The pseudo half-lives have been taken as the times graphically interpolated from the chronometric data when Q is one-half the value of Q at 20 minutes. The values of P are shown in the last column of Table III, at various values of E_0, to be nearly constant for a 5-fold variation in E_0. This is in agreement with the predictions of Equation 9 for the first order kinetics.

Conclusions.—The experimental evidence is consistent with first order kinetics for enzyme inactivation and is not consistent with three-halves order kinetics. It is thus no longer necessary to assume the stepwise inactivation mechanism proposed by Asimov and Dawson but there is insufficient evidence at the present time to propose another mechanism. Regardless of the kinetic interpretations, the usefulness of their chronometric equation for assay purposes remains unimpaired.

Acknowledgment.—We wish to thank Dr. Hans Lineweaver and Dr. Sigmund Schwimmer for helpful discussions concerning this research.

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[CONTRIBUTION FROM THE BASIC SCIENCES RESEARCH DEPARTMENT, U. S. NAVAL CIVIL ENGINEERING RESEARCH AND EVALUATION LABORATORY]

The Reaction of Certain Substituted Furfurals with Aniline and Aniline Hydrochloride

BY RICHARD W. DRISKO AND HERBERT MCKENNIS, JR.

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An investigation has been conducted to determine whether a number of substituted furfurals in reaction with aniline and aniline hydrochloride undergo ring cleavage as does furfural. Both 5-chloro-2-furaldehyde and 5-bromo-2-furaldehyde yielded bis-(phenylamino) compounds, and no cleavage of the furan nucleus of the parent aldehydes was noted. 5-Nitro-2-furaldehyde, under the same conditions, gave the corresponding anil in good yield. Thiophene-2-aldehyde was found to combine with aniline to form an anil which did not react further with aniline hydrochloride.

Since Stenhouse[1] first reported that aniline, aniline hydrochloride and furfural react together to form a deep purple compound, the structure of this compound and the mechanism of its formation have had widespread investigation.[2-8] The evidence thus far indicates that the formula (I) of Zincke and Mülhausen[4] is consistent with the properties of the compound

$$(C_6H_5NH_2CH{=}CHCH{=}C(OH)CH{=}NC_6H_5)^+Cl^- \quad \text{I}$$

Youngburg and Pucher[9] found that methyl-2-furaldehyde[10] reacts with aniline acetate to give a light yellow color that does not appreciably interfere in the determination of furfural. Experiments in our laboratory have shown that aniline hydrochloride reacts slowly with 5-methyl-2-furaldehyde in ethanol to form a deep red color. The anil of 5-methyl-2-furaldehyde has been prepared and has also been found to react slowly with aniline hydrochloride to form a deep red color. This suggested that certain nuclear substituted furaldehydes and their anils would undergo ring cleavage in a manner similar to the cleavage of furfural itself.

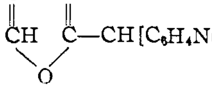
When 5-chloro-2-furaldehyde and 5-bromo-2-furaldehyde were treated with aniline and aniline hydrochloride, an entirely different reaction was found to occur. It was further noted that the same product was formed when two moles of aniline was used instead of one mole each of aniline and aniline hydrochloride.

In considering the possible structures of the products obtained, it was noted that Schmid and Becker[11] reported that acetaldehyde and propionaldehyde react with 2-aminopyridine in alcohol to form products which gave the correct analyses for compounds which may be assumed to have formulas II and III, although a rigid proof of structure was not given.[12,13]

$$CH_3CH(NHC_5H_4N)_2 \quad \text{II} \qquad CH_3CH_2CH(NHC_5H_4N)_2 \quad \text{III}$$

A similar reaction has been reported by Kondo and Ochiai[14] who found that methylal combines with β-phenylethylamine in the presence of hydrochloric acid to form diphenylethylaminomethane.

Fischer[15] has found that dimethylaniline reacts with furfural in the presence of zinc chloride to form a compound (IV) of the triphenylmethane type.



IV

The absorption spectra of the compounds obtained from the 5-halo-2-furaldehydes and aniline (Fig. 1) suggested the same type of structure for the two compounds. Elementary analyses showed

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[1] J. Stenhouse, *Ann.*, **156**, 197 (1870).

[2] H. Schiff, *Ann.*, **201**, 355 (1880); **239**, 349 (1887).

[3] W. König, *J. prakt. Chem.*, **72**, 555 (1905).

