uncorrected but were taken in a Fisher melting point apparatus with a set of Anschütz thermometers which gave correct melting points with various pure reagents.

DEPARTMENT OF CHEMISTRY
HOLY CROSS COLLEGE
WORCESTER, MASSACHUSETTS

RECEIVED JUNE 1, 1936

COMMUNICATIONS TO THE EDITOR

SYNTHETIC SUBSTRATES FOR PROTEIN-DIGESTING ENZYMES

Sir:

Knowledge regarding the specificity of those enzymes which hydrolyze intact proteins (peptic, tryptic and catheptic proteinases) is meager. In general it is assumed that these enzymes react exclusively on high molecular substrates.

Recently it has been possible to study the specificity of proteinases with the aid of synthetic substrates. Such substrates have been found in this Laboratory for the catheptic enzymes, papain, liver-cathepsin and bromelin. The authors have now observed the splitting of α-hippuryl-lysine-amide by tryptic proteinase.

α-Hippuryl-ε-carbobenzoxy-lysine methyl ester was converted into α-hippuryl-ε-carbobenzoxy-lysine amide, m. p. 212°, with the aid of methanolic ammonia. *Anal.* Calcd. for $C_{23}H_{28}N_4O_5$: C, 62.7; H, 6.4; N, 12.7. Found: C, 62.6; H, 6.7; N, 12.8. This amide was hydrogenated catalytically, yielding α-hippuryl-lysine-amide which was isolated as the strongly hygroscopic hydrochloride, m. p. 248°. *Anal.* Calcd. for $C_{15}H_{23}N_4O_3Cl$: C, 52.5; H, 6.8; N, 16.3. Found: C, 52.0; H, 7.0; N, 15.9.

The tryptic proteinase was prepared according to E. Waldschmidt-Leitz and A. Purr [*Ber.*, **62**, 2217 (1929)]. The preparation contained no dipeptidase, aminopeptidase, and no carboxypeptidase; however, protaminase probably was present (Table I).

In contrast to HCN-papain, which splits only one peptide bond, tryptic proteinase splits two. After a complete splitting, hippuric acid was isolated from the digest (over 70% of the theoretical amount). Therefore, the splitting also must have liberated lysine and ammonia. That the free ε-amino group is an essential condition for the enzymic hydrolysis is shown by the fact that the above mentioned α-hippuryl-ε-carbobenzoxy-lysine amide is not hydrolyzed under the conditions of our experiments. The hydrolysis of our substrate by tryptic proteinase is remarkable since tryptic proteinase is supposed to react exclusively on anionic substrates.

TABLE I

ENZYMIC HYDROLYSIS OF α-HIPPURYL-LYSINE-AMIDE AT 40°

(Titration of liberated carboxyl groups)

Enzyme	Time, hrs.	Hydrolysis in % of one peptide bond
Tryptic proteinase, *p*H 8.8	22	123
	72	200
Tryptic proteinase, *p*H 8.8	18	121
	42	175
HCN-Papain, *p*H 5.0	5	58
	24	80
	49	85

It is intended to continue this research by studying the action of pure tryptic proteinases.

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

MAX BERGMANN
WILLIAM F. ROSS

RECEIVED JULY 10, 1936

STEROLS. VI. SYNTHETIC PREPARATION OF OESTRONE (THEELIN)

Sir:

The evidence for the accepted structure of oestrone has recently been reviewed [L. F. Fieser, "Chemistry of Natural Products Related to Phenanthrene," A. C. S. Monograph Series, No. 70]. We have been able to prepare a well crystallized compound from ergosterol which by analysis, derivatives and mixed melting points, is identical with oestrone isolated from pregnancy urine. It has been previously shown that ergosterol may be converted into 3-hydroxy-nor-allo-cholanic acid [Chuang, *Ann.*, **500**, 270 (1933); Fernholz