Obtaining and purifying esculin acetates through reactions catalyzed by Novozyme 435

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INTRODUCTION

Esculin is a glycosylated coumarin whose range of bioactivity has already been demonstrated in murine models. However, these classes of molecules have low solubility in both hydrophilic and hydrophobic media, which hinders their industrial application. Acylation reactions allow coumarins to become more lipophilic by incorporating acyl radicals into these compounds, consequently enhancing their solubility. Furthermore, it has been proven that the insertion of carbon chains into coumarins and flavonoids can improve biological activity. Biocatalytic processes are widely used to acylate molecules due to the characteristic selectivity of enzymes, with a special focus on transesterification reactions that can yield excellent results. In this regard, the objective of the present study was to promote the enzymatic acylation of esculin.

METHODS

- 15 mg of esculin (Sigma-aldrich)
- 1 mL of vinyl ester (Sigma-aldrich)
- 1 mL of solvent (THF)
- 10% lipase to total substrate mass

Stirring: 200 rpm (Orbital shaker);
Temperature: 60°C.
Reaction times: from 0h to 8h (50µL aliquots)

Scale-UP (qudruplicate)

Liquid Cromatography analysis: HPLC-DAD and LC-MS (274 nm)

Kinetic analysis data

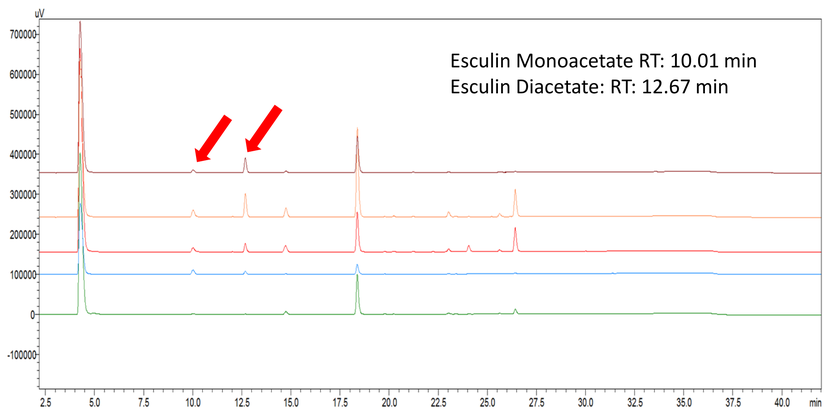
Purification by high-speed countercurrent chromatography

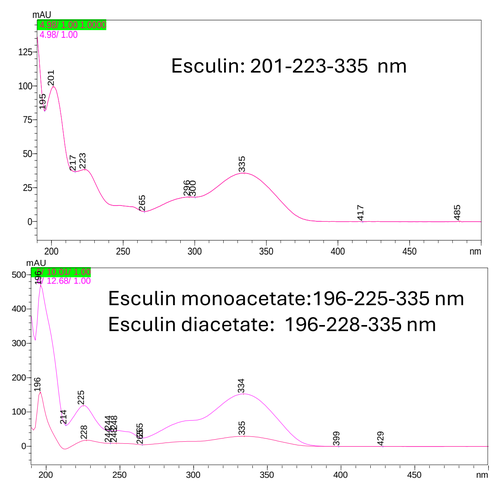
Structural elucidation by nuclear magnetic resonance

