NOTES

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

By Charles E. Becker and Quentin M. Geiman

Received January 14, 1954

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.

Acknowledgment.—This work was sponsored by the Commission on Enteric Infections, Armed Forces Epidemiological Board, and was supported by a contract between the Office of the Surgeon General, Department of the Army, and Harvard University. The authors also wish to thank Margaret A. St. Lawrence, Jeanette Weeks and Barbaranne P. Schleyer for technical assistance.

Department of Tropical Public Health
Harvard School of Public Health
Boston 15, Mass.

(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

By Hewitt G. Fletcher, Jr., Harry W. Diehl and Robert K. Ness

Received February 12, 1954

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]

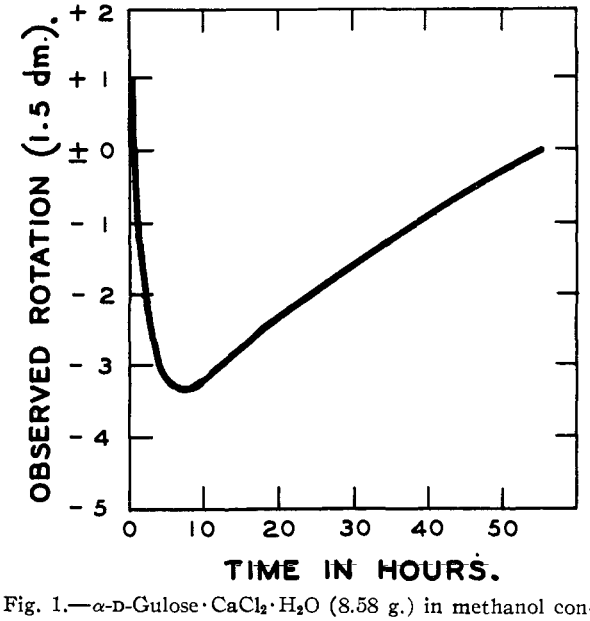

Fig. 1.—α-D-Gulose·$CaCl_2$·H_2O (8.58 g.) in methanol containing 5% hydrogen chloride (120 ml.) at 20°.

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.

substance having the analysis of a methyl hexoside and physical properties which distinguish it from the known methyl D-gulopyranosides. With aqueous sodium metaperiodate the new methyl hexoside gives free iodine—behavior characteristic of an aldohexofuranoside. Hockett, Nickerson and Reeder[3] have shown that with respect to their behavior with lead tetraacetate the aldohexofuranosides fall into two classes. Those like methyl α-D-mannofuranoside, having a *cis* pair of hydroxyls within the ring, consume one mole of lead tetraacetate with great rapidity; further oxidation is relatively slow because of hemi-acetal formation, formaldehyde being liberated in traces if at all. Others, like ethyl β-D-galactofuranoside, with a *trans* pair of hydroxyls within the ring, consume more than two moles of lead tetraacetate with considerable speed and liberate in the process a mole equivalent of formaldehyde. If the new methyl aldohexofuranoside has the gulose configuration it should fall in the first of these two classes. As may be seen in Fig. 2, experiment confirmed this, the oxidation curves for the new substance and for methyl α-D-mannofuranoside being identical within the experimental error. No formaldehyde was detected. The new substance is, therefore, methyl D-gulofuranoside; because of its relatively high negative rotation, $[\alpha]^{20}D$ −108° (H_2O), it is tentatively designated as the β-D-isomer.

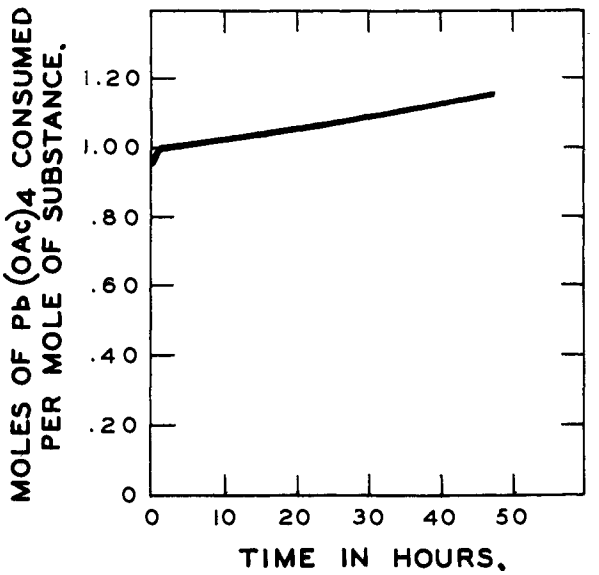

Fig. 2.—Oxidation of methyl α-D-mannofuranoside and methyl β-D-gulofuranoside by an excess of lead tetraacetate in glacial acetic acid at 25°.

