Acetyl Content of Marinobufagin, Arenobufagin and Acetyl-marinobufagin

FOR marinobufagin, first isolated by Abel and Macht[1] from *Bufo marinus* and afterwards by Deulofeu and Mendive[2] from *B. paracnemis*, Slotta and Neisser[3] suggested the formula $C_{27}H_{38}O_6$, at variance with $C_{24}H_{32}O_5$ accepted by Jensen in many of his papers. They supposed that marinobufagin could contain one propionyl group.

The determination of the volatile acid content of several samples of marinobufagin by the Kuhn and Roth method[4] has given values lower than 1 per cent, so that acetyl or propionyl groups seem to be excluded from its constitution. Analysis of the samples gave carbon and hydrogen values according with Jensen's formula $C_{24}H_{32}O_5$.

When the same method was applied to acetyl marinobufagin[5], the acetyl content was found to be about 18 per cent, pointing to two acetyl groups in the molecule (calculated 17·76 per cent). Two easily esterifying alcoholic groups seem to be present (primary or secondary). On the basis of its elementary analysis, acetyl-marinobufagin has always been considered a mono-acetyl derivative. This difference will be further investigated.

From *B. arenarum*, Chen, Jensen and Chen[6] isolated a substance melting at 220° (correct.), to which formula $C_{25}H_{34}O_5$ was assigned. Jensen[7] states that the acetyl group is present, as by alkali hydrolysis acetic acid was detected as the silver salt.

We have isolated from the crude venom of *B. arenarum* another compound melting at 231–233° with a formula $C_{24}H_{32}O_6$ (elementary analysis) and without acetyl group (less than 1 per cent). Only further research on both compounds will explain the relationship between them.

That the method employed for the determination of acetyl groups is reliable results from the work of Wieland, Hesse and Hüttel[8] on bufotalinine, where correct acetyl values were obtained.

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Substrate Specificity of Yeast Zymase

IN a recent paper[1] further and seemingly conclusive proof was given that hydrolysis of maltose to glucose is not a necessary condition of maltose fermentation by living yeast cells. It may be noted that one of the findings upon which this conclusion was rested has now been independently confirmed[2]. Results since obtained suggest that not only is the mechanism of maltose fermentation *in vivo* different from, but also that it is not inclusive of, the glucose fermentation mechanism.

(1) It has been found that maltose fermentation by maltase-poor baker's yeast is strongly inhibited by the presence of methyl α-glucoside, but that under the same conditions glucose fermentation is unaffected.

The inhibiting action is not due to an inhibition of maltase since fermentation by maltase-rich brewery yeast and hydrolysis by maltase preparations of either baker's or brewery yeast are not subject to inhibition by methyl α-glucoside under the same conditions. It follows, therefore, that the mechanism of maltose fermentation includes a component which is not present in glucozymase and is not maltase.

(2) Conditions have been realized in which maltose ferments more rapidly than glucose. It is known that in aqueous or dilutely buffered medium, brewery yeast may ferment maltose and glucose at about the same rate. High salt concentrations depress the fermentation-rate of both sugars but have been found to do so more markedly in the case of glucose. The fermentation rate ratio of maltose to glucose in concentrated solutions of phosphate-citrate or other buffer salts may still be near 1·0 when fresh yeast is used, but far exceeds 1·0 when yeast which has been allowed to age is used.

The markedly superior fermentability of maltose in high buffer concentrations is proof that maltozymase is not merely maltase plus glucozymase. The same finding also offers support, though it does not itself constitute conclusive proof, for the view that maltozymase does not include glucozymase.

This conclusion is not opposed to any known fact of maltose fermentation, and finds further support in several observations which reveal primary differences in the fermentation mechanisms of maltose and glucose[3,4,5]. Also it should be mentioned that the *p*H-activity curve of maltose fermentation by baker's yeast is not given by glucose, and, as has been confirmed by us, is essentially different also from the *p*H-activity curve of either cell-bound or free yeast maltase[2,1].

The question may therefore be put whether the time-honoured term 'alcoholic fermentation of sugar' does not in fact cover processes which are different for different substrates.

It is intended to give a fuller discussion of this problem, details of the present experiments and a report of experiments on the alcoholic fermentations of sucrose, raffinose, and lactose in a later paper.

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A Decalcification Fluid

SINCE my former communication[1], I have had opportunity to conduct a number of experiments with the sodium hexametaphosphate decalcifying fluid reported.

Using the formulæ given by Gray[2], I find that a 25 per cent solution of the sodium hexametaphosphate is miscible in all the basal fixing solutions mentioned,