# LETTERS TO THE EDITORS

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## Specific Inhibition of Esterase in Ester-Hydrolysing Enzyme Systems

IN trying out several emulsifying agents having at a fairly neutral *p*H a stabilizing effect upon emulsions of olive oil, monobutyrine, ethylbutyrate, methylbutyrate and ethylpropionate, the following observations were made. Gummi arabicum activates on one hand the cleavage of olive oil to a very remarkable degree, but exerts no influence upon the cleavage of monobutyrine. It causes on the other hand a very sharp inhibition of the saponification of such esters where glycerol is substituted by lower alcohols. This inhibitory effect amounted in several instances (depending upon the source of the lypolytic enzymes employed) to 100 per cent, and in no case was finally less than 65 per cent. In the tests with olive oil, the activating effect of gummi arabicum proved to be dependent upon the stability of the emulsion, which on its part depends principally upon the procedure of preparation and to a much less degree upon the absolute amount of gummi arabicum added. Thus, in several instances, where no stable emulsions were obtained, the activating effect was either nil or very small.

The concentration of the substrates employed in the enzyme tests (see above) was in all cases 0·001 moles, while the concentration of olive oil was adjusted, according to its saponification value, to contain the same amount of saponifiable linkages. The amount of gummi arabicum added was in all cases half the amount by weight of the substrates (commercial gummi arabicum was employed).

The enzymes used were the glycerol extracts from pancreatin (Parke, Davis and Co.), glycerol extracts from worker maggots of the honey bee of different ages, from organs of adult worker bees, and beef liver juice obtained in the usual way with the hydraulic press.

To illustrate the results obtained, some examples are given in the accompanying tables. Concentration of substrate: 1 millimole contained in 10 ml. phosphate-buffer 7·2 (Sörensen). The extracts of the enzymes were prepared by grinding the biological materials with 90 per cent glycerol in a mortar (10 gm. glycerol per 1 gm. of substance) and leaving them overnight at 30° C. The undissolved part was then centrifuged off and the extract diluted with buffer solution *p*H 7·2 (Sörensen) as described below. Values of additional cleavage are corrected by the blanks and given in ml. of *n*/20 NaOH (Sörensen's formol titration). All tests were carried out at 37° C.

TABLE 1. ENZYME: 0·5 BEEF LIVER JUICE 1 : 5 (DILUTED WITH BUFFER SOLUTION) ADDED TO 20·5 ML. OF SUBSTRATE SOLUTION

| Substrate | Time of action | Additional cleavage in 4 ml. solution | |
|---|---|---|---|
| | | with gummi arabicum | without |
| Methylbutyrate | 30 min. | 0·30 | 0·60 |
| | 90 min. | 0·45 | 1·25 |
| | 22 hr. | 0·98 | 4·55 |
| Ethylbutyrate | 30 min. | 0·20 | 0·30 |
| | 90 min. | 0·35 | 0·65 |
| | 22 hr. | 0·73 | 4·43 |
| Ethylpropionate | 30 min. | 0·05 | 0·40 |
| | 90 min. | 0·25 | 0·95 |
| | 22 hr. | 1·35 | 4·18 |

TABLE 2. ENZYME: 3 ML. GLYCEROL EXTRACT FROM WORKER MAGGOTS OF THE HONEY BEE, 5 DAYS OF AGE, 3 : 2 (DILUTED WITH BUFFER SOLUTION) ADDED TO 10 ML. OF SUBSTRATE SOLUTION

| Substrate | Time of action | Additional cleavage in 5 ml. of solution | |
|---|---|---|---|
| | | with gummi arabicum | without |
| Methylbutyrate | 23 hr. | 0·50 | 2·15 |
| Ethylbutyrate | ,, | 0·25 | 4·35 |
| Ethylpropionate | ,, | 0·15 | 2·10 |

TABLE 3. ENZYME: 3 ML. GLYCEROL EXTRACT FROM WORKER MAGGOTS OF THE HONEY BEE, 8 DAYS OF AGE. 3 : 2 (DILUTED WITH BUFFER SOLUTION) ADDED TO 10 ML. OF SUBSTRATE SOLUTION

| Substrate | Time of action | Additional cleavage in 5 ml. solution | |
|---|---|---|---|
| | | with gummi arabicum | without |
| Methylbutyrate | 23 hr. | 0·05 | 1·40 |

TABLE 4. ENZYME: 3 ML. GLYCEROL EXTRACT FROM PANCREATIN 1 : 2 (DILUTED WITH BUFFER SOLUTION) ADDED TO 10 ML. OF SUBSTRATE SOLUTION

| Substrate | Time of action | Additional cleavage in 5 ml. solution | |
|---|---|---|---|
| | | with gummi arabicum | without |
| Methylbutyrate | 23 hr. | 2·10 | 7·40 |
| Ethylbutyrate | 23 ,, | 0·85 | 8·00 |
| Monobutyrine | 22 ,, | 3·40 | 3·35 |
| Olive oil | 22 ,, | 1·75 | 0·85 |

It thus appears that there are at least two distinctly different enzymes (or enzyme systems) present in these glycerol extracts: (1) a lipase, hydrolysing esters of glycerol, which is not inhibited by gummi arabicum; and (2) an esterase, hydrolysing esters of lower alcohols than glycerol, which is inhibited by addition of gummi arabicum.

