whereas drying under vacuum at temperatures around 100° produced decomposition. Consequently, for analysis, the crystalline products were dissolved in chloroform and precipitated with light petroleum ether or *n*-hexane; this procedure yielded products apparently free of retained solvents. All the compounds were yellow in color, and all gave water-soluble hydrochlorides with the exception of the derivatives butyl through octyl.

Some activity against Sarcoma 37 in mice was exhibited by all of the compounds.[6]

Experimental

The colchicine used had a m.p. of 158–159° cor. and was purified[7] by us from a commercial product employing chromatography over activated alumina. The amines were the best grades of Eastman Kodak Co., Sharples Chemicals Inc., and Fischer Scientific Co. with the exception of hexylamine (Eastman Kodak Co., practical), and of diethanolamine (Carbon and Carbide Chemicals Corp., practical); methylamine, ethylamine and dimethylamine were used as the concentrated aqueous solutions.

N-(β-Chloroethyl)-colchiceinamide.—To a solution of 0.43 g. (0.001 mole) of N-(β-hydroxyethyl)-colchiceinamide in 200 cc. of dry, thiophene-free benzene, cooled to 20°, was added dropwise with vigorous stirring 0.1 cc. of purified[8] thionyl chloride. After standing overnight in the refrigerator, the supernatant liquid was decanted off, the residue washed with benzene by decantation, and the crude yellow product dried in a vacuum desiccator; yield 0.45 g. (94%). The compound was purified by dissolving in chloroform, precipitating with twenty volumes of absolute ether, washing the solid with ether and drying in vacuum at 55°. The amorphous substance is hygroscopic.

Attempts to prepare the N,N-bis-(β-chloroethyl) derivative from N,N-bis-(β-hydroxyethyl)-colchiceinamide in a similar fashion yielded an extremely hygroscopic gummy product which always contained less than the theoretical amount of chlorine and could not be satisfactorily purified.

(6) To be reported in the *Journal of the National Cancer Institute.*

(7) J. N. Ashley and J. O. Harris, *J. Chem. Soc.*, 677 (1944).

(8) L. F. Fieser, "Experiments in Organic Chemistry," 2nd edition, D. C. Heath and Co., Boston, Mass., 1941, p. 381.

LABORATORY OF CHEMICAL PHARMACOLOGY
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
U. S. PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA, MARYLAND

Amino Acid Composition of Crystalline Inorganic Pyrophosphatase Isolated from Bakers Yeast

BY WERNER HAUSMANN

RECEIVED JULY 11, 1951

Inorganic pyrophosphatase has recently been isolated by Dr. M. Kunitz in crystalline form.[1,2] It appeared of interest to investigate the amino acid composition of the new enzyme qualitatively and quantitatively by hydrolysis and chromatography of the hydrolysate.

Qualitative Determination.—A good qualitative picture of the amino acid spectrum of the protein was obtained by paper chromatography.

Five mg. of air-dried crystalline pyrophosphatase was hydrolyzed in 1 ml. of 6 *M* HCl for 24 hours at 110°, in a sealed, evacuated Pyrex glass tube. The hydrolyzate was then evaporated to dryness at 50° and 9 mm. pressure, redissolved in 1 ml. of distilled water and evaporated again in order to remove excess HCl. The remaining mixture of amino acid hydrochlorides was dissolved in 0.5 ml. of distilled water to give a concentration corresponding to 10 γ of original protein per μl.

Twenty μl. (200 γ) of this solution was subjected to two dimensional paper chromatography on Whatman No. 1 filter paper, using the ascending technique.[3] The solvent system used first for running along the longer edge of the paper consisted of 150 ml. of redistilled secondary butanol +60 ml. of 3% aqueous ammonia. This was done twice before turning the paper and running it once along the short paper edge in the second solvent system: 150 ml. of distilled secondary butanol +30 ml. of 88% aqueous formic acid +20 ml. of water. After drying, the paper was held in a horizontal position and sprayed with ninhydrin solution. After five minutes, when the paper looked dry, the cystine region was sprayed with Folin reagent.[4] The area of arginine was sprayed with Sakaguchi solution.[5] Control runs proved that these two specific color reactions were positive even after ninhydrin treatment, if applied immediately.

For comparison a synthetic mixture of 20 amino acid hydrochlorides was prepared, and chromatographed in exactly the same manner. The result is illustrated in Fig. 1. Proline and hydroxyproline give yellow spots. For more accurate comparison the hydrolyzate was run together with standard samples of the 16 amino acids indicated. No new spots appeared.