The new glycoside was characterized further as its very readily crystalline tetraacetate, a substance which was first isolated in small yield after acetylation of the mother liquor remaining from the preparation of methyl α-D-gulopyranoside according to Isbell.[2]

Several years ago a monobenzylidene derivative of methyl β-D-gulopyranoside was isolated fortuitously in this Laboratory. This substance has now been shown, through the fact that it consumes one mole of periodate, to be methyl 4,6-*O*-benzylidene-β-D-gulopyranoside and has been further characterized through the preparation of a crystalline dibenzoate. From methyl α-D-gulopyranoside a similar 4,6-*O*-benzylidene derivative has been prepared. It may be noted, in passing, that this substance should, on oxidation with periodate, give the same dialdehyde as that from methyl 4,6-*O*-benzylidene-α-D-galactopyranoside provided the asymmetric carbon atom attached to the phenyl group has the same configuration in each case. However, methyl 4,6-*O*-benzylidene-α-D-galactopyranoside apparently exists in two modifications: one with m.p. 177–178° and $[\alpha]^{15}D$ +162.1° ($CHCl_3$)[4] and another of m.p. 171° and $[\alpha]^{20}D$ +144° ($CHCl_3$).[5] The latter form which we obtained on benzalating methyl α-D-galactopyranoside gave, upon oxidation with sodium metaperiodate, a solution showing a significantly different rotation than that obtained when methyl 4,6-*O*-benzylidene-α-D-gulopyranoside was so oxidized. It seems probable, then, that the two substances differ in configuration at the carbon atoms attached to the phenyl group.

Experimental[6]

Methyl β-D-Gulofuranoside.—The powdered, crystalline calcium chloride compound of D-gulose (8.58 g., α-D-gulose·$CaCl_2·H_2O$[1]) was added to 120 ml. of methanolic hydrogen chloride (5% HCl w./w.) and the mixture stirred until solution was complete. The clear, colorless liquid, kept at 20°, was observed polarimetrically and tested at regular intervals with Fehling solution. After 6.25 hr. the reducing power had nearly vanished and the rotation in a 1.5-dm. tube had come to −3.17°; the reaction was then halted by the addition of 50 g. of silver carbonate. After filtration, solvent was removed *in vacuo* and the nearly colorless sirup dissolved in 100 ml. of methanol. The resulting solution was filtered through a thin layer of decolorizing carbon and concentrated *in vacuo* to a honey-like sirup which, from its solution in 11 ml. of 1-propanol, afforded 1.25 g. of nearly pure methyl β-D-gulofuranoside. After concentration, the mother liquor afforded 0.75 g. more product of nearly equal purity, raising the total yield to 37%. Recrystallization from 3 parts of 1-propanol gave with little loss pure methyl β-D-gulofuranoside melting at 100–101° and showing $[\alpha]^{20}D$ −108° (water, *c* 0.82).

Anal. Calcd. for $C_7H_{14}O_6$: C, 43.29; H, 7.27. Found: C, 43.52; H, 7.05.

A sample (97.3 mg., 0.000501 mole) of the glycoside was oxidized with lead tetraacetate in glacial acetic acid using the technique of Hockett and McClenahan.[7] At the same time a similar sample (99.5 mg., 0.000512 mole) of methyl α-D-mannofuranoside was also oxidized. The data for the consumption of oxidant as a function of time are plotted in Fig. 2, both glycosides behaving in an identical manner within the experimental error.

Methyl β-D-Gulofuranoside Tetraacetate.—One gram of methyl β-D-gulofuranoside was acetylated with acetic anhydride and pyridine in the usual manner to yield 1.55 g. (83%) of tetraacetate. Recrystallized from ether–pentane it melted at 75–76° and showed $[\alpha]^{20}D$ −65.0° (*c* 0.77, $CHCl_3$).

(3) R. C. Hockett, M. H. Nickerson and W. H. Reeder, III, THIS JOURNAL, **66**, 472 (1944).

