LETTERS TO THE EDITORS

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Molecular Weight of α-Amylase

PROF. KURT H. MEYER and his co-workers[1,2] have recently succeeded in the crystallization of α-amylase from pig's pancreas. The molecular weight of the substance, kindly supplied by Prof. Meyer, has been determined from sedimentation and diffusion experiments. The dried substance was dissolved in a buffer solution of *p*H 8·4, with a molarity of 0·2 in sodium chloride, 0·02 in boric acid and 0·005 in borate.

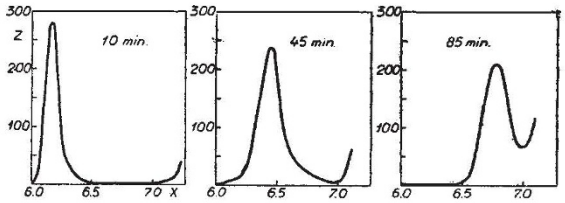

Fig. 1. SEDIMENTATION CURVES FOR α-AMYLASE

Ultracentrifuge measurement. The sedimentation diagrams of Fig. 1 were obtained by centrifuging at 65,000 r.p.m. The diagrams show one definite peak, characteristic of a uniform substance[3]. The concentrations of amylase used are shown in the table.

Conc. of amylase %	s_{20} (in *S* units)
0·09	4·63
0·18	4·32
0·22	4·44
0·26	4·50
0·30	4·38
0·66	4·64

The value of the sedimentation constant is evidently independent of the concentration of amylase. The average value is 4·50 *S*.

Diffusion measurement. The determination of the diffusion constant was carried out according to Lamm's scale method[4,5] in a diffusion cell of steel[6]. The resulting curves are shown in Fig. 2.

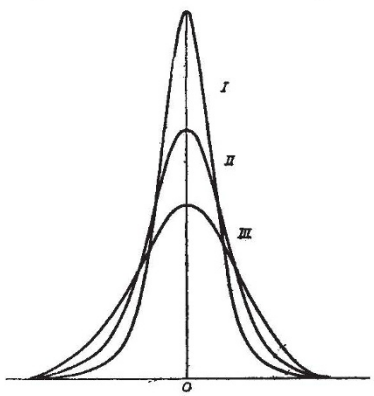

Fig. 2. DIFFUSION CURVES
I, after 20,100 sec.; II, after 42,700 sec., and III, after 80,900 sec.

The diffusion constant was calculated by two independent methods. Values of $D_A = 7{\cdot}89 \times 10^{-7}$ and $D_m = 8{\cdot}19 \times 10^{-7}$ were obtained. Hence, the agreement between the two methods of calculation is good. The average value of $D = 8{\cdot}05 \times 10^{-7}$ is used in the following.

The molecular weight was calculated according to Svedberg's formula: $M = \frac{R\,T\,s_{20}}{D(1 - V\rho)}$, where R is gas constant, T is absolute temperature, s_{20} is sedimentation constant, D is diffusion constant, V is partial specific volume of the solute, ρ is density of the solvent.

The value of V was 0·70 ± 0·02, kindly determined by Prof. C. Drucker. Using these values, the molecular weight of this α-amylase is 45,000.

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Sept. 2.

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[2] Fischer, E. H., "La purification et l'isolement de l'α-amylase de pancréase", Thèse No. 1094 (Genève, 1947).
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[4] Lamm, O., *Nova Acta Reg. Soc. Sci. Upsal.*, Ser. iv, **10**, No. 6 (1937).
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Structure of Starch: Mode of Attachment of the Side-chains in Amylopectin

ESTIMATIONS of the proportion of glucose residues present as end-groups in the amylopectin component of starch have shown that some 5 per cent of the glucose residues occur as terminal groups. Since amylopectin has a high molecular weight, of the order of 10^6, a branched-chain structure must be present in the molecule. The main features of the structure are indicated diagrammatically in Fig. 1[1], where (*A*), (*B*), (*C*), etc., are chains containing on

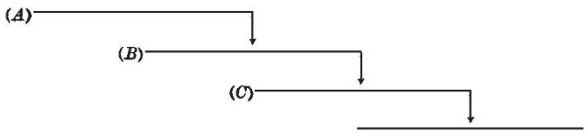

Fig. 1

the average 20–24 1 : 4-linked α-gluco-pyranose residues, each chain being united through C_1 of its first glucose residue to one of the glucose residues in another chain. The location in the chain of this glucose residue is not known, but it is clear that it must have three of its hydroxyl groups, namely, those at C_1, C_4 and at the carbon atom involved in the junction, occupied by linkages to other glucose residues. Two hydroxy groups remain free, and it follows that an investigation of the dimethyl glucose obtained on hydrolysis of a completely methylated amylopectin should enable the mode of linkage to be ascertained. Experiments on these lines showed that 2 : 3 dimethyl glucose was present in the hydrolysis products[2], and it was inferred that some at least of the side-chain linkages were through C_6 of the glucose residues concerned. Uncertainty is caused, however, by the experimental difficulty encountered in the complete methylation of amylopectin and by the fact that trimethyl glucose tends to undergo some demethylation in contact with methanolic hydrogen chloride. An effort to minimize these difficulties was made by using for the methanolysis a fully methylated but highly disaggregated sample of starch of low mole-