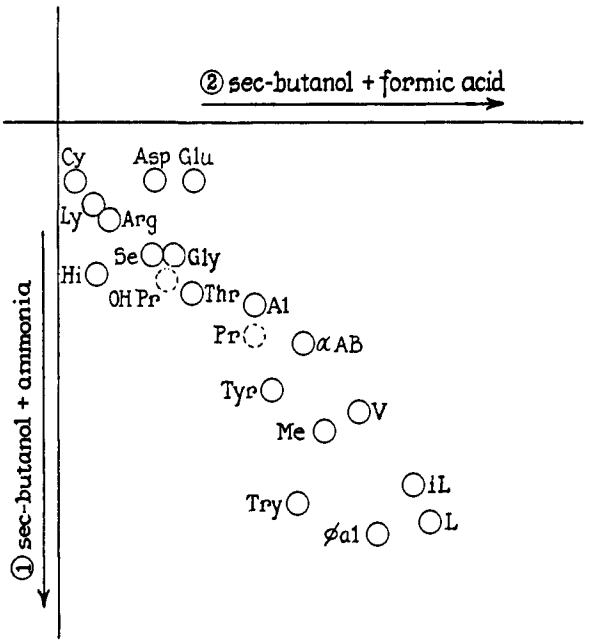


Fig. 1.—Synthetic mixture of amino acid hydrochlorides.

Cystine could not be detected either by ninhydrin or Folin treatment.

Reaction of the intact protein with *p*-dimethylaminobenzaldehyde[6] indicated the presence of tryptophan. This was confirmed by paper chromatography of a $Ba(OH)_2$ hydrolyzate.

Paper chromatography has revealed that crystalline inorganic pyrophosphatase is a protein containing the following 17 amino acids: aspartic acid, glutamic acid, lysine, arginine, histidine, serine, threonine, proline, methionine, tyrosine, tryptophan, glycine, alanine, valine, leucine, isoleucine and phenylalanine. Cystine and hydroxyproline could not be detected.

Quantitative Determination.—The quantitative amino acid composition was determined on the above mentioned hydrolyzate in HCl by chromatography on Dowex 50 columns,[7] and the fractions were analyzed by the colorimetric ninhydrin method.

Figures 2 and 3 represent patterns obtained by the 100-cm. and the 15-cm. columns, respectively. The qualitative

(1) M. Kunitz, THIS JOURNAL, **73**, 1387 (1951).

(2) M. Kunitz, *J. Gen. Physiol.*, **35**, 423 (1952).

(3) R. J. Williams, and H. Kirby, *Science*, **107**, 481 (1948).

(4) O. Folin and J. M. Looney, *J. Biol. Chem.*, **51**, 421 (1922).

(5) E. Jorpes and S. Thoren, *Biochem. J.*, **26**, 1504 (1932).

(6) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1249 (1949).

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

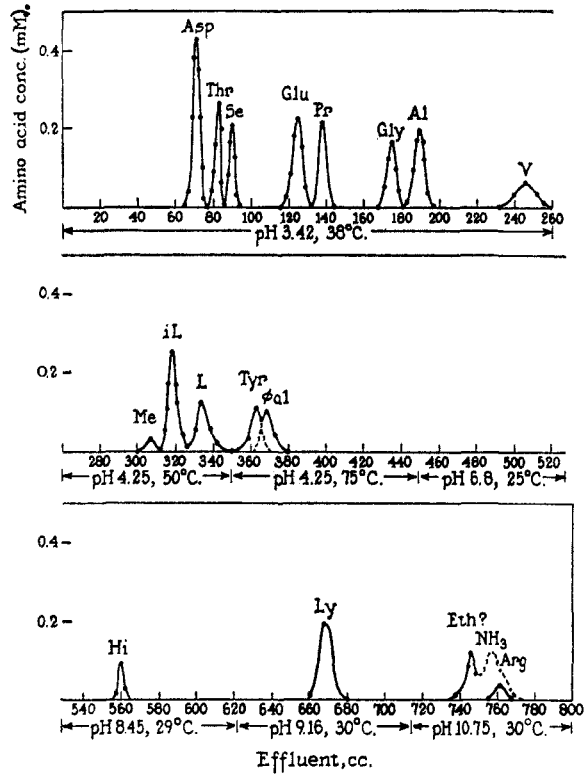


Fig. 2.

