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Enzymatic Esterification of Betulinic Acid

S. Omar, F. H. Ahmad, H. Hassan, N. H. Faujan and **M. Basri**

Department of Chemistry, Faculty of Science and Environmental Studies, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT Esterification of betulinic acid with 1-decanol in chloroform was described. It gave the desired betulinic acid ester. The esterification as expected occurred at C-28 position. The conversion gave high yield of the desired ester after 24 hours of reaction. The reaction was carried out at 37 ^{0}C in horizontal water bath shaker. Lipozyme, an immobilized lipase, was used as the catalyst. The product was analysed using spectrocopic data. To the best of our knowledge this is the first report in which betulinic acid ester was prepared using enzymatic reaction.

ABSTRAK Pengesteran asid betulinik dengan 1-dekanol dalam kloroform telah dikaji. Tindakbalas ini menghasilkan asid betulinik ester seperti yang dijangkakan. Pengesteran berlaku pada kedudukan C-28. Tindakbalas ini menghasilkan ester yang tinggi selepas tindak balas dilakukan selama 24 jam pada suhu 37 ^{0}C. Lipozim digunakan sebagai mangkin dalam tindak balas ini. Hasil yang didapati telah dianalisa dengan menggunakan data spektroskopi. Penemuan ini adalah yang pertamanya dilaporkan di mana ester betulinik asid berjaya disediakan dengan tindak balas enzim.

INTRODUCTION

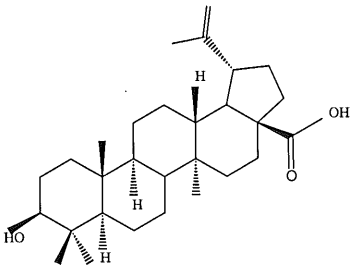
Betulinic acid **1**, 3β-hydroxy-lup-20(29)-ene-28-oic acid is a pentacyclic tritepene. Betulinic acid could be isolated from the leaves of *Syzigium claviflorum.* Although betulinic acid has several botanical sources, it can be chemically derived from betulin, a substance found in abundance in the outer bark of white birch trees (*Betula alba*). *Betulinic acid was* found to inhibit the HIV replication in H9 *lymphocyte cells* [1] and selectively kill human melanoma cells while leaving healthy cells alive [2, 3].

Enzymes are delicate lifelike substance found in all living cells. Enzymes differ in two ways from inorganic catalyst, such as acids, metals, and metal oxides. Firstly, enzyme are very large molecules that, with a very few exceptions, are proteins. Secondly, enzymes are very specific in their action.

Lipases are used in organic synthesis for several reactions such as hydrolysis, esterification, thioesterification and amidation. In our laboratory we observed that lipozyme can catalyse the esterification reaction of some aromatic acids in high yields [4]. Thus; we now applied such methodology to the synthesis of betulinic acid ester without pre-protected at C-3 position and our preliminary results are presented in this communication.

MATERIAL AND METHODS

Crude betulinic acid was subjected to silica gel column chromatography using dichromethane, as the eluent and concentrated to one tenth of their volume using rotary evaporator and kept in a refrigerator for overnight. Fine colourless crystals were separated out from the elutes.



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To a solution of betulinic acid in chloroform was added a stock solution of 1-decanol in chloroform and lypozime. The mixture was then shaken for 24 hours at 37 ^{0}C. The lypozime was filtered off

and the solvent was then removed by rotary evaporator. The white crystalline solid obtained was then dried in the dessicator. The pure product was subjected to silica gel column chromatography using dichromethane, as the eluent

RESULTS AND DISCUSSION

The IR spectrum of the desired product showed the absorbance for hydoxyl group at ν_{max} 3420-3000 cm^{-1}, carbonyl group at ν_{max} 1686 cm^{-1} and olefinic bond at ν_{max} 1644 cm^{-1}, respectively. The absorbance at ν_{max} 884 cm^{-1} was due to CH out of plane bend.

The white solid was diluted in chloroform and analyzed using GC-MS Spectroscopy. The GC-MS spectrum gave only single peak in which having molecular ion at *m/z* 598 (M^++2) suggesting the presence of the desired betulinic acid ester; $C_{40}H_{68}O_3$. The spectrum shown base peak at *m/z* 43.

Its ^{1}H-NMR spectrum in $CDCl_3$ showed signals at δ 4.60 and 4.73 corresponds to the two hydrogen of the olefinic bond at C-29. The signal that resonance at δ 3.01 was assigned for hydrogen at C-19, whereas signals at δ 0.76, 0.82, 0.94, 0.97, 1.27, and 1.69 were due to the presence of six methyl groups in the pentacyclic skeleton.

Further details (included on its bioactivities studies) on this product are on progress and will be presented elsewhere.

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