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CN5172 Individual Term Paper

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Upstream Processing of Prednisolone

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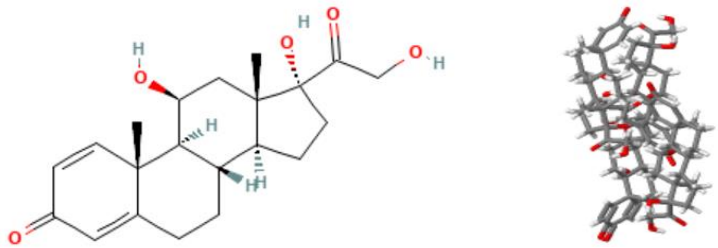
Contents:

Introduction:

Prednisolone($C_{12}H_{28}O_5$) is a steroid medication that falls under the class of corticosteroids. Unlike Anabolic steroids, that are primarily used for increasing muscle mass and reducing fat, corticosteroids are primarily used as an anti-inflammatory medication. Corticosteroids refer to steroids produced by the adrenal cortex, and their production is regulated stringently by the Adrenocorticotropic hormone(ACTH)[1]. Within the wide range of Corticosteroids, Prednisolone is classified as a glucocorticoid - impacting carbohydrate, lipid, and protein metabolism while displaying anti-inflammatory, anti-allergy and desensitizing effects due to its nature as an immunosuppressant. Prednisolone is also closely linked to Prednisone, the latter being the biologically inert precursor to the former with conversion to prednisolone from prednisone occurring primarily in the liver. As such, prednisolone is the preferred form of medication to patients whose liver activity is severely impaired.



Figures 1 and 2: Prednisolone 2D and 3D Structures

Medical usage and mechanism of Action

Prednisolone is a drug available via prescription only, and is used in the treatment of Asthma, Nephrotic syndrome, autoimmune diseases such as rheumatoid arthritis and Lupus, as well as some skin conditions such as eczema and psoriasis. It is usually prescribed in the form of a liquid (Dilute and/or concentrated) to be ingested orally. It is typically taken together with food in order to reduce the chances of stomach upsets following ingestion[2].

Upon ingestion, prednisolone decreases inflammation via suppression of the migration of polymorphonuclear leukocytes and reversing increased capillary permeability. Upon entering its target cells post-surface receptor attachment, it binds and activates specific nuclear receptors, facilitating the inhibition of proinflammatory cytokine production by altering gene expression. This reduction in cytokine production leads to a corresponding decrease in the number of floating lymphocytes, additionally stimulating apoptosis in sensitive tumour cell

populations. These effects combined lead to the suppression of the immune response near active areas, while also restricting the spread of any infection in sensitive areas. Due to its nature as an immunosuppressant, continuous use has been linked with multiple side-effects such as muscle weakness, easy bruising, and difficulties in sleeping, requiring careful regulation of dosage by doctors to maximise the beneficial effects on the patients without excessively compromising the immune system[3].

Discovery and Production:

Prednisolone was discovered by Arthur Nobile and W Charney at Schering Corporation in 1950. It was discovered when attempting to synthesise hydrocortisone by hydrolysing the acetate groups in hydrocortisone 11,21 diacetate while maintaining the integrity of the side chains present. Due to the inability to effectively carry out the hydrolysis chemically, *Corynebacteria Simplex* was used to carry out the hydrolysis which yielded prednisolone instead of Hydrocortisone. It was discovered in 1954 and approved for use just under a year later in 1955[4].

In the discovery of Prednisolone, *Corynebacteria Simplex* was used. However, in future processes, other bacterial types like *Arthrobacter Simplex* were used. Due to the absence of detailed literature on the characteristics and preparation of *Corynebacteria Simplex*, the work of Kawabata and Nakagawa in 1990 will be used as the case study to examine cell line development and production of prednisolone. In their paper, prednisolone was synthesized by immobilized *Arthrobacter Simplex ATCC 6946* (3-ketosteroid-Δ^1-dehydrogenase activity) by capture on the surface of unwoven cloth coated with Cross-linked poly-(N-benzyl-4-vinylpyridinium bromide) containing styrene (BVPS), as well as by capture on the dry surface of beads of the insoluble variant of the BVPS polymer.

Cell cultivation and Immobilization:

Cells were precultured at 33°C for 24h with growth media containing peptone and beef extract. After extensive dilution with yeast extract followed by sterilization, they were cultured under the same conditions as preculture with cortisol (dissolved in methanol) being added midway through culture, in order to activate the targeted enzyme, 3-ketosteroid-Δ^1-dehydrogenase. The cell suspension was then washed with a pH 7.0 Potassium Phosphate Buffer solution before being sent for immobilization.