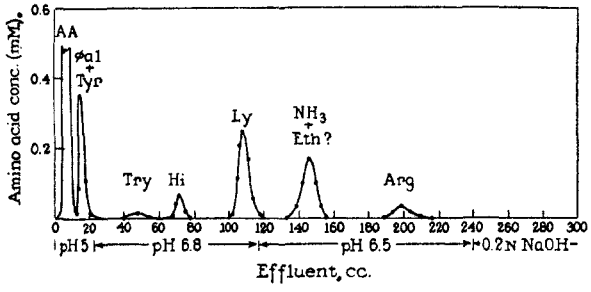


Fig. 3.

composition found by paper chromatography was confirmed and an as yet unidentified ninhydrin positive peak which seemed to be differentiated from the ammonia peak, appeared near the ethanolamine position on the 100-cm. column. On the short column this possible component may be included in the ammonia peak and the ammonia figure is therefore questionable. Cystine was found to be present in traces only, or not at all. The ammonia–arginine doublet was analyzed by evaporating the ammonia from every second tube.

Table I gives the amounts of the single amino acids in per cent. amino acid residue and per cent. nitrogen in the original protein, dried for 3 hours at 100°.

TABLE I

Amino acid	G. amino acid residue per 100 g. protein	Nitrogen, %
Aspartic acid	12.1	1.5
Alanine	4.9	1.0
Ammonia[a]	1.5	1.2
Arginine	3.3	1.2
Glutamic acid	9.7	1.1
Glycine	2.9	0.7
Histidine	2.2	0.7
Isoleucine	8.9	1.1
Leucine	6.0	0.8
Lysine	10.9	2.4
Methionine	1.3	0.1
Phenylalanine	6.2	.6
Proline	6.4	.9
Serine	3.1	.5
Threonine	4.8	.7
Tryptophan[b]	3.6	.5
Tyrosine[c]	6.0	.5
Valine	4.1	.6
Total	97.9	16.1
Found N (Kjeldahl)		16.2

[a] Tentative figure, perhaps including an additional component. [b] Determined spectrophotometrically by Kunitz. 2.5% was found chromatographically in the acid hydrolyzate. [c] This value checks closely with that of Kunitz, determined by the ultraviolet absorption method.

Acknowledgment.—I wish to thank Dr. M. Kunitz for providing the material for this investigation and Miss E. A. Jacobs for her technical assistance.

THE LABORATORIES OF THE
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, N. Y.

The Effect of α-Fluorine Atoms on S_N1 Reactivity[1]

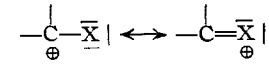
BY JACK HINE AND DONALD E. LEE

RECEIVED JANUARY 2, 1952

It has been previously shown that in comparison to hydrogen, both chlorine and bromine atoms increase the S_N1 reactivity[2] of other halogen atoms attached to the same carbon atom.[3] It is now found that the corresponding effect of α-fluorine atoms is very much smaller than that of α-chlorine or bromine and that it may even be of a deactivating nature.

In 50% aqueous acetone, benzodifluorochloride solvolyzes less than one-fifth as fast as benzyl chloride. If benzyl chloride, under the conditions used, hydrolyzes entirely by the S_N1 (carbonium ion) mechanism, then α-fluorine atoms do decrease the S_N1 reactivity. However, there are several facts which suggest that this reaction may be at least partly S_N2 (bimolecular displacement). One is the great reactivity of benzyl chloride in reactions known to be S_N2 in mechanism. Another is the fact that while the replacement of the first chlorine of benzotrichloride by hydrogen causes a 33-fold decrease in reactivity, the replacement of the second causes only a 5-fold decrease in reaction rate (the situation with benzyl bromide is even more striking).[3] Hence the effect of α-fluorine on S_N1 reactivity in comparison to that of α-hydrogen, while probably not large, is not discernible from the present work.

Although increasing ease of double bond formation and the resultant increased carbonium ion



(1) From an M.S. thesis submitted by Donald E. Lee to the Graduate School of the Georgia Institute of Technology.

(2) For the significance of the terms, S_N1 and S_N2, see I. Dostrovsky, E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 173 (1946), and earlier papers.

(3) J. Hine and D. E. Lee, THIS JOURNAL, **73**, 22 (1951).