500 MHz

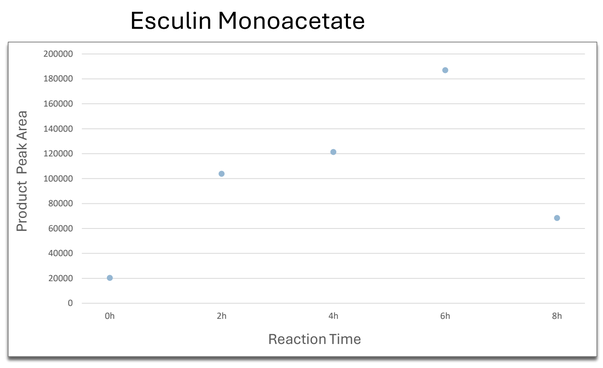
RESULTS AND DISCUSSION

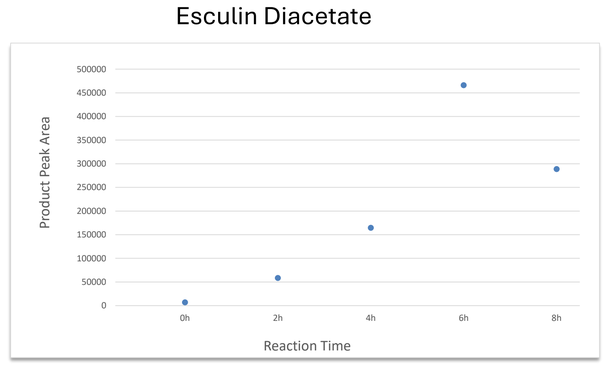
- **HPLC-DAD analysis**



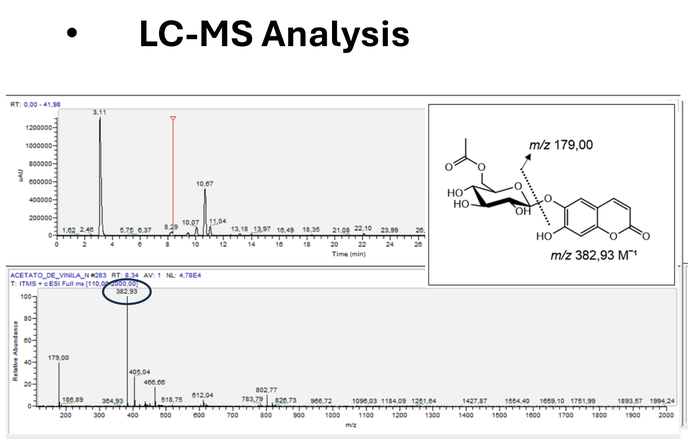


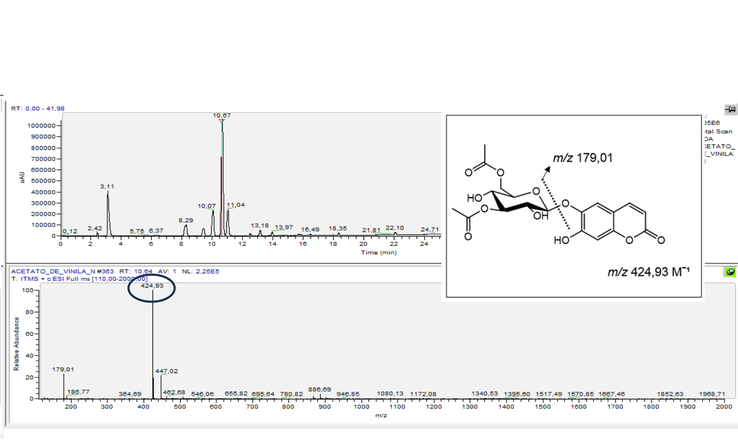
- **Kinetic analysis**





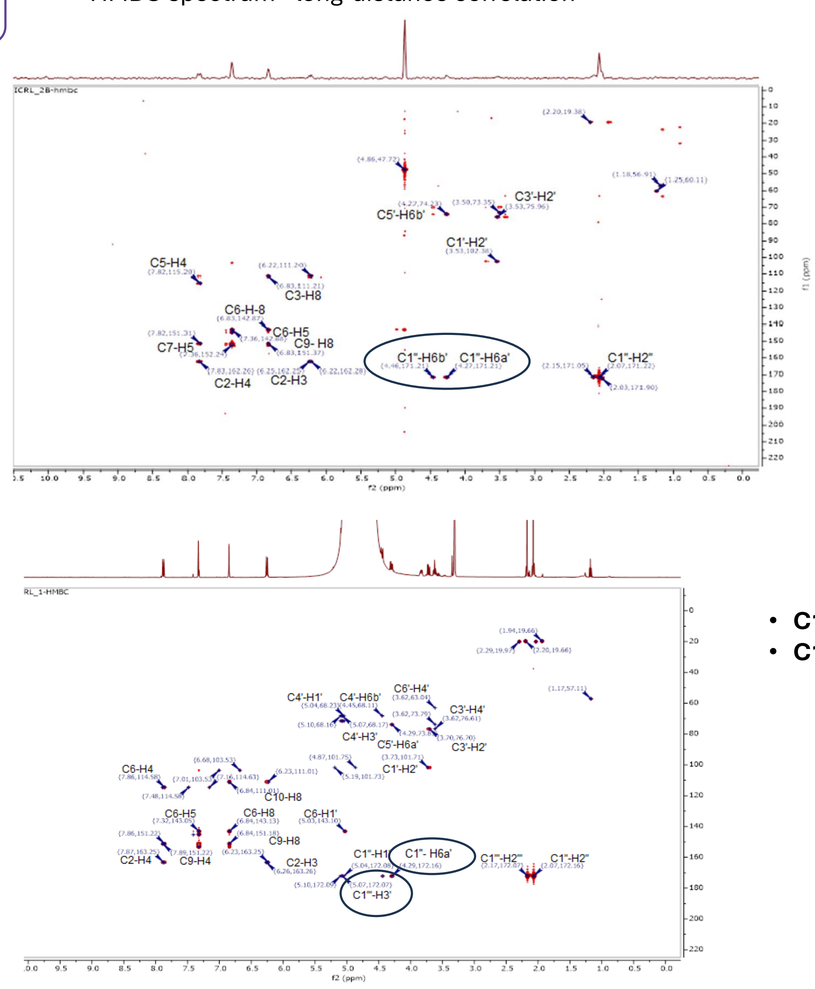
- **LC-MS Analysis**



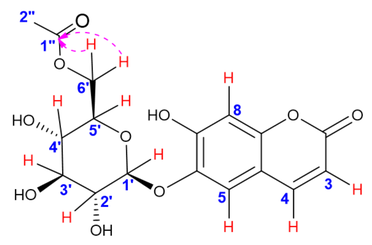


- RMN analysis

HMBC spectrum - long-distance correlation



C1''-H6a' e C1''-H6b'- Proves acylation at the 6'OH position of the glycosidic moiety



- **C1'''-H3':** acetylation at the 3'-OH position
- **C1''-H6a':** acetylation at the 6'-OH position