ated. The crude product was isolated as described above and then distilled. A forerun of 6.6 g. was followed by the main fraction b.p. 158–162° at 1.7 mm. The 52.5 g. (80%) of ketimine thus obtained melted at 121–123°. Crystallization from absolute alcohol gave pure material m.p. 123–124°.

Data pertaining to the anils prepared in this study are recorded in Table I.

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Trypsin Hydrolysis of Lysine Ethyl Ester

BY HAROLD WERBIN AND ANN PALM

In a recent review Neurath and Schwert[1] have discussed the specificity requirements of synthetic substrates for trypsin. L-Lysine ethyl ester (LyEE) which possesses the necessary configuration, (1) a lysyl side chain, (2) a susceptible ester linkage and (3) a polar group (NH_2) alpha to the carbonyl of the susceptible bond, has been found to be hydrolyzed by trypsin. The rate of hydrolysis was measured by using the Hestrin technique[2] for the quantitative determination of esters. Figure 1 shows that the hydrolysis follows zero order kinetics at *p*H 7.88 and 25.0°. The calculated rate constant was found to be a linear function of the enzyme concentration as illustrated in Fig. 2.

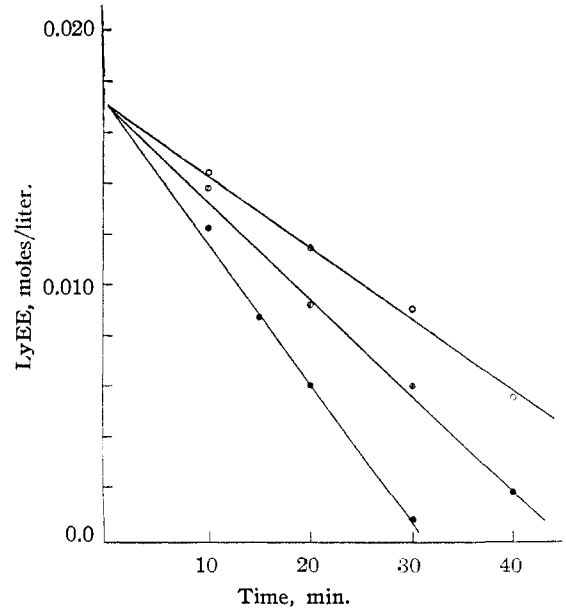

Fig. 1.—Zero order hydrolysis of 0.017 *M* lysine ethyl ester by trypsin at 25° and *p*H 7.88: ○, 2.73 × 10^{-3} mg. TPN/ml.; ◐, 3.66 × 10^{-3} mg. TPN/ml.; ●, 5.46 × 10^{-3} mg. TPN/ml.

Recently Iselin, *et al.*,[3] have employed a colorimetric procedure similar to the one described below to measure the hydrolysis of hydroxamides by chymotrypsin, while the use of the Hestrin method to measure the extent of hydrolysis of 1-

(1) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).
(2) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
(3) B. M. Iselin, H. T. Huang and C. Niemann, *ibid.*, **183**, 403 (1950).

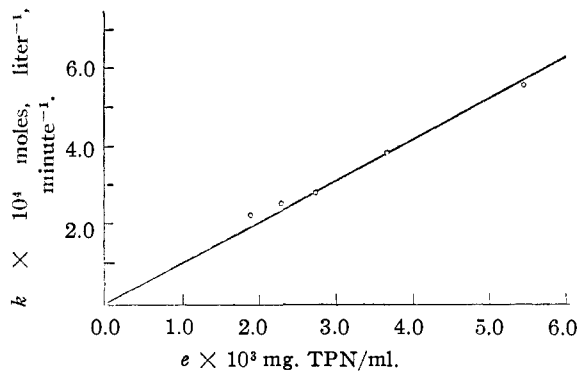

Fig. 2.—Specific rate constant for the hydrolysis of lysine ethyl ester by trypsin.

arginine methyl ester[4] by trypsin and of 1-tyrosine ethyl ester[4] by chymotrypsin has been described by Werbin.[5] Financial aid from the Dazian Foundation for Medical Research is acknowledged.

Experimental

Substrate.—LyEE was prepared by passing dry hydrogen chloride gas through a mixture of 6.0 g. of lysine monohydrochloride and 210 ml. of absolute ethanol until all the lysine had dissolved. The addition of hydrogen chloride was stopped, the solution was refluxed for 2 hours, and placed in the refrigerator overnight. On the following day it was distilled under reduced pressure and the residual oil was reësterified. After removal of the ethanol, crystals appeared and recrystallization from 25 ml. of absolute ethanol yielded 5.0 g. of white crystals, decomposing at 144°. Akabori and Kaneko[6] report 143.5–144.5°.

Kinetic Study.—To 2.0 ml. of 0.06 *M* LyEE and 2.0 ml. of 0.3 *M* phosphate buffer incubated for 10 minutes at 25° ± 0.02 was added 2.0 ml. of crystalline trypsin dissolved in hydrochloric acid solution of *p*H 3.0. The stopwatch was started at the time of half-addition of the trypsin. At the desired time 1.0 ml. of reaction mixture was added to 2.0 ml. of alkaline hydroxylamine solution following the procedure employed by Hestrin.[2] The ferric chloride solution used to develop the color was 0.4 *M*. The transmission of the solution at 520 mμ was read on a brociner-mass photoelectric colorimeter 5 minutes after the addition of the ferric chloride. A blank run demonstrated that there was no spontaneous hydrolysis of the ester.

(4) These substrates were suggested by Dr. Harry Goldenberg.
(5) H. Werbin, Ph.D. Thesis, Polytechnic Institute of Brooklyn, June, 1950.
(6) S. Akabori and T. Kaneko, *Bull. Chem. Soc. Japan*, **11**, 208 (1936).

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Preparation of 1,1,3,3-Tetrachloropropane

BY A. M. WHALEY[1] AND H. W. DAVIS

There are twenty-nine possible chloropropanes, two of which do not have physical properties listed in the literature. Both of these compounds, namely, 1,1,3,3-tetrachloropropane and 1,1,1,3,3,3-hexachloropropane, have all the halogen atoms located on the end carbon atoms, so that it is not easy, if at all possible, to make these chlorides by processes involving addition of chlorine to a double bond; of interest, however, is the fact that the 1,1,1,3,3-pentachloropropane has been pro-

(1) Halogen Chemicals Inc., 616 King St., Columbia, S. C.