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The Betulin Project

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2014

# The Betulin Project: Introduction 2014

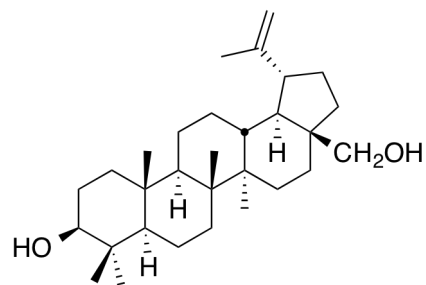
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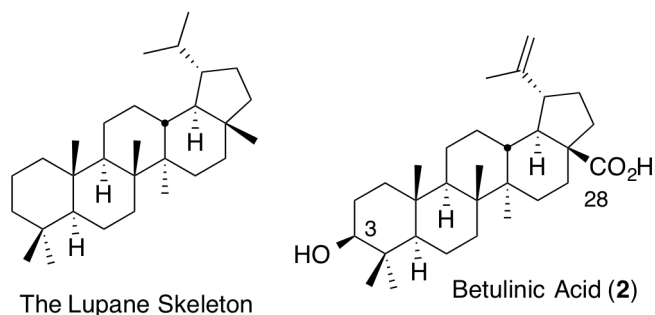
## Betulin Project

Betulin (**1**) is a lupane triterpene natural product that is most commonly found in the bark of birch trees (*Betula sp.*). In fact, the outer bark of the white and paper birches (*Betula alba* and *B. papyrifera*, respectively) are reported to contain up to 30% betulin.<sup>1</sup> The bark of the white birch will provide the source of betulin for this project. Innumerable claims have been made as to the biological activities of betulin, which is widely considered to be a protective compound for the birch. These claims have been difficult to verify, though given the high abundance of betulin in the bark of these two species, it is difficult to imagine that such an expenditure of bioenergy does not have a advantageous purpose if the species was to successfully compete in nature. Given it's highly nonpolar character, and lack of functionality other than the primary and secondary alcohols, as well as the alkene, the role of betulin in the survival of the paper and white birch may be simply to serve as a protective coating, not unlike the coatings of paint one applies to a home to protect the external walls from the various forces of nature, both passive and predatory. Thus, in addition to being highly water resistant, betulin simply doesn't "taste good" to potential birch predators. In fact, when one walks through a deciduous forest in New England and comes across a long-fallen white or peper birch, it is readily apparent that the tree rots from the inside out, not through the bark. The betulin-protected coating remains in tact long after the internal matrix of the birch tree has crumbled into decay.

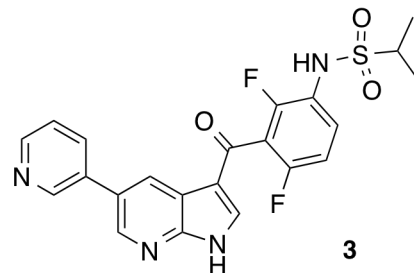


Betulin (**1**)

While natural products chemists have long been attracted to terpenoids in general, triterpenes of the lupane class, to which betulin belongs, have been a keen focus of many research groups. Lupane triterpenes have numerous representatives with important biological activities, most appropriately for this project perhaps being betulinic acid (**2**). Betulinic acid has undergone intense scrutiny from both natural product and synthetic chemists due to its confirmed activity against melanoma,<sup>2</sup> the most virulent and often deadly form of skin cancer. Until recently, there were no



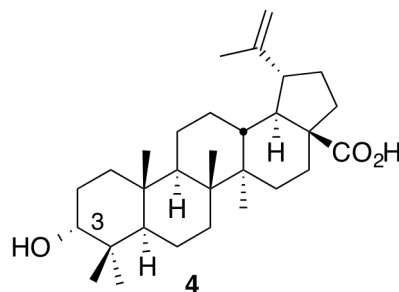
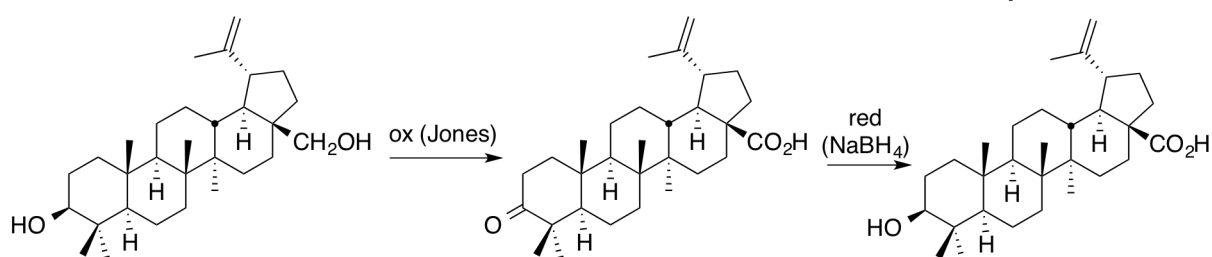
chemotherapeutic agents available to treat melanoma, and even the best of treatments now available are only marginally successful. For example, a recently approved drug for melanoma, vemurafeniv (**3**), is 77 - 81% effective in keeping melanoma in check after metathesis for only about 7 months, at a cost of \$112,000/year, for those patients with B-RAF mutation (about half of all melanoma cases). Further expanding the interest in



