G-200 produced a 543-fold-purified preparation. The homogeneity of the purified cycloisomerase was established by analytical polyacrylamide-gel electrophoresis in sodium dodecyl sulphate and also by analytical ultracentrifugation.

A sedimentation coefficient, $s_{20,w}^0$, of $8.75 \pm 0.18S$, with a concentration coefficient, k_s , of 9.4 g/ml, was measured for 3-carboxy-*cis-cis*-muconate cycloisomerase. The apparent weight-average molecular weight was determined by the high-speed equilibrium-sedimentation method of Yphantis (1964) as 191 000. The molecular radius of the enzyme had a value of 4.626 nm when determined by the method of Ackers (1967). Substitution of this value into the equation given by Oncley (1941) gave a molar frictional ratio of 1.22 for the enzyme.

The quaternary structure of 3-carboxy-*cis-cis*-muconate cycloisomerase was studied by analysing the dissociated enzyme. The presence of a single protein band on disc electrophoresis in the presence of sodium dodecyl sulphate indicated that the enzyme probably consisted of an aggregate of low-molecular-weight polypeptide chains of identical molecular weight. In the presence of 5M-guanidine hydrochloride and 0.01% (v/v) 2-mercaptoethanol, the sedimentation coefficient, $s_{20,w}^0$, fell to 1.84S. High-speed equilibrium sedimentation under these conditions indicated an apparent weight-average molecular weight of 25 000 for the dissociated enzyme. Calibrated polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate by the method of Weber & Osborn (1969) predicted a value of $23\,000 \pm 3\,000$ (mean $\pm$ S.E.M.) for the minimum polypeptide molecular weight.

We conclude therefore that the 3-carboxy-*cis-cis*-muconate cycloisomerase molecule is composed of eight subunits of identical molecular weight.

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The Kinetic Properties of 3-Carboxy-*cis-cis*-muconate Cycloisomerase

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The 3-carboxy-*cis-cis*-muconate cycloisomerase of *Aspergillus niger* L 6 (Cain *et al.*, 1968) catalyses the lactonization of 3-carboxy-*cis-cis*-muconate in an isomerization that produces the corresponding (1→4)-lactone, 3-carboxymuconolactone. Enzymically, however, the reaction is characteristic of a lyase, consisting of the addition of the carboxyl oxygen of the 1-carboxyl group and a proton to the 3,4-olefinic bond. The system was analysed by the use of initial-velocity and inhibition studies.

The initial velocity of the reaction was found to increase linearly with the enzyme concentration. The relationship between the initial velocity and the substrate concentration was found to obey the Michaelis-Menten equation up to a concentration of 70 μ M, above which increasing substrate inhibition was observed. The K_m , determined by extrapolation of the linear portion of a double-reciprocal plot, was 57.2 μ M in 0.1M-sodium acetate buffer, pH 6.0. The V_{max} and K_m of the reaction were determined over a range of H^+ concentrations between pH 4.5 and 7.5. Plots of $\log V_{max}$ versus pH displayed a bell-shaped curve with two inflexions, corresponding to molecular ionization constants of approx. 5.1 for an unprotonated group and 6.5 for a protonated group. The plot of $\log(V_{max}/K_m)$ versus pH, however, displayed a wave form. The affinity of the enzyme for the substrate appeared to be greatly increased by a group with a molecular ionization constant of 5.0. A group with pK 6.6 was responsible for the rapid decrease in affinity above pH 6.0.

A preliminary investigation into the inhibition of 3-carboxy-*cis-cis*-muconate cycloisomerase by inorganic anions was performed. The initial velocity at different H^+ concentrations was measured in the presence of a 10 mM solution of the sodium salt of the anion under consideration. Inhibition was found to be a general phenomenon, with all the anions tested except chloride producing some perturbation of the profile of the plot of initial velocity versus pH.

