THE FORMATION OF S-METHYLCYSTEINE FROM MIMOSINE BY *LEUCAENA* SEEDLING EXTRACTS

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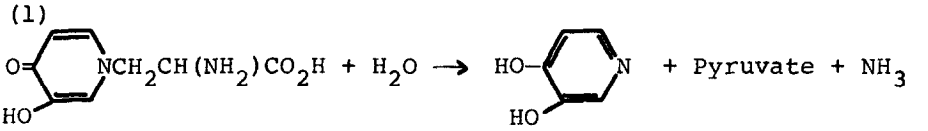
SUMMARY. An extract of *Leucaena* seedlings has been used to demonstrate the enzymic synthesis of S-methylcysteine from mimosine and methyl mercaptan: 3,4-dihydroxypyridine represents the other reaction product. The synthesis probably involves the initial elimination of a β-proton and a negative dihydroxypyridine ion; an α-aminoacrylate moiety bound to the enzyme at this stage then reacts with methyl mercaptan, or certain other mercaptans, to form the corresponding S-substituted cysteines.

Many S-substituted derivatives of cysteine have been found as components of the free amino acid pool of higher plants[1,2]. In addition to thioether derivatives, numerous related sulphoxides also have been isolated and characterized[2,3].

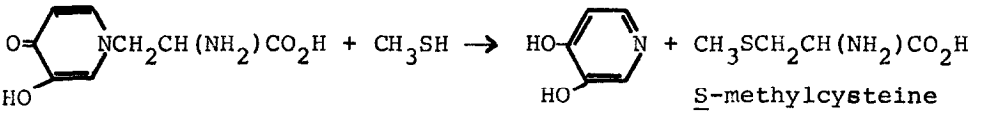
During an investigation of the enzymic synthesis of mimosine /β-(3-hydroxypyrid-4-one)-α-aminopropionic acid/ by extracts of *Leucaena* seedlings, we have demonstrated the formation of S-methylcysteine from mimosine and methyl mercaptan, a reaction that yields dihydroxypyridine as the other product (reaction 2). The reaction may be compared with the enzymic degradation of mimosine (reaction 1) by *Leucaena* seedling extracts which Smith and Fowden[4] earlier showed to give dihydroxypyridine, pyruvic acid and ammonia: both reactions probably involve a β-elimination step.

(1)



mimosine dihydroxy-pyridine

(2)



S-methylcysteine

Crude enzyme preparations were obtained from seedlings of Leucaena leucocephala, grown in the dark for 4 days at 30°. The testas were removed and the seedlings were homogenized in 0.1M potassium phosphate buffer, pH 8.0 (1 ml/4 g seedlings). After expressing through fine muslin, the extract was centrifuged at 25,000 g for 20 min. The supernatant was applied to a column of Sephadex G-25 (fine) to obtain an enzyme solution free from low mol. wt. substances, 0.1M phosphate (pH 8.0) being used for elution.

Reaction mixtures contained crude enzyme solution (0.2 ml), mimosine (5 μ mole) and methyl mercaptan (15 μmole) in a final volume of 0.8 m. (pH 8.0). The mixtures were incubated for 1.5 hr at 30° when the reaction was stopped by addition of 3 vol. of ethanol. Precipitated protein was removed by centrifuging and the supernatant was examined simultaneously. At pH 8.0 in the absence of enzyme preparation, S-methylcysteine was not formed chemically from mimosine and methyl mercaptan.

Amino acids present in the final reaction mixture were separated on paper chromatograms developed in butan-1-ol-acetic acid-water (90, 10, 29 by vol.) and

in butan-1-ol-pyridine-water (1, 1, 1 by vol.). Both solvents indicated the presence of a product, reacting positively with ninhydrin and iodoplatinate, that was inseparable from added S-methylcysteine (R_f values of 0.28 and 0.44, respectively, were determined for these solvents). The product's identity as S-methylcysteine was confirmed using an automatic amino acid analyser (Shibata AA-500 instrument, 150 cm. column, 50°, 0.2N citrate buffer, pH 3.25, flow rate 0.513 ml/min.). Under these conditions, both the product and an authentic sample of S-methylcysteine were eluted after 400 min. at an otherwise clear position on the amino acid elution profile (reference peaks of proline and glycine eluted at 380 and 470 min., respectively). S-methylcysteine formation under these conditions can be explained according to a reaction scheme previously proposed by Morino and Snell[5], and extended to cover the reaction mechanism of β-tyrosinase action by Yamada et al.[6]. Mimosine is considered to undergo a β-elimination reaction releasing H^+ and a negatively-charged elimination dihydroxypyridine ion, leaving an α-aminoacrylate moiety bound to the Leucaena enzyme. Hydrolysis then yields pyruvate and NH_3 (reaction 1), and regenerates the enzyme, but in the presence of methyl mercaptan, addition across the double bond of the α-aminoacrylate residue occurs giving S-methylcysteine almost exclusively (reaction 2). When 2-mercaptoethanol, mercaptoacetic acid, 3-mercaptopropionic acid or allyl mercaptan replaced methyl mercaptan in the reaction mixture, the corresponding S-substituted cysteines were identified tentatively as

reaction products.

It is possible to envisage an enzyme-catalyzed addition to α-aminoacrylate of compounds other than mercapto derivatives to yield a series of β-substituted alanines, and a detailed survey of such reactions is in progress. So far, no evidence to suggest the formation of 3,4-dihydroxyphenylalanine has been obtained in experiments in which catechol replaced the mercaptan in reaction mixtures.

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