ENZYMATIC PREPARATION OF (4- HYDROXYPHENYL) ACETIC ACID-1 -^{14}C.

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4-Hydroxyphenylacetic acid (HAA) is an intermediate in microbial ring-opening of several aromatic compounds (e.g. ref. 1) and a precursor of rumen tyrosine (2). In tracer studies of these reactions carboxyl-labelled HAA is used with the advantage that interference from labelled decarboxylation products is avoided.

The commercially unavailable HAA-1-^{14}C is conveniently prepared by the action of snake venom L-amino acid oxidase on L-tyrosine(side-chain-2-^{14}C) since - in the absence of catalase - the keto acid formed is further oxidized to the desired compound (3). Yields approach 50% and the product is essentially pure HAA as judged by chromatographic methods.

EXPERIMENTAL

In a typical experiment 6 ml dialyzed enzyme solution (for details see ref. 4) containing 2 mg/ml of dried Crotalus adamanteus venom (Sigma) in Tris-HCl buffer (0.4 M, pH 7.8 at 25 °C) and 50 microCi D,L-tyrosine(side-chain-2-^{14}C) (The Radiochemical Centre, Amersham) with a specific activity of 48.8 mCi/mmol plus 2.0 mmol cold L-tyrosine in 1 ml Tris-HCl buffer were incubated at 37°C for 27 hrs. under gentle agitation. Conc. HCl (1 ml) was used as stop reagent, the precipitate removed by centrifugation, and the supernatant extracted with ether (2 x 20

ml). The extract was washed with water (2 x 15 ml), evaporated, redissolved in a few drops of ethanol, and chromatographed on a silica gel plate in chloroform saturated with 50% acetic acid. Radioscanning showed a minor peak at the origin and a major one which migrated with authentic HAA. The latter spot was scratched off and the product extracted with ethanol (3 x 2 ml).

The yield was 12 microCi (48% based on L-isomer) with a calculated specific activity of 9.95 mCi/mmol. The product was autoradiographically pure after paper chromatography in benzene : ethyl methyl ketone (18:2) saturated with 2% formic acid; n-butanol : acetic acid : water (12:3:5); and acetic acid : water (3:1). Traces of labelled tyrosine, 4-hydroxyphenylpyruvic acid, or unknown by-products were not found.

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