Inhibition by organic anions was examined in detail. The organic monocarboxylic acids showed no effects on the reaction velocity, whereas di- and tri-carboxylic acids were invariably inhibitory. Inhibition was competitive and the inhibition constant, K_i , was determined by the method of Dixon (1953) for 17 organic acids. The position of the carboxyl groups

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in the inhibitor molecule was found to have a profound effect on the inhibition constant of the acid. Saturated dicarboxylic acids, with the exception of oxalate, were less inhibitory as the molecular weight increased. A *cis* effect was observed in the inhibition by unsaturated organic acids; compounds with two *cis*-carboxyl groups were much more inhibitory than the corresponding *trans*-isomers. This position effect was investigated quantitatively by measurement of the distances between carboxyl groups in space-filling models of the inhibitors. When these distances were plotted against the inhibition constant of the inhibitor, the degree of inhibition was observed to increase linearly with the increasing proximity of the carboxyl groups.

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Inhibition of Blood Platelet Function by Cytochalasins: Effects on Thrombosthenin and on Glucose Metabolism

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The cytochalasins are mould metabolites that inhibit cytokinesis and cell movement (Carter, 1967). Cytochalasin B can also inhibit platelet aggregation (White, 1971) and clot retraction (Shepro *et al.*, 1970; White, 1971). An attempt has been made to correlate the effects of cytochalasins A and B on platelet function with biochemical changes.

Citrated pig platelet-rich plasma at 37°C was used. Platelet aggregation was initiated with ADP (10 μ M) and clotting with bovine thrombin (10 N.I.H. units/ml) in both cases 5 min after addition of cytochalasin. Cytochalasin A inhibited aggregation completely at 20 μ g/ml (42 μ M), but cytochalasin B only partially at 100 μ g/ml (209 μ M). Clot retraction was inhibited completely by 4 μ g/ml of cytochalasin A and by 20 μ g/ml of cytochalasin B. Cytochalasin A (but not cytochalasin B) reacted with added cysteine, which blocked its effects.

Thrombosthenin (platelet actomyosin) was extracted from pig platelets (Grette, 1962; Haslam & Mustard, 1969). The effects of 0.1 mM-ATP on the turbidity of stirred suspensions of thrombosthenin in 60 mM-KCl at pH 7.0 were recorded. In the presence of 4 mM-MgCl₂, ATP cleared the suspension. This effect was inhibited by cytochalasin A (5–50 μ g/ml) but not by cytochalasin B (100 μ g/ml). With 15 mM-

MgCl₂, ATP caused a rapid superprecipitation which was similarly inhibited by cytochalasin A but not by cytochalasin B. Thrombosthenin was also isolated from platelets that had been incubated with cytochalasin A (4 or 20 μ g/ml) for 5 min and then washed. Only with 20 μ g/ml were the clearing and superprecipitation phenomena markedly inhibited. Thus only the inhibition of aggregation by cytochalasin A was associated with marked changes in thrombosthenin.

Washed rabbit platelets depleted of endogenous substrates rapidly form ¹⁴CO₂ from [6-¹⁴C]glucose (McElroy *et al.*, 1971). In this system cytochalasin A at 4 μ g/ml and at 20 μ g/ml inhibited ¹⁴CO₂ formation by about 30 and 70% respectively, whereas these concentrations of cytochalasin B inhibited by 91 and 98%. Thus the relative effectiveness of cytochalasins A and B was reversed in these experiments.

The biological activities of cytochalasin B have been attributed to disruption of contractile microfilaments (Schroeder, 1970; Wessells *et al.*, 1971). The effect of cytochalasin B on glucose metabolism and its lack of effect on thrombosthenin suggest that this conclusion may be premature.

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Amino Acid Sequences in Penicillo-pepsin: Evidence for Homology with Porcine Pepsin and Chymosin

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Penicillo-pepsin is an extracellular acid protease secreted by *Penicillium janthinellum*. Its purification and properties have been reviewed (Šodek & Hofmann, 1971). Earlier it was shown that the amino acid sequence around the active-site aspartic acid residue is almost identical with that of porcine pepsin (Šodek & Hofmann, 1970). In further sequence studies peptides have now been obtained from tryptic, chymotryptic and thermolytic digests by separation on