Immobilization was carried out at room temperature. For the method cloth coated with soluble BVPS, the cell suspension prepared was passed through a glass column containing the rolled coated cloth for 20h before any free circulating cells were purged using potassium phosphate buffer solution. For the method involving insoluble beads, a similar procedure was carried with the exception being that the coated cloth was replaced with polymer beads arranged in a packed bed structure within the column, with the cells being immobilized within the insoluble beads at the end of the procedure[5].

Immobilization is a well-known technique for improving consistency of biocatalyst performance. However, whole cell immobilization was carried out in this procedure instead of biocatalyst(enzyme) immobilization due to the comparative advantage of surface immobilization allowing for efficient transport of desired products and raw materials (e.g. Oxygen) even those with high molecular weights, while maintaining constant exposure of the cells to the substrate.

Fermentation processing:

In Kabawata and Nagakawa's work, immobilized cells under each of the 2 techniques were then primarily utilised in continuous production methods. In the case of the *Arthrobacter Simplex* used, the precursor for prednisolone was cortisol instead of hydrocortisone, and methanol was added during the production process to stabilize the concentration of cortisol, which is insoluble otherwise in water, with potassium phosphate buffer solution also added to maintain stable pH conditions. The continuous method involved the use of a jacketed 12mm diameter, 14cm long glass column.

Spectrophotometric analysis of the liquid showed a drastic increase in yields from maximally 22% in the packed bed system to approximately 90% in the cloth based system, with enzyme activity within the immobilized cells remaining high for approximately 5 days before dropping off significantly. The provision of adequate aeration was also seen as an additional limiting factor in ensuring the consistency and stability of the production process.

Novel Technologies and Evaluative concerns:

More recently, newer methods of synthesizing prednisolone and its derivatives have been proposed by researchers. This involves either using new bacteria or by adding additional stages of bioconversion or chemosynthesis as required. In the efforts to improve the yield,

and processing of prednisolone. Here, the two main techniques of interest: Immobilization, as well as the variation of cell lines for bioconversion, will be discussed.

Immobilization:

In Kawabata and Nagakawa's work, the principle of immobilization was explored using different techniques of immobilization. However, more modern methods also have an increasing incidence of whole cell immobilization incorporated into their procedure. In fact, for prednisolone synthesis in particular, even during a mixed culture preparation method, the medium containing all cultures immobilized outperformed the remaining media with incomplete immobilization of cells, in comparison to the synthesis of hydrocortisone using the same combination of cells and the same bioconversion mechanism, in which the highest performing configuration had one set of cells immobilized and the other set of cells freely floating[6]. The methods of immobilization have changed over time however, from simple entrapment using insoluble polymer beads to the entrapment within a PVP/PEO copolymer hydrogel polymerized by radiation, which contributed to excellent yields of approximately 90% with 98.8% bioconversion observed[7]. With one of the key concerns with immobilization being the ability to sustainably manufacture and utilize enough immobilization media, these advancements in immobilization techniques and technology shows promise as a tool which could be in mainstream use in the future. However, none of the immobilization techniques mentioned were displayed in an industrial scale of manufacture. With there being a marked improvement in performance in immobilized cells against free cells for this process[6], more research must be done in order to find better methods to sustainable scale up immobilization techniques shown to significantly improve prednisolone production.

Variation of cell lines for Bioconversion:

From the initial discovery using *Corynebacteria Simplex* to the latest methods using a mixed culture method involving even fungi (*Cunninghamella echinulata*) alongside newer bacteria (*Bacillus sphaericus),*[6] there have been a multitude of methods proposed for the bioconversion stage of prednisolone production, on the basis of their high performance. However, for industrial production purposes, the chosen bioconversion method should involve cells that are able to provided consistent bioconversion performance over a range of temperatures in order to prevent significant impacts on production efficiency due to inevitable fluctuations in operating conditions throughout the long production process. As

such, research similar to that done in Kabawata and Nagakawa's work must be carried out, where the production of prednisolone was measured across a range of temperatures which indicated sharp changes in production over a relatively small range of temperatures (5°C)[5], rendering the bacteria used, *Arthrobacter simplex* unsuitable for scale-up manufacturing of prednisolone.

Conclusion:

Due to the limited available literature on scale-up processing of prednisolone, the production of prednisolone on a smaller, laboratory scale was considered, along with the potential differentiating factors between the variety of available methods to distinguish a method viable for scale-up processing among the rest. Due to its potent, yet widespread impact on the human body, this is an essential drug for production, with a lot of room for optimization as shown. In fact, the synthesis of prednisolone in a continuous process as explored earlier is also an area that has much potential for optimization, and can set a precedent for a new wave of technology converting previously antiquated batch processing methods into more modern and sustainable continuous manufacturing processes for pharmaceutical products. As such, further research especially into optimizing the production of prednisolone on a scale-up basis is definitely warranted, with true progress just on the horizon.

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