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The natural occurrence of β-hydroxyaspartic acid

The formation of β-hydroxyaspartic acid *in vitro* by a transamination reaction between oxaloglycolate and glutamate has been established by studies carried out in this laboratory[1] as well as by the independent work of GARCIA-HERNANDEZ AND KUN[2]. We wish to report the isolation of β-hydroxyaspartic acid from pancreatic digests of casein. The isolation procedure involved the removal of the aromatic amino acids from the hydrolysates by adsorption on charcoal[3] and successive chromatography of the resulting solutions on columns of Dowex-1 formate and Dowex-50, hydrogen form, with [^{14}C]aspartic acid as a column marker.

The isolated compound has been identified by the following enzymic reactions. Incubation of the isolated hydroxyaspartate and α-ketoglutarate with the transaminase preparation from sheep brain[1] resulted in glutamate formation (Table I).

The isolated amino acid (20 μmoles) in the presence of 50 μmoles of [^{14}C]carbamyl phosphate (specific activity, 12,800 counts/min/μmole) and the transcarbamylase preparation from normal rat liver was enzymically converted into a single radioactive compound (total radioactivity, 47,800 counts/min) which on column and paper chromatography was identical with known N-carbamylhydroxyaspartate (ureidomalate)[5].

Further identification of the isolated compound was achieved by chromatographic studies. The isolated amino acid gave the same R_F as authentic hydroxyaspartate in three different solvent systems. The dinitrophenyl derivative of the isolated compound was prepared[6]. Paper chromatography[7] of the derivative gave an R_F (0.24) identical with that of a sample of authentic N-dinitrophenylhydroxyaspartate. Additional studies were carried out on the automatic amino acid analyzer. The isolated compound, synthetic hydroxyaspartate (a mixture of four isomers[8]) and *erythro*-β-hydroxy-L-aspartate[9] were added separately to a synthetic mixture of

TABLE I

TRANSAMINATION OF ISOLATED HYDROXYASPARTIC ACID

The reaction systems contained 20 μmoles hydroxyaspartate, 20 μmoles α-ketoglutarate, where indicated, 20 μg pyridoxal phosphate, 4 mg protein and 50 μmoles phosphate buffer, pH 7.4, in a total vol. of 1.8 ml. After incubating for 1 h at 37°, 0.5 ml 4 *N* $HClO_4$ was added. The deproteinized solutions were adjusted to pH 7 with 4 *N* KOH and the precipitated $KClO_4$ removed by centrifugation. Aliquots of the supernatant solutions were passed over 1 × 22 cm Dowex-1 formate columns. Glutamate and hydroxyaspartate were separated by elution with 0.05 *N* formic acid. Fractions containing the individual amino acids were analyzed by the ninhydrin method[4]. Independent assays for hydroxyaspartate were made by the periodate method[1].

Reactants	*Hydroxyaspartate (μmoles) by:*		*Glutamate (μmoles) by:*
	Ninhydrin	*Periodate*	*Ninhydrin*
Isolated hydroxyaspartate	19.1	19.3	0
Isolated hydroxyaspartate + α-ketoglutarate	10.4	11.3	8.8
Authentic hydroxyaspartate	19.5	19.1	0
Authentic hydroxyaspartate + α-ketoglutarate	11.2	11.2	8.3

the known amino acids. Elution analysis of the three mixtures was made. In each case, a single discrete peak, preceding the aspartic acid peak by 36 to 37 ml of effluent volume, was observed. It is of interest to note that this elution area corresponds in general to that of the unknown compound reported by MOORE *et al.*[10] to occur in protein-free extracts of rat liver.

On the basis of these enzymatic and chromatographic studies the isolated compound has been identified as β-hydroxyaspartic acid. Preliminary experiments in progress indicate that this compound may be isolated from acid-hydrolyzed casein.

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