aureus* and *Escherichia coli*. It has been reported that betulin and some of its biosynthetic derivatives, e.g. betulinic acid, betulinic aldehyde, acetyl-betulinic aldehyde, allobetulin and lupeol are present in birch barks with betulin content varying from 10 to 30% (w/w) [5]. Betulin is used as a photo stabilizer for mechanical and chemical pulps [6], while betulinic acid is presently undergoing preclinical development as distributed above.

In this study, we used sycamore (*Platanus occidentalis*) tree outer bark as raw material and extracted betulinic acid with more than 5% yield and 95% purity using hot water extraction followed by solvent extraction. Betulinic acid was obtained as a solid by removing the solvent with a rotary evaporator and it was characterized by comparing IR, NMR and MS spectra with those of an authentic sample.

Experimental

Preparation of betulinic acid

Sycamore outer bark falls down by itself or can be peeled off by hand easily without cutting down the tree. It was collected and crushed as a sample source. It was boiled in

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water for 1 h, which was replaced with new water and boiled for another a half hour. After filtration, it was extracted thrice with hydrophilic organic solvents (e.g. methanol, acetone, ethanol, 2-propanol) for 24 h at room temperature. The filtrates were combined and evaporated and the collected products used for analysis.

Melting point measurement

Melting point was measured by the Fisher-Johns Melting Point Apparatus (Series No. 4006, Fisher Scientific Co.) and HaakeBuchler Melting Point Apparatus (App. No. 89 65 315).

IR spectra measurement

Fourier-transform infrared (FTIR) spectra were obtained directly from the products using the high attenuated total reflectance technique in a Bruker Tensor 27 FT-IR Spectrometer. The spectra were recorded in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} over 16 scans.

NMR spectra measurement

Acetone- d_6 (d , 99.9%) (Cambridge Isotope Laboratories, Inc., GC) was used as a solvent. A 10 mg of the sample was put into a 1 mL vial, dissolved in 1 g acetone- d_6 solutions and stirred until no precipitation was confirmed. It was then put into a measurement tube for analysis.

Mass spectra measurement

By direct introduction, mass spectra (MS) of the products were measured at 75 eV and at the minimum temperature (source temperature: 170°C, sample temperature: 180°C) required for volatilization.

Results and discussion

Using this simple method and trying to find a better extraction solvent, we were surprised to find that the yields of betulinic acid were 5.70% by methanol extraction, 5.43% by acetone extraction, 5.46% by ethanol extraction, and 5.57% by 2-propanol extraction, respectively. The average yield was 5.54%. The purity of the product was tested to compare with 95% authentic compound on TLC (solvent mixture, hexane:ethyl acetate:acetic acid 7:3:0.1 v/v) and it was much cleaner than the authentic compound; therefore it was concluded to be over 95% pure. Additionally, we tested the purity by using GC (capillary column EC-1, 0.25 mm \times 30 m, operation conditions: 200°C initial temp., 1 min hold, rate 5°C/min, final temp. 250°C, 20 min hold, TMS), and found its purity to be 96.2%. The

melting point (Both methanol and acetone extracts were measured.) was over 300°C using a manual melting point apparatus (max 300°C). Additional testing using a digital melting point apparatus, showed it to be 300–303°C (295°C in Aldrich Chemical catalog, 294°C in TCI Co. catalog, and 316–318°C in Encyclopaedia Chimica). The structure of betulinic acid is shown in Fig. 1.

IR spectra of the product

In Fig. 2, the absorption at 3428 cm^{-1} based on $-\text{OH}$; the absorption at 2923, 2868 cm^{-1} based on $-\text{CH}$, $-\text{CH}_3$, $-\text{COOH}$

