



# Theoretical and vibrational spectral analysis of betulin natural extract from Birch tree

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## ABSTRACT

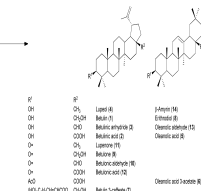
Most of the pentacyclic triterpenes species from extract possess an extremely important antitumor activity, with a low toxicity [1, 2]. Therefore, their structure-function relationship is of highly importance in the field. In this respect, the Fourier transform-Raman (FT-Raman) and FT-IR spectra of birch bark natural extract from *Betula Pendula Roth* were analyzed with the intention of gaining a deeper insight into the structure and vibrational spectra of betulin and of further suggesting the possibility of cyclodextrin complexation [3]. Betulin (lup-20(29)-ene-3 $\beta$ ,28-diol), found mainly as crystalline deposits in the outer layer of the bark, can be extracted by sublimation or extraction using organic solvents, as proved from our earlier study [4], in which the best solvent was found to be dichloromethane [3]. The promising biological activity of this compound has led us to perform detailed spectroscopic and theoretical DFT investigation on its molecular structure. The theoretical spectra of betulin resemble well the experimental ones, acquired using a Bruker spectrometer as shown in the Fig. 1. The most intense bands in the Raman spectrum were 1195 cm<sup>-1</sup> (assigned to mixed CH bending, CH<sub>3</sub> rocking and C-CH<sub>3</sub> stretching), 1440 cm<sup>-1</sup> (attributed to CH<sub>2</sub> and CH<sub>3</sub> bending), 1645 cm<sup>-1</sup> (assigned to CH<sub>2</sub>-CH<sub>3</sub> bending and H<sub>3</sub>C-CH<sub>3</sub> stretching) and the very strong band at 2927 cm<sup>-1</sup> (assigned to CH, CH<sub>2</sub> and CH<sub>3</sub> stretching). To achieve a precise assignment of Raman bands observed experimentally and for a detailed understanding of the optimized geometry of the molecular structure, we employed density functional calculations. First of all, a geometry optimization calculus was employed using Gaussian 03 software package at the B3LYP/6-31G(d) level of theory. Afterwards, a frequency theoretical calculus was performed using the same basis set, in order to predict the IR and Raman spectra of the molecule. No negative frequency modes were obtained, proving that a true minimum on the potential energy surface was found.

## Introduction

Because of its wide range beneficial medicinal activities, the bark of birch tree has been the subject of respect and admiration since ancient times, as well as the subject of science and industry in the modern world. The outer bark of birch tree is rich in pentacyclic triterpenoid compounds such as betulin, betulinic acid, betulinic aldehyde, lupeol, oleanolic acid, betulin 3-caffeate and other minor components. The biological activities of pentacyclic triterpenes are known to have a wide-range of activities, some of them possessing anti-virus, anti-inflammatory, anticancer and other useful properties. Betulin (lup-20(29)-ene-3 $\beta$ , 28-diol), prevails within the composition of triterpenes from the birch bark.

In this study, FT-Raman and FT-IR spectroscopy were employed in order to characterize the structure of betulin, obtained from vegetal sources (birch tree outer bark, birch tree leaves) by sublimation or extraction with chemical solvents (methanol, dichloromethane, chloroform). The promising biological activities of this compound encourage us to perform detailed spectroscopic and theoretical investigations on its molecular and vibrational structure.

## Materials



Betulin was purchased from Extrasynthese, France as white crystalline powder of analytical grade purity.

Molecular formula : C<sub>30</sub>H<sub>50</sub>O<sub>2</sub>

Molecular weight: 442.72 g/mol<sup>-1</sup>

Melting point: 256-257<sup>o</sup> C

## Computational details

Quantum chemical calculations were employed for the optimization of the molecular structure of betulin using the Gaussian 03 software package at the B3LYP level of theory. For the expansion of the orbitals the splitting valence basis set 6-31 G(d) of Pople's group was used. After the molecular optimization, a frequency theoretical calculus was performed using the same basis set, in order to predict the IR and Raman spectra of the molecule. The computed optimized geometry and the corresponding parameters are confirmed to be a minimum energy conformation, considering the fact that no negative frequency modes were obtained.

## Instrumentation

FTIR and FT-Raman spectra have been recorded using an Equinox 55 Bruker spectrometer with an integrated FRA 106 S Raman module. An Nd: YAG laser operating at 1064 nm line was employed for the excitation of the samples. The power of laser was set to 400 mW.

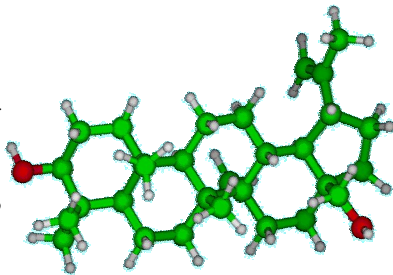
An attenuated total reflectance (ATR) module has been coupled to the Equinox 55 FTIR Bruker spectrometer for the recording of the FTIR absorbance spectra in the 4000-650 cm<sup>-1</sup>. The spectral resolution was 4 cm<sup>-1</sup> and 40 scans were accumulated for each powder sample.

