



# 橘皮酵素之酯水解反應

Regiospecific Ester Hydrolysis  
by Orange Peel Esterase

環工2A   化生二

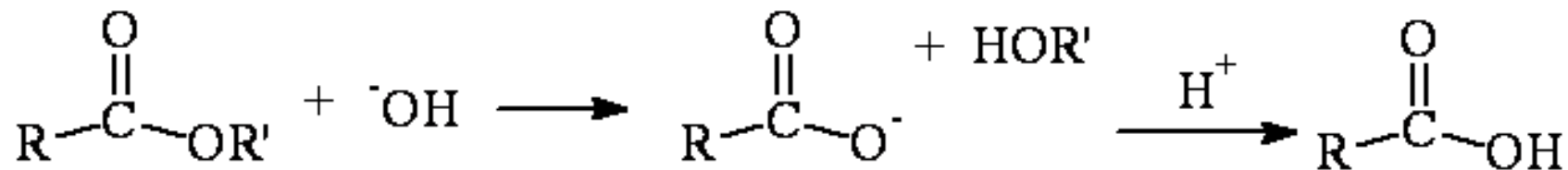
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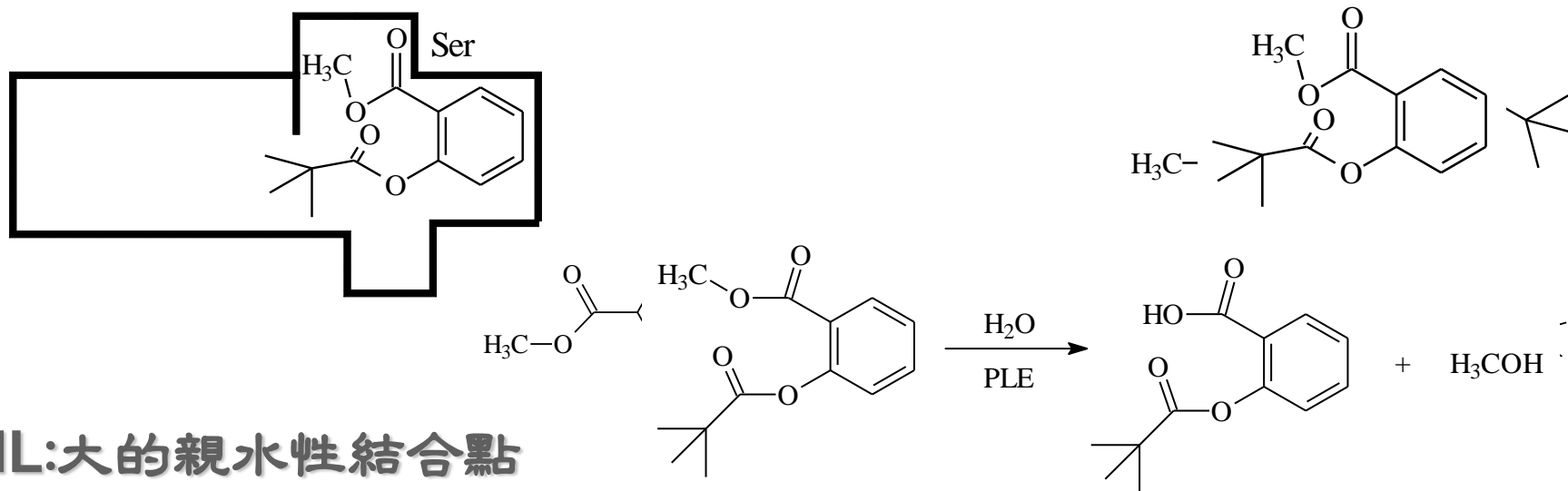
## 1. Ester hydrolysis :