[4] T. Zincke and G. Mülhausen, *Ber.*, **38**, 3824 (1905).

[5] E. R. Riegel and M. Hathaway, THIS JOURNAL, **63**, 1835 (1941).

[6] G. Williams and C. L. Wilson, *J. Chem. Soc.*, 506 (1942).

[7] C. F. Koelsch and J. J. Carney, THIS JOURNAL, **72**, 2285 (1950).

[8] W. M. Foley, Jr., G. E. Sanford and H. McKennis, Jr., publication in preparation.

[9] G. E. Youngburg and G. W. Pucher, *J. Biol. Chem.*, **61**, 741 (1924).

[10] These investigators did not state which of the isomeric methyl-2-furaldehydes was employed in their studies, but the subject matter of the paper would suggest that 5-methyl-2-furaldehyde was used by them.

[11] L. Schmid and B. Becker, *Monatsh.*, **46**, 675 (1925).

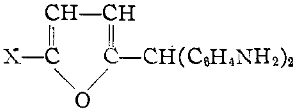
[12] M. M. Sprung, *Chem. Revs.*, **26**, 297 (1940), has reviewed much of the earlier literature on related compounds.

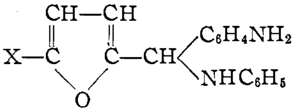
[13] A. Senier and W. Goodwin, *J. Chem. Soc.*, **81**, 280 (1902), discuss rearrangements at bis-(phenylamino)-methyl compounds under rather mild conditions.

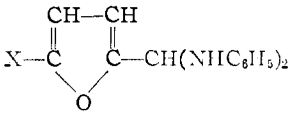
[14] H. Kondo and H. Ochiai, *J. Pharm. Soc. Japan*, **495**, 913 (1923); *C. A.*, **17**, 3032 (1923).

[15] O. Fischer, *Ann.*, **206**, 141 (1880).

that the empirical formulas were $C_{17}H_{15}ON_2X\cdot H_2O$. Loss of weight upon drying indicated the presence of one molecule of water of crystallization. For the anhydrous products formulas V, VI and VII were considered.


V


VI


VII

Several attempts were made to diazotize the chloro product. The results in all cases were entirely negative. This appeared to exclude the possibilities of structures V and VI, and attention was directed toward the 2-[bis-(phenylamino)]-methyl-5-halofuran structure (VII).

Hydrolytic decomposition of the chloro product to yield aniline was accomplished by heating in 50% sulfuric acid and subsequent steam distillation of the reaction mixture which had been rendered alkaline. For each mole of compound thus treated, 1.6 moles of aniline was obtained on the basis of titration. The identity of the resultant product was confirmed by acetylation and comparison with a known sample of acetanilide. From this experiment and the preceding discussion, it was concluded that formula VII is correct.[16]

Under conditions comparable to those employed for the preparation of the bis-(phenylamino)-methyl compound from the 5-halo-2-furaldehydes, 5-nitro-2-furaldehyde yielded the anil as the reaction product. The anil was the product obtained when the nitro compound was treated with aniline alone or aniline and aniline hydrochloride in equimolar amounts.

Neither the anil nor thiophene-2-aldehyde itself gave a red color upon treatment with aniline hydrochloride.

Experimental

Anil of 5-Methyl-2-furaldehyde.—5-Methyl-2-furaldehyde (3.30 g., 0.03 mole) was added to aniline (2.79 g., 0.03 mole) in a Claisen flask (25 ml.). The mixture was heated until a water phase separated. The water was removed by distillation under slightly reduced pressure, and the anil was distilled from the residue at 112–114° (1 mm.) to yield a light yellow oil (4.68 g.). The oil was easily frozen with ice and had m.p. 17°.

Anal. Calcd. for $C_{12}H_{11}NO$: C, 77.81; H, 5.99. Found: C, 77.80; H, 5.88.

Reaction of Anil of 5-Methyl-2-furaldehyde with Aniline Hydrochloride.—The anil of 5-methyl-2-furaldehyde was added to a saturated solution of aniline hydrochloride in absolute alcohol. The solution slowly turned to a deep red color. Aniline hydrochloride, however, was the only product isolated after the mixture had stood 20 hours. Under similar conditions aniline hydrochloride was found to react almost instantaneously with the anil of furfural. In a test-tube experiment a drop of 5-methyl-2-furaldehyde was added to a drop of glacial acetic acid and a drop of aniline in absolute alcohol (5 ml.). The solution slowly turned red.

