NOTES

Amino Acids Found in Protein from *Entamoeba histolytica*[1]

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As part of a study of the chemical composition and metabolism of the dysentery amoeba, *Entamoeba histolytica*, we have isolated protein from amoeba trophozoites and analyzed that protein for its amino acid content. Table I shows the results of duplicate analyses. Separate analyses will be made to detect any tryptophan present and to identify the S-containing compounds in the protein. We believe that this represents the first insight into the chemical composition of *E. histolytica*.

Table I

Amino Acids Found in Protein from *E. histolytica*[a]

Aspartic acid	13.6	12.6	Isoleucine	2.5	2.1
Threonine	6.2	5.8	Leucine	10.8	9.8
Serine	6.7	6.1	Tyrosine	0.4	0.8
Glutamic acid	14.6	15.2	Phenylalanine	10.1	7.1
Proline	7.6	6.2	Histidine	1.8	2.0
Glycine	5.3	4.9	Lysine	11.6	13.8
Alanine	9.1	9.1	Amide-NH_3	1.2	1.0
Valine	2.2	2.4	Arginine	6.7	6.9

[a] Expressed as g. of amino acid per 100 g. of protein.

Experimental

Production of Amoebae.—One hundred ninety-five million trophozoites of *E. histolytica* (HK-9 strain) were obtained from a series of perfusion jar (PJ) cultures.[2]

Isolation of Protein.—The amoebae together with associated bacteria and insoluble rice starch were harvested from the PJ cultures with a wash liquid that contained only inorganic salts and acetate in the concentrations used for the culture medium. Soluble material in the pooled harvests was diluted out to 1 in 50,625 by centrifuging and washing the amoebae suspension 4 times for 7 min. at 26° and 895 × *g*. Fifty-six % of the amoebae originally harvested were recovered after the 4th centrifugation. These amoebae were lysed by 2 centrifugations for 30 min. at 4° and 2,435 × G. The supernatant fluid was removed from the residue, and the protein from the lysed amoeba in the supernatant fluid was precipitated with 10% trichloroacetic acid (TCA). Amoeba suspensions harvested from the PJ cultures in which the amoeba were lysed prior to the low-speed centrifugation gave no precipitate with 10% TCA when carried through this separation procedure.

The amoeba protein was extracted with 10% TCA at 90° and 3:1 ethanol–ether at 60° and dried; total yield, 16.6 mg. (moisture-free, corrected for 10.8% ash). This protein contained 16% N[3] and 1.38% S.[4]

Analysis of Protein.—The protein (usually about 1.75 mg.) was hydrolyzed with 6 *N* HCl and chromatographed on 12% cross linked, 200 to 400 mesh Dowex-50.[5] The amino acids in the eluate fractions were determined with ninhydrin.

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(1) A preliminary report has been given (C. E. Becker and Q. M. Geiman, *Federation Proc.*, **12**, 175 (1953)).

(2) Q. M. Geiman and C. E. Becker, *Ann. N. Y. Acad. Sci.*, **56**, 1048 (1953).

(3) A. Hiller, J. Plazin and D. D. Van Slyke, *J. Biol. Chem.*, **176**, 1401 (1948).

(4) Parr Instrument Company, Manual 121, page 37 (1950).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

Methyl β-D-Gulofuranoside and Certain Other Derivatives of D-Gulose

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When the crystalline addition compound of D-gulose, α-D-gulose·$CaCl_2$·H_2O,[1] is dissolved in methanol containing 5% by weight of hydrogen chloride and the resulting solution observed polarimetrically at 20°, data are obtained from which a curve such as shown in Fig. 1 may be plotted.[2]

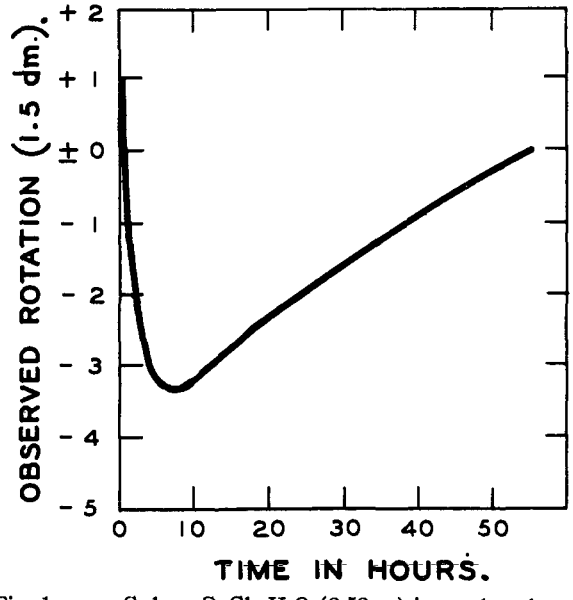

Fig. 1.—α-D-Gulose·$CaCl_2$·H_2O (8.58 g.) in methanol containing 5% hydrogen chloride (120 ml.) at 20°.

Periodic tests with Fehling solution reveal that after about seven hours, when the solution is near its maximum levorotatory value, the reducing power of the reaction mixture has very nearly vanished. If, at about this time, the acid and calcium chloride are removed with silver carbonate and the solution concentrated, there is obtained a sirup which, from 1-propanol, affords in 37% yield a readily crystalline

(1) H. S. Isbell, *J. Research Natl. Bur. Standards*, **5**, 741 (1930).

(2) The slow dextromutarotation eventually attained a constant value of +3.83° after 485 hr. Doubtless the product then consisted chiefly of a mixture of the two anomeric methyl D-gulopyranosides similar to that obtained by Isbell [*J. Research Natl. Bur. Standards*, **8**, 1 (1932)] using a higher temperature and somewhat weaker acid.