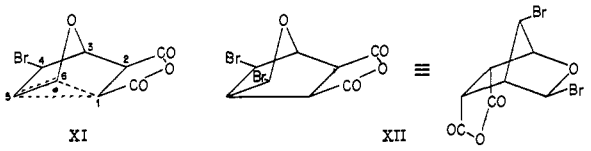
quiring stereospecific approach of bromine to C_5 from the *exo*-direction, and or (II) the unstable α-bromoether (XII), ionic rearrangement of which to IIB is in precise steric and electronic analogy to the change camphene hydrochloride → isobornyl chloride.[6]



XI XII

The formation of *cis*-dibromide by either or both of these paths appears to be unique in the literature.

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PARTICIPATION OF ATP AND COENZYME A IN THE ENZYMATIC DECARBOXYLATION OF MALONIC ACID[1]

Sir:

Malonic acid was previously shown to be an intermediate metabolite of uracil degradation by bacterial enzymes.[2,3] More recently decarboxylation of malonic acid was observed with dried bacterial cells and crude extracts.[4] It has now been found that the enzymatic decarboxylation of malonic acid requires adenosinetriphosphate (ATP) and coenzyme A (CoA) and an activated form of malonate is proposed as an intermediate.

Pseudomonas fluorescens strain TR-23,[5] a strictly aerobic microörganism, was grown for about 20 hours at 26°, with constant shaking, in a medium containing 1% NH_4Cl, 0.5% disodium malonate, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.1% Difco yeast extract. Cell-free extracts were prepared by grinding the washed cells with alumina (Alcoa A-301) in the presence of reduced glutathione (1.5 mg. of the sodium salt per g. of wet cells), extracting with 6 parts of 0.02 *M* phosphate buffer (*p*H 7.0), and centrifuging at 25,000 × *g* for 30 minutes.

A reaction mixture (2.0 ml.) containing 0.1 ml. of the crude extract (1.43 mg. protein), 100 μM. KF, 20 μM. reduced glutathione (sodium salt), 10 μM. $MgCl_2$, 200 μM. sodium acetate buffer (*p*H 5.8), 100 units CoA, 10 μM. ATP (sodium salt), 50 γ cocarboxylase and 100 μM. sodium malonate was incubated under pure nitrogen at 30° for 30 minutes. In the complete system 28.8 μM. of carbon dioxide was evolved. When ATP and CoA were omitted, only 1.9 μM. of carbon dioxide was produced. Pretreatment of the extracts with both Dowex-1[6] and charcoal[7] caused a more pronounced difference. With 0.1 ml. of the treated extract (0.86 mg. protein) the complete system yielded 13.5 μM. of carbon dioxide, whereas when either CoA, or ATP or both were omitted, 2.6, 0.9 and 0.2 μM. of carbon dioxide was produced, respectively. Neither Mg^{++} nor cocarboxylase affected the rate of the reaction under these conditions. There was no carbon dioxide production when malonate was omitted or the extract was treated at 100° for 5 minutes.

A reaction mixture (prepared as described above but with tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.0, instead of acetate buffer) containing 1.0 ml. of the crude extract and 200 μM. of hydroxylamine, yielded 7.4 μM. of hydroxamic acid derivatives[8] in the presence of ATP and CoA, whereas only 0.15 μM. was formed in the absence of the added cofactors. These hydrosamic acid derivatives were tentatively identified by paper chromatography (Whatman No. 3 with water-saturated butanol as solvent[9]) as (1) acethydroxamic acid (R_f: 0.51–0.53) and as (2) malonmonohydroxamic acid[10] (R_f: 0.36–0.38).

Thus the mechanism of malonate decarboxylation appears to involve activation of malonate (probably as malonyl CoA) as a primary step, analogous to the mechanism of succinate decarboxylation recently proposed for anaerobic microörganisms.[11,12] It has not yet been established whether the decarboxylation occurs at the activated carboxyl group to form an active one carbon compound and free acetate or whether the other carboxyl group is decarboxylated to produce active acetate and carbon dioxide. Since crude extracts were found to form hydroxamic acid derivatives from acetate, propionate, and succinate under the conditions described above, purification of the enzymes involved appears to be necessary to elucidate this point.

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(13) The excellent technical assistance of Mrs. Natalie A. Fraser is gratefully acknowledged.

A MODEL FOR THE CONFIGURATION OF SULFHYDRYL GROUPS IN PROTEINS

Sir:

The differing reactivity of protein –SH groups and, especially, the marked increase in their reactivity upon denaturation of the protein, has been the subject of much speculation. We wish to report two sets of observations which suggest an explanation for this phenomenon.

In the first series of experiments three cysteine-