Thus, substrates such as methylbutyrate and ethylbutyrate, usually recommended as standard substrates for the measurement of the activity of pancreatic lipase, seem, *de facto*, to undergo cleavage not by the lipase itself but by an esterase associated with the latter.

Further investigation into the nature of this inhibitory effect is proceeding.

A detailed report will be given elsewhere.

P. J. FODOR

Department of Biological and Colloidal Chemistry,
Hebrew University,
Jerusalem.
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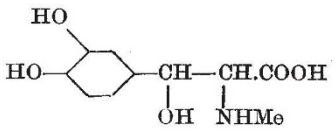
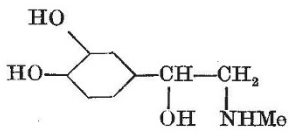
## Adrenaline Carboxylic Acid (N-Methyl-β-(3 : 4-dihydroxyphenyl)-serine)

THIS hitherto unrecorded amino-acid (I) is of considerable pharmacological interest in view of its intermediate relationship to adrenaline (II) and to 'dopa' (III): at the suggestion of Dr. H. Blaschko, we have consequently investigated its preparation.



(I)



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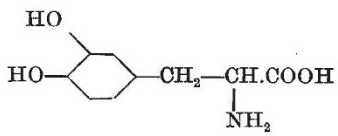

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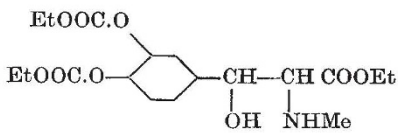

(IV)

The following synthesis has now been accomplished. Dicarbethoxy-protocatechuic aldehyde was condensed with sarcosine ethyl ester under the influence of sodium in ether[1] to give ultimately N-methyl-β-(3 : 4-dicarbethoxy-dihydroxyphenyl)-serine ethyl ester (IV). Since the hydrochloride of this compound was a viscous syrup, it was converted to the oxalate, m.p. 147° (decomp.), which on recrystallization dissociated to give the monohydrated hydrogen oxalate, m.p. 157° (decomp.). Considerable difficulty was experienced in the attempted alkaline hydrolysis of salts of (IV). Hydrolysis was, however, smoothly effected in good yield with negligible oxidation by boiling with dilute acetic acid, and the amino-acid (I), recrystallized from aqueous alcohol, formed cream-coloured crystals, m.p. 233° (decomp.) (Found: C, 53·1; H, 5·5; N, 6·1 per cent. $C_{10}H_{13}O_5N$ requires C, 52·9; H, 5·7; N, 6·2 per cent). No indication of the presence of more than one racemate was obtained.

Further work is required before the mechanism of the above condensation is elucidated, but certain interesting points have emerged. Rosenmund and Dornsaft[1] adduced evidence that the condensation of benzaldehyde with glycine ethyl ester involves the initial formation of a Schiff's base, $CH_2[N{:}CHPh]COOEt$, which then condenses with a second molecule of the aldehyde to form $PhCH(OH).CH[N{:}CHPh]COOEt$, from which the initial benzaldehyde residue is ultimately hydrolysed, giving the acid $PhCH(OH).CH(NH_2)COOH$. We find that our condensation does not succeed unless two molecules of aldehyde are used for each molecule of sarcosine ester. This suggests that the reaction may proceed through the stages $CH_2[NMe.CH(OH)R]COOEt \rightarrow R'CH(OH)CH[NMe.CH(OH)R]COOEt \rightarrow R'CH(OH)CH(NHMe)COOEt$, where $R$ represents the 3 : 4-dicarbethoxy-dihydroxyphenyl group. It is noteworthy that we have been unable to condense veratric aldehyde with sarcosine ester, in spite of a wide variety of conditions employed, and the condensation appears to be critically influenced by the groups used to protect the two phenolic groups.

The examination of the amino-acid (I) is being undertaken in the Department of Pharmacology at Oxford. The description of our chemical work will appear elsewhere.

F. G. MANN
C. E. DALGLIESH

University Chemical Laboratory,
Cambridge.
Aug. 6.

[1] Cf. Rosenmund and Dornsaft, *Ber.*, **52**, 1734 (1919).