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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RECEIVED OCTOBER 16, 1950

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.

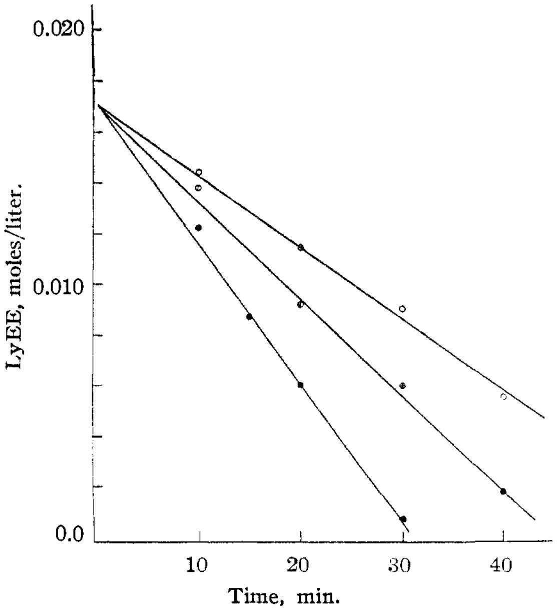

Fig. 1.—Zero order hydrolysis of 0.017 *M* lysine ethyl ester by trypsin at 25° and *p*H 7.88: O, 2.73 × 10^{-3} mg. TPN/ml.; ◐, 3.66 × 10^{-3} mg. TPN/ml.; ●, 5.46 × 10^{-3} mg. TPN/ml.

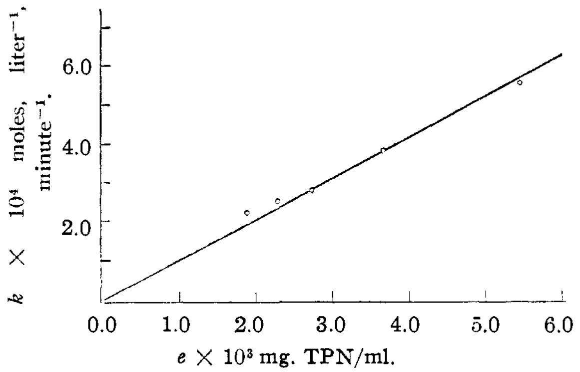

Fig. 2.—Specific rate constant for the hydrolysis of lysine ethyl ester by trypsin.

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-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.

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(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).

(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).

DEPARTMENT OF BIOCHEMISTRY
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RECEIVED AUGUST 16, 1950

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-

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

duced by the action of chlorine on 1,1-dichlorocyclopropane.[2]

In the course of a fundamental study of the properties of the chloropropanes, we have prepared and characterized the missing 1,1,3,3-tetrachloropropane. The final step was the chlorination of 1,1,3-trichloropropane followed by fractionation to separate the three possible tetrachlorides, 1,1,1,3-, b.p. 157.5°; 1,1,3,3-, b.p. 162°; 1,1,2,3-, b.p. 178.3°.

Experimental

1,1,3-Trichloropropane was prepared, independently of, but by a method similar to that of Stitz,[3] from β-chloropropionaldehyde and PCl_5. Eight moles (1180 g.) of this trichloride diluted with 1000 g. of carbon tetrachloride was chlorinated at 55–60° in a two-liter flask fitted with a gas dispersion disc, stirrer, thermometer and condenser. Chlorine was passed in slowly until the gain in weight was 221 g., corresponding to 80% monochlorination, 10 hours being required for this operation. The reaction mixture was distilled using a 2.5 × 100 cm. column packed with glass helices, at atmospheric pressure until the carbon tetrachloride was removed and finally at 100 mm. pressure. The yield of 1,1,3,3-tetrachloropropane, boiling range 94–98° (100 mm.) was 320 g. or 30%. Properties of the purified material were: b.p. 161.9° (756 mm.); f.p. −27° (uncor.); d^{20}_4 1.4689; d^{25}_4 1.4612; n^{20}_D 1.4848; n^{25}_D 1.4813.

Anal. Calcd. for $C_3H_4Cl_4$: Cl, 77.97. Found: Cl, 77.19.

Acknowledgment.—The authors are grateful to Miss Darien Dorn and Mr. R. J. Breazeale who assisted in this work.

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[2] P. G. Stevens, THIS JOURNAL, **68**, 620 (1946).

[3] F. Stitz, *Österr. Chem.-Ztg.*, **48**, 186 (1947); *C. A.*. **44**, 7226 (1950).