## 2. Saponification:



### 3. PLE (豬肝酯化酵素)之立體專一性:



HL:大的親水性結合點

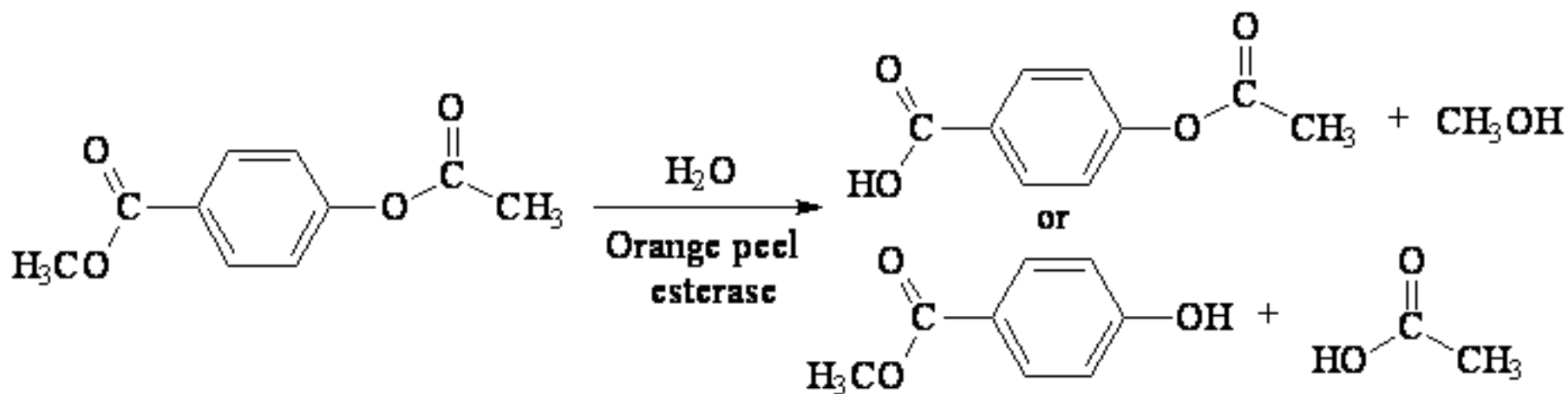
HS:小的親水性結合點

PF:前端極性基結合點

PB:後端極性基結合點

Ser:活性位置

#### 4. regiospecific ester hydrolysis:

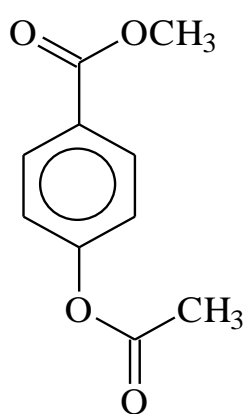


**(3) Standard TLC: ( elute with ether/hexane=1:1)**

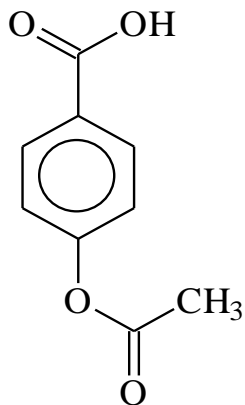
**a diester solution (methyl 4-acetoxybenzoate)**

**b 0.01g 4-acetoxybenzoic acid + 0.5mL acetone**

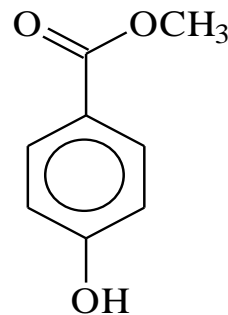
**c 0.01g methyl 4-hydroxybenzoate + 0.5mL acetone**



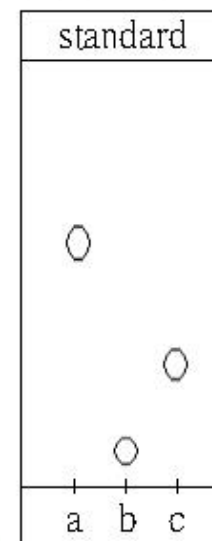
a



b



c



#### (4) Orange peel extract

(2顆)orange peel + 200mL buffer

(50mM trisodium citrate含2.3% NaCl→pH=5.5)

[buffer: 14.7g trisodium citrate + 23g NaCl + 962mL H<sub>2</sub>O]



blender (note:旋至定位再啓動)



centrifuge (note:對稱重量不得相差0.1g以上)



keep it in ice bath



## (5) Reactions:

add 0.3mL diester solution in a flask



add 2.0mL buffer and 0.7mL orange peel



mark the TLC plate with pencil



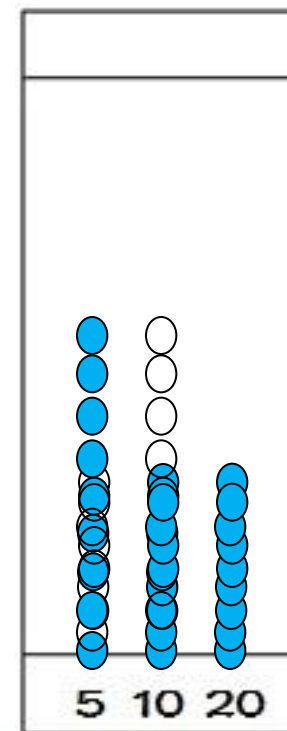
TLC test after 5min 10min and 20min



elute



circle the dots



1. 點完先照UV燈,以確定濃度不會太稀。
2. 沖堤液面不可超過起點線。
3. 沖堤過程中,人不可離開,一到達終止線,要馬上取出。
4. 毛細管要平整。
5. 每次使用,沖堤液接要重配,因為ether易揮發。





1. 檢查兩片TLC片，拍照上傳 Zuvio，測量各點移動距離，回去算Rf值。
2. 實驗問題：2，3

問題2. 用橘皮酵素水解 methyl 4-acetoxybenzoate (diester)會得到何種產物?

問題3. 追蹤反應中,5min,10min和20min的TLC片有何不同? 此變化代表何種意義?



*The End !*

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