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Abstract

Obtaining and purifying esculin acetates through reactions catalyzed by Novozyme 435 †

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Abstract: 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. 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. Vinyl acetate was used as the acylating agent, and Novozyme 435, an immobilized lipase, served as the catalyst, with both reagents solubilized in THF. The biocatalytic transformation occurred at a temperature of 60°C, six hours after the start of the reaction. The products were isolated using high-speed countercurrent chromatography. The chemical structures of esculin monoacetate and diacetate were determined through ^{1}H and ^{13}C and two-dimensional Nuclear Magnetic Resonance, as well as liquid chromatography coupled with mass spectrometry. The formation of the products was monitored by thin-layer chromatography and high-performance liquid chromatography coupled with diode arrays at regular time intervals between 0 and 8 hours of the reaction to obtain retention time data for kinetic analysis. The λmax values of esculin monoacetate were found to be 196, 225, and 334 nm, while those of esculin diacetate were 196, 228, and 335 nm. The evaluated methods confirmed that one reaction product was esterified at the 6'OH position (R_T: 10.01 min and m/z 382.93 $[M-H]^-$) of the glycosidic portion of esculin, and the other at both the 6'OH and 3'OH positions (R_T: 12.67 min and m/z 424.93 $[M-H]^-$) in that moiety of the molecule.

Keywords: Biocatalysis; Esculin acetates; Liquid chromatography

Supplementary Materials:

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