## Results and discussions

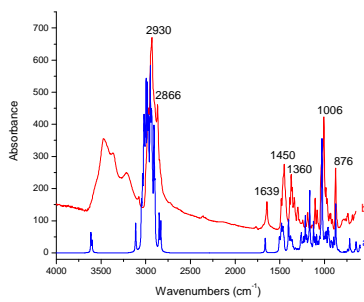
### Geometry optimization

Chemically betulin is a triterpenoid of lupane structure. It comprises of four six-member rings and one five-member ring.

One hydroxyl group is present in C14 position, one CH<sub>2</sub>OH group in C68 position, five methyl groups in C13, C1, C5 and C22 positions and one CH<sub>2</sub>CH<sub>3</sub> group (terminal group) in C78 position.



### FT-IR spectra

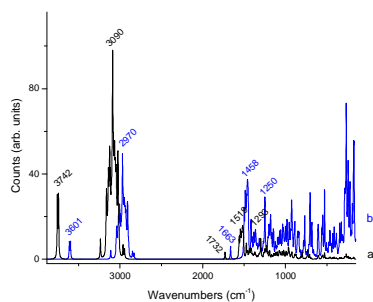


FTIR theoretical (a) and experimental (b) spectra of betulin. Excitation 1064 nm, Nd:YAG laser

The broad band at 2930 cm<sup>-1</sup> appears due to asymmetrical CH<sub>2</sub> stretching from the CH<sub>2</sub>OH group, while its shoulder, the 2866 cm<sup>-1</sup> can be assigned to CH<sub>3</sub> stretching in the first ring. The 1639 cm<sup>-1</sup> band can be assigned to C=C stretching and CH<sub>2</sub> bending in the terminal methyl group. The 1450 cm<sup>-1</sup> appears because of bending vibrations of the methyl and of the CH<sub>2</sub> groups in the rings, while the 1370 cm<sup>-1</sup> band can be assigned to bending vibrations of the CH<sub>3</sub> groups. The band at 1006 cm<sup>-1</sup> can be assigned to C-O stretching vibrations in the CH<sub>2</sub>OH group. 875 cm<sup>-1</sup> appears due to wagging vibrations of the CH<sub>2</sub> in the terminal group.

### FT-Raman

Theoretical Raman spectra of betulin (a) before and (b) after scaling the frequencies. Raman activities were converted to relative Raman intensities using a scaling factor of  $f = 0.9614$ . In the scaled spectrum, the wavenumbers are shifted towards right (especially in the big wavenumbers region ~ 100 cm<sup>-1</sup>) and the relative intensity of the bands is weaker in the big wavenumbers region and higher in the small wavenumbers region.



A linear correlation between calculated harmonic frequencies (B3LYP/6-31G(d)) and the observed position of the main Raman peaks of betulin was computed for finding the right scaling factor. The slope of the straight line gives a scaling factor of 0.9606 which is very close to the one used to calculate the scaled theoretical frequencies.

Raman theoretical (a) and experimental (b) spectra of betulin. Excitation 1064 nm.

In the fingerprint region, the Raman spectrum presents intense bands at 1645 cm<sup>-1</sup> assigned to C=C stretching and CH<sub>2</sub> bending in the terminal methyl group, 1484, 1464 and 1440 cm<sup>-1</sup> which have been assigned to CH<sub>2</sub> and CH<sub>3</sub> bending vibrations, 1195 cm<sup>-1</sup> band attributed to C-H and O-H bending and C-H twisting vibrations and 701 cm<sup>-1</sup> assigned to twisting vibrations in the terminal group.

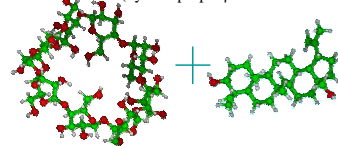
The hydroxyl groups present in C16 and C68 positions have significant importance. Analyzing the way they influence the Raman and IR spectra and which vibrational modes are amplified when they are excited is of great value in the case of studying the interaction mode of betulin with other molecules, such as cyclodextrines. The terminal group CH<sub>2</sub>CH<sub>3</sub> and the hydroxyl groups have significant influence on some medium and strong bands that appear in the vibrational spectra of betulin.

## Conclusions

FT-IR and Raman spectra of betulin pentacyclic triterpene have been presented and a tentative assignment of Raman modes has been proposed. In order to reveal betulin's structural and characteristic features, quantum chemical calculations and vibrational spectral studies have been performed.

This study creates perspectives for further research in cancer diagnosis, based on investigating the inclusion complexes made of HPGCD (hydroxypropil- $\gamma$ -cyclodextrin) and betulin.

Kneading product of betulin and HPGCD cyclodextrin



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## Selected References

- [1] C. A. Dehelean, C. Tatu, S. Cîntă Pânzaru, C. Peev, C. Soica, G. Tanasie, S. Anghel, Toxicology Letters, 164, 1, (2006) S228.
- [2] S. C. Pinzaru, N. Leopold, W. Kiefer, "Vibrational spectroscopy of betulinic acid HIV inhibitor and of its birch bark natural source", Talanta, 57, 2002, 625-631
- [3] C. M. Soica, C. A. Dehelean, C. I. Peev, G. Coneac, A. T. Gruia, "Complexation with HPGCD of some pentacyclic triterpenes. Characterization of their binary products", Farmacia, vol. LVI, 2, 2008, 182-190
- [4] C. A. Dehelean, S. Cîntă Pinzaru, C. I. Peev, C. Soica, D. S. Antal Characterization of birch tree leaves, buds and bark dry extracts with antitumor activity", J Optoelectr. Adv. Mat. 9, 3, 2007, 783-787