(4) E. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **28**, 1 (1945). G. J. Robertson and R. A. Lamb [*J. Chem. Soc.*, 1321 (1934)] reported m.p. 170–172° and $[\alpha]^{15}D$ +166.5° ($CHCl_3$) while A. C. Maehly and T. Reichstein [*Helv. Chim. Acta*, **30**, 496 (1942)] and C. Tamm [*ibid.*, **32**, 163 (1949)] give m.p. 171 and 170–172°, respectively.

(5) These values were determined in the course of the present work using material which gave satisfactory analyses for carbon and hydrogen. R. E. Reeves [THIS JOURNAL, **71**, 1737 (1949)] has reported m.p. 168–169° and $[\alpha]^{25}D$ +142° ($CHCl_3$) for methyl 4,6-*O*-benzylidene-α-D-galactopyranoside.

(6) All melting points are corrected.

(7) R. C. Hockett and W. S. McClenahan, THIS JOURNAL, **61**, 1667 (1939).

Anal. Calcd. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found: C, 49.72; H, 6.07.

Methyl 4,6-*O*-Benzylidene-α-D-gulopyranoside.—Methyl α-D-gulopyranoside·$CaCl_2·2H_2O$[1] (6.2 g.) was dissolved in water and the solution deionized by successive passage through columns of Amberlite IR-120[8] and Duolite A-4.[9] The effluent was concentrated *in vacuo* to a sirup which was dried by azeotroping ethanol therefrom. The sirup was then dissolved in 14 ml. of 1% methanolic hydrogen chloride and the solution treated with 2 ml. of freshly distilled benzaldehyde. After 10 days at +5° the crystalline product (1.4 g., 27%) was removed and recrystallized from 5 parts of absolute alcohol. The pure substance melted at 147–148° and rotated in chloroform (*c* 0.90) $[\alpha]^{20}D$ +79.8°.

Anal. Calcd. for $C_{14}H_{18}O_6$: C, 59.56; H, 6.43. Found: C, 59.24; H, 6.31.

A sample (0.6212 g.) of the product described above was oxidized in aqueous solution with sodium metaperiodate. After 23 hr. analysis showed the consumption of 1.08 moles of oxidant per mole of substance taken. In a 4-dm. tube the oxidation mixture rotated +1.63° while a completely parallel oxidation of an equal quantity of the form of methyl 4,6-*O*-benzylidene-α-D-galactopyranoside, which melts at 171° and shows $[\alpha]^{20}D$ +144° (*c* 0.57, $CHCl_3$), gave a rotation of +2.35°.

An attempt to obtain the dibenzoate of the above compound in crystalline form failed.

Methyl 4,6-*O*-Benzylidene-β-D-gulopyranoside.—Methyl β-D-gulopyranoside[10] (300 mg.) was dissolved in 2 ml. of hot methanol, the solution cooled and treated with 1.2 ml. of 1% methanolic hydrogen chloride and 0.17 ml. of freshly distilled benzaldehyde. After 2 days at +5° the solution was concentrated to a crystalline magma which was extracted with water. Extraction of the aqueous extracts with methylene chloride, followed by concentration of the organic solvent gave a crystalline residue which, from 1:1 absolute alcohol–pentane, gave slender rods (27.8 mg., 6.4%) melting at 177–178°. Recrystallization failed to change this value. The pure substance rotated $[\alpha]^{20}D$ −87.8° in chloroform (*c* 1.01).

Anal. Calcd. for $C_{14}H_{18}O_6$: C, 59.56; H, 6.43. Found: C, 59.69; H, 6.57.

A sample of the compound described above was oxidized in aqueous solution with a slight excess of sodium metaperiodate. After 18 hr. at room temperature 1.13 moles of oxidant had been consumed per mole of substance taken.

Methyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranoside.—Benzoylation of methyl 4,6-*O*-benzylidene-β-D-gulopyranoside with benzoyl chloride and pyridine in the usual manner gave from alcohol lath-shaped needles melting at 141–146°. Recrystallization from 10 parts of absolute ethanol afforded material melting at 155–156° and showing $[\alpha]^{20}D$ −57.3° in chloroform (*c* 0.61).

Anal. Calcd. for $C_{28}H_{26}O_8$: C, 68.56; H, 5.34. Found: C, 68.70; H, 5.61.