DEPARTMENT OF CHEMISTRY
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COLUMBIA, SOUTH CAROLINA
RECEIVED OCTOBER 5, 1950

Reciprocal Solubility of 4,6-Dimethyl-1,2-pyrone and Water

BY RICHARD H. WILEY AND NEWTON R. SMITH

In studies involving reaction of 4,6-dimethyl-1,2-pyrone in water solution, it was observed that this unsaturated lactone possessed lower and upper consolute temperatures between 0 and 100°. Although this compound has been known for a number of years,[1] no comment has been made on this phenomenon. This report presents data on the solubility of 4,6-dimethyl-1,2-pyrone in water which establish upper and lower consolute temperatures.

The occurrence of an upper and lower consolute temperature of organic compounds in water solution has been observed in only a few cases; these compounds include amines,[2] ketones,[3] alcohols[4] and glycol monoethers.[5] This apparently is the first reported occurrence of both upper and lower consolute temperatures for an ester or lactone. The solubility relations between 4,6-dimethyl-1,2-pyrone and water are unusual for this reason and also because the region which consists of two phases occupies such a small area on the phase diagram. The lower and upper consolute temperatures are 59.7 ± 0.5° and 86.3 ± 0.5°, respectively, as shown in Fig. 1. In systems containing concentrations below 25% and above 50% of the pyrone by weight, the two liquids are completely miscible above 51.5°, the melting point of the pyrone. At room temperature the solid pyrone crystallizes from sufficiently concentrated solutions slowly on standing or rapidly if seeded with the pyrone.

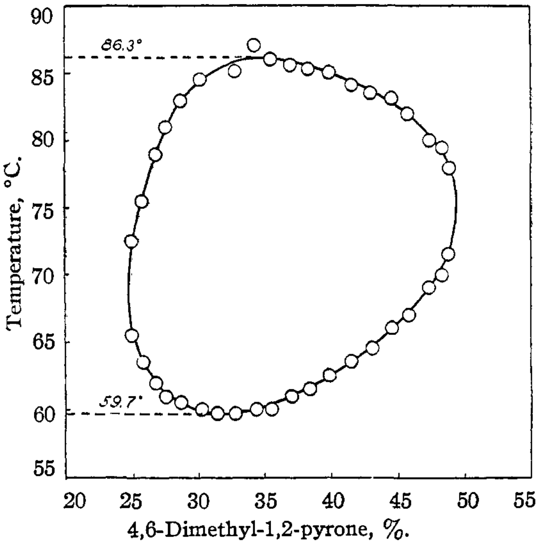

Fig. 1.—The 4,6-dimethyl-1,2-pyrone–water system.

Within the accuracy of the experiment the solubility curve is smooth except for one point on the higher solution curve when an upper consolute temperature of 87° was obtained at a concentration of 34.3% pyrone. The temperatures were read at a point of definite separation of phases. This separation was preceded by a marked opalescence. The exact temperature at which the opalescence occurs was difficult to determine and was obscured by a change from a water white to a yellow color as the experiment progressed.

The usual explanation for this phenomenon is that below the lower critical temperatures compound formation of the hydrogen bond type increases the solubility of the organic compound.[6] This is probably the explanation of the solubility behavior of the dimethylpyrone. There is a possibility that since the dimethylpyrone is easily hydrolyzed the phenomenon may be due to a reversible hydrolysis of the lactone to the corresponding acid. This requires that the pyrone precipitate from cold and the acid from hot solutions. This is not too likely as one would expect the acid to be soluble in the concentration ranges involved at the high temperatures and it is known that the solubilities of ethyl ether and ethyl acetate increase with decreasing temperature.

Experimental

4,6-Dimethyl-1,2-pyrone, prepared by the decarboxylation of isodehydroacetic acid,[1] was distilled repeatedly and

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[1] Hantzsch, *Ann.*, **222**, 9 (1883).

[2] Flaschner, *J. Chem. Soc.*, **95**, 668 (1909).

[3] Kohnstamm and Timmermans, *Verslag Akad. Wetenschappen Amsterdam*, **21**, 783 (1913).

[4] Timmermans, *Arch. Néerland sci.*, **6**, 147 (1922).

[5] Cox, Nelson and Cretcher, THIS JOURNAL, **49**, 1080 (1927).

[6] Glasstone, "Textbook of Physical Chemistry," second ed., D. Van Nostrand Company, Inc., New York, N. Y., 1946, p. 728.