betulinic acid were later reports of its activity against HIV.<sup>3</sup>

The excitement triggered by the anti-melanoma activity of betulinic acid has long been tempered by its very poor bioavailability caused by its very high level of lipophilicity (it is very hydrocarbon-like). Medicinal chemists have been preparing derivatives for a long time in an attempt to discover equally effective analogues, but with better water solubility, and hence better bioavailability. In addition, these efforts to alter or “decorate” around the betulinic acid structure have led to a better understanding of which functional groups of **2** are required for bioactivity. For example, it is now known that the C-28 carboxylic acid is required (betulin itself is not active against melanoma, for example, and neither are esterified betulinic acid derivatives), and the C-3  $\alpha$ -stereoisomer **4** is significantly less active than **2**. In addition, the activity of betulinic acid has spurred numerous investigations for the synthetic conversion of **1**, which is very abundant and inexpensive, into **2**, which is relatively scarce and expensive (Scheme 1). This

**Scheme 1.** Conversion of betulin to betulinic acid.



conversion, however, is not stereoselective at C-3 in the reduction step. A direct oxidation of betulin to betulinic acid has also been reported.<sup>4</sup>

In this project you will begin with the isolation of betulin from the bark of the white birch collected locally (Harvard, MA), then use the betulin you've isolated to make derivatives that ultimately derivatize and remodel the basic structure. A primary goal in this chemistry will be the incorporation of nitrogen into the betulin framework. The derivatives targeted are designed to both illustrate basic reactions and principles in organic synthesis, as well as to build a small library of betulin derivatives for biological screening. Ultimately, the goal is to have you develop your own ideas that extend the chemistry carried out this semester in a direction that intrigues you. These further laboratory explorations could pursue other analogues of betulinic acid that would improve bioavailability (this is a popular endeavor, so one needs to be aware of the literature before pursuing such goals), further probe structural requirements for bioactivity (the bioactivity need not be “anticancer”, but any activity discovered in a biological screen), or even remodel the basic lupane skeleton of betulin itself to generate new, complex “unnatural” or “remodeled” natural products. You may also wish to pursue the conversion of betulin to betulinic acid, then use betulinic acid as the starting material for further medicinal chemistry pursuits.

You will be given a minimal outline of the goals of each lab, then be required to write out the procedure you intend to implement in the lab itself prior to the start of the pre-lab

session, during which we will discuss the procedure(s) to employed. **Follow the notebook format as detailed for the regular organic chemistry lab.** It is certainly possible that more than one of your procedures you've uncovered from the literature will be implemented in the lab by if more than one set of protocols looks reasonable. Good luck, and should your experiments fail, the chemistry department will, of course, disavow any prior knowledge of your mission.

Vocabulary:

triterpene  
terpenoid  
functional group  
alcohol  
alkene  
lupane triterpene  
bioavailability  
lipophilicity  
hydrocarbon  
esters  
stereoisomer

Questions: (40 pts total)

- 1) What is the structure of the methyl ester of betulinic acid?
- 2) Why does high lipophilicity reduce bioavailability?
- 3) What is cLogP?
- 4) What is the difference between a lupane triterpene and an oleanane triterpene?
- 5) What is the structural difference between **2** and **4**?

In this project, the following experiments may be carried out, though as with all research projects, variations in the experiments depends upon the outcome of the previous experiment. These goals are illustrated in accompanying PowerPoint presentation, which also points out procedures attempted last year (Fall, 2013) which did not work well, or at all!

Experiment #1 – Isolation of Betulin from the Bark of *Betula alba*

Experiment #2a – Monoacetylation of Betulin

Experiment #2b – Rearrangement of Betulin to Allobetulin

Experiment #3 – Oxidation of Betulin Monoacetate (Betulone Monoacetate)

Experiment #4 – Oxime Formation from Betulone Monoacetate

Experiment #5 – Beckman Rearrangement of Betulone Monoacetate Oxime

## Experiment #6 – Reduction of A-Ring Lactam

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<sup>1</sup> For excellent reviews of betulin: (a) Jāāskelāinen, P. *Pap. Puu* **1981**, *63*, 599-603. (b) Eckerman, C.; Ekman, R. *Pap. Puu* **1985**, *67*, 100-106. (c) Hayek, E. W. H.; Jordis, U.; Moche, W.; Sauter, F. *Phytochemistry* **1989**, *28*, 2229-2242.

<sup>2</sup> (a) Pisha, E.; Chai, H.; Lee, I.-S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Das Gupta, T. K.; Pezzuto, J. M. *Nature Med.* **1995**, *1*, 1046-1051. (b) Fulda, S.; Friesen, C.; Los, M.; Scaffidi, C.; Mier, W.; Benedict, M.; Nu~ez, G.; Krammer, P. H.; Peter, M. E.; Debatin, K.-M. *Cancer Res.* **1997**, *57*, 4956-4964.

<sup>3</sup> (a) Fujioka, T.; Kashiwada, Y.; Kilkuskie, R. E.; Cosentino, L. M.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Chen, I.-S.; Lee, K.-H. *J. Nat. Prod.* **1994**, *57*, 243-247. (b) Lee, K.-H.; Morris-Natschke, S. L. *Pure Appl. Chem.* **1999**, *71*, 1045-1051.

<sup>4</sup> Csuk, R.; Schmuck, K.; Schaefer, R. *Tetrahedron Lett* **2006**, *47*, 8769-8770.