Acknowledgment.—We wish to thank Mr. Edward W. Tracy and Mr. John T. Sipes for preparing the D-gulose calcium chloride compound. Drs. Horace S. Isbell and Harriett L. Frush kindly furnished seeds of the anomeric methyl D-gulopyranosides and their tetraacetates. Analytical data were obtained in the Institutes' Microanalytical Laboratory under the direction of Dr. William C. Alford.

NATIONAL INSTITUTE OF ARTHRITIS AND
METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
BETHESDA 14, MARYLAND

(8) A product of Rohm & Haas Co., Philadelphia, Pa.

(9) A product of the Chemical Process Co., 901 Spring St., Redwood City, Calif.

(10) This substance, first prepared by Isbell (ref. 1) by boiling α-D-gulose·$CaCl_2·H_2O$ with methanolic hydrogen chloride, was found to be more readily accessible through the following series of reactions: α-D-gulose·$CaCl_2·H_2O$ → D-gulopyranose pentaacetate → tetra-*O*-acetyl-D-gulopyranosyl bromide → methyl β-D-gulopyranoside tetraacetate → methyl β-D-gulopyranoside.

The Stereochemistry of the Reaction of Aluminum Bromide with α-Phenethyl Aryl Ethers

BY HAROLD HART AND RAYMOND J. ELIA

RECEIVED JANUARY 8, 1954

An unusual result was obtained recently by Tarbell and Petropoulos[1] in a study of the action of aluminum bromide on benzyl phenyl ether. In several solvents, a conversion of the ether to *o*-benzylphenol (55%) and phenol (40%) occurred *within five seconds, even at −40°*. This rapid reaction was followed by a slower one, in which *o*-benzylphenol was converted to phenol. In both reactions, the benzyl group also entered into various transformations with the solvent. Because no *p*-benzylphenol was isolated, the authors suggested that the transition state had to be so constructed as to permit an *intramolecular* shift of the benzyl group, as well as cleavage of the ether to form a benzyl carbonium ion.

Having recently prepared several optically active α-phenethyl aryl ethers and established their configurations,[2] and also the configurations of the related α-phenethylphenols,[3] we decided to investigate the stereochemistry of the reaction described by Tarbell.

It was first established that *dl*-α-phenethyl phenyl ether reacted with aluminum bromide in chlorobenzene in a fashion analogous to the rapid reaction of benzyl phenyl ether. The α-phenethylphenols were analyzed by a spectrophotometric procedure[4] and found to be 85% *o*- and 15% *p*-isomer. From *dl*-α-phenethyl 2,6-xylyl ether, however, only the cleavage product was obtained (2,6-xylenol, 94%). Thus, when both *ortho* and *para* positions are available, rearrangement occurs predominantly to the *ortho* carbon, but when this position is blocked, cleavage is the principal result. (−)-α-Phenethyl phenyl ether gave (+)-α-phenethylphenols, predominantly the *ortho* isomer. This result implies retention of configuration.[2,3] The same stereochemical result was obtained with α-phenethyl *p*-tolyl ether, (−)-ether giving (−)-*o*-α-phenethyl-*p*-cresol. Since in this case the product consisted of only one isomer, a calculation of the extent of retention of optical purity was possible. Using rotation established previously,[2,3] we estimate that migration occurred with about 76% retention of optical purity. These stereochemical results are in accord with the proposed intramolecularity of the rearrangement. The reaction may be analogous to S_Ni displacements.

Experimental

General Procedure for the Rearrangement.—The rearrangements were carried out according to the procedure described by Tarbell and Petropoulos[1] for isolating their initial reaction products. Solutions of the ether (about 0.09 mole) and aluminum bromide (about 0.21 mole) in chlorobenzene (total volume about 800–900 ml.) were mixed at room temperature. To the red solution thus formed, there was added, within 10 seconds, 500 ml. of water to quench the reaction. Petroleum ether was added to aid in separating layers. Phenolic products were extracted from the or-

(1) D. S. Tarbell and J. C. Petropoulos, THIS JOURNAL, **74**, 244 (1952).

(2) H. Hart and H. S. Eleuterio, *ibid.*, **76**, 519 (1954).

(3) H. Hart and H. S. Eleuterio, *ibid.*, **76**, 516 (1954).

(4) H. Hart, *Anal. Chem.*, **24**, 1500 (1952).