Spectrophotometric Method for Estimating Enzymatic Synthesis of Butyl Oleate

RAMAKRISHNAN and NEVGI[1] prepared an acetone powder from castor seed which affected 39% synthesis of butyl oleate in five days. HOFSTEE[2] has recently worked out an assay system to estimate esterase spectrophotometrically using Salycilate ester as substrate. The author has worked out a similar assay system to estimate the synthesis of butyl oleate spectrophotometrically.

Experimental. Acetone-dried lipase powder was prepared from castor seed according to RAMAKRISHNAN and NEVGI's method[1] and used for the experiments. N-butyl alcohol, oleic acid and petroleum ether (B.P. 40–60°) were used. The absorption was studied in a BECKMAN spectrophotometer. The results of the various experiments are given in the following Tables.

Table I

The reaction mixture consisted of castor lipase: 0·1 g; N. Butyl alcohol: 0·005 M; oleic acid: 0·005 M; petr. ether: to make up the vol. to 3 cm³; incubation for 2 h at 37°C

Set No.	Wave lengths mμ	Absorbance
1	390	0·075
2	395	0·085
3	400	*0·092*
4	405	*0·092*
5	410	0·090
6	415	0·070
7	420	0·065
8	430	0·062
9	440	0·055
10	450	0·045
11	500	0·014
12	540	0·009

In all the experiments, petroleum ether, alcohol and enzyme blanks had negligible absorptions. The absorbance was proportional to the concentration of ester.

Table II

The reaction mixture consisted of castor lipase 0·2 g, different amounts of alcohol and acid and petroleum ether to make up the volume to 3 cm³, incubated for 2 h at 37°C and read at 400 mμ against blank containing all except the enzyme

Set No.	Concentration of alcohol (M)	Concentration of oleic acid (M)	Absorption	% Synthesis
1	0·001	0·005	0·037	2·4
2	0·002	0·005	0·073	4·8
3	*0·005*	*0·005*	0·184	*12·0*
4	0·008	0·005	0·184	12·0
5	0·005	0·001	0·038	2·4
6	0·005	0·002	0·070	4·6
7	0·005	0·008	0·182	11·9

From the above results, it can be seen that butyl oleate shows an absorbance at 400–405 and it is proportional to the concentration of ester. Mixture consisted of 0·005 M acid and alcohol, 0·2 g of lipase and petroleum ether to make up the volume to 3 cm³ and incubated for 2 h at 37°C gives 12% butyl oleate synthesis. This method seems to be a very simple and handy one and similar assay systems can be constructed for estimating the synthesis of different esters.

Table III

The reaction mixture consisted of 0·005 M alcohol and acid, and different amounts of enzyme and petroleum ether added to make up the volume to 3 cm³ incubated for 2 h at 37°C and read against blank at 400 mμ

Set No	Concentration of enzyme	Absorption	% Synthesis
1	0·1	0·092	6·0
2	0·2	0·184	12·0
3	0·3	0·180	11·7

Table IV

The reaction mixture consisted of 0·2 g of castor lipase, 0·005 M each of alcohol and acid, and petroleum ether added to make up the volume to 3 cm³, incubated for different amounts of time and read against blank at 400 mμ

Set No.	Incubation time h	Absorption	% Synthesis
1	½	0·100	6·5
2	1	0·152	9·9
3	1–½	0·178	11·6
4	2	0·184	12·0
5	2–½	0·184	12·0
6	3	0·182	11·9

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Zusammenfassung

Es wird eine einfache spektrophotometrische Methode für die Bestimmung der enzymatischen Synthese von Butyloleat beschrieben.

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Enzymatic Conversion of Testosterone to Androstenedione by Human Serum

Recent studies on the *in vitro* metabolism of testosterone by human tissues have shown that active metabolism of this steroid is carried on by a variety of normal and malignant human tissues[1]. These tissues include target-organs (prostate) as well as non-target-organs.

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