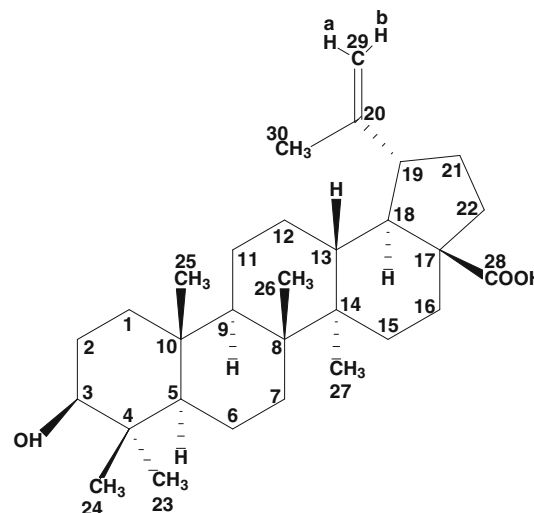


Fig. 1 Structure of betulinic acid

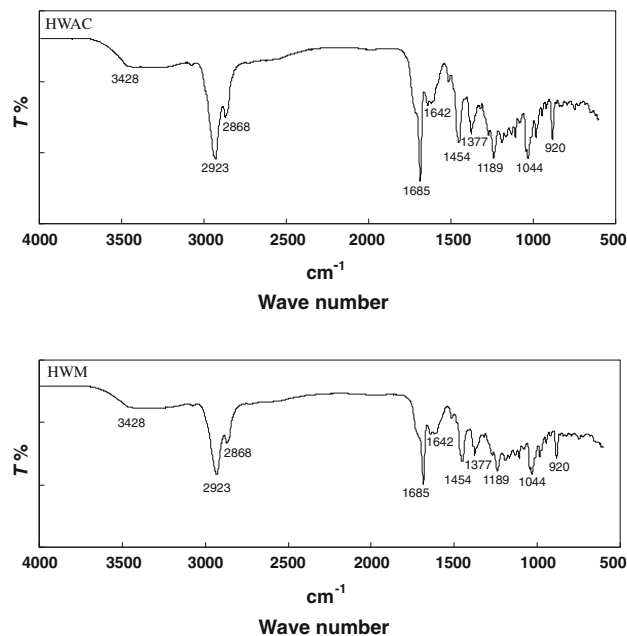


Fig. 2 FT-IR spectra of HMWAC and HWM from the Sycamore bark (HWAC hot water–acetone extracts, HWM hot water–methanol extracts)

Fig. 3 ^1H -NMR and ^{13}C -NMR spectra of HWAC from the Sycamore bark ^1H -NMR spectra (proton number consists with Fig. 1) and ^{13}C -NMR spectra (carbon number consists with Fig. 1)

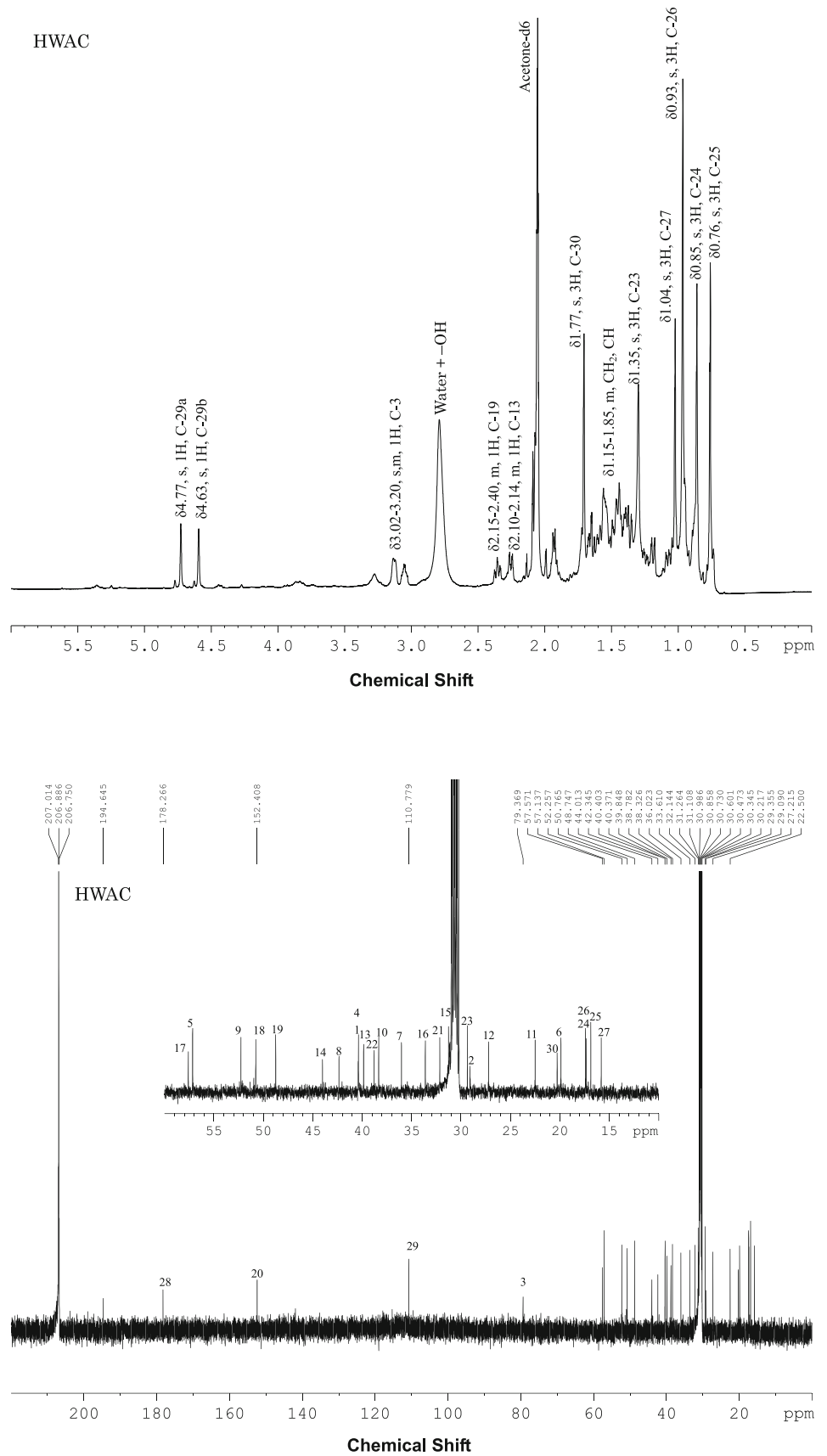
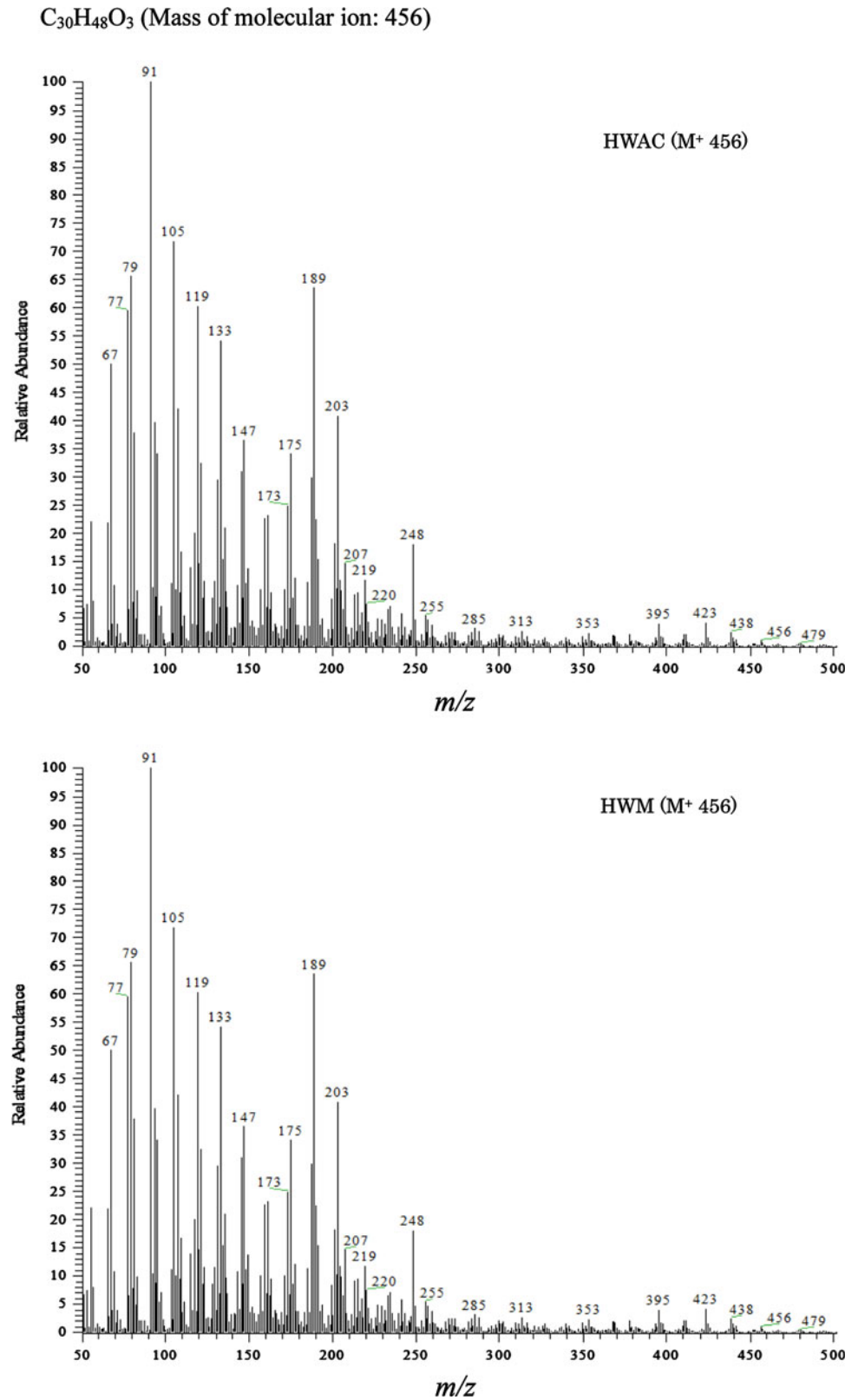


Fig. 4 MS spectra of HWAC and HWM from the sycamore bark $C_{30}H_{48}O_3$ (mass of molecular ion: 456)



and 1685 cm^{-1} based on $-\text{COOH}$; 1642 cm^{-1} based on $-\text{C}=\text{CH}_2$ can be observed clearly both in the HWM and HWAC. All the other absorptions matched those for the betulinic acid in the organic compound database of AIST in Japan.

NMR spectra of the product

Both $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra are shown in Fig. 3, which were exactly matched to the spectra of betulinic acid in the database of Aldrich Chemical Co.

MS spectra of the product

Mass spectra are shown in Fig. 4. Both acetone and methanol extracted samples showed the same features. All of them matched to the spectra of betulinic acid in the organic compound database of AIST in Japan.

Conclusions

In this work, a new and simple method for obtaining betulinic acid from the outer Sycamore tree bark has been indicated. Hot water extraction prior to organic solvent

extraction is the key in this process. We think that water resistance contents were cleaned by the hot water extraction and betulinic acid thus became easy to elute into an organic solvent. This method is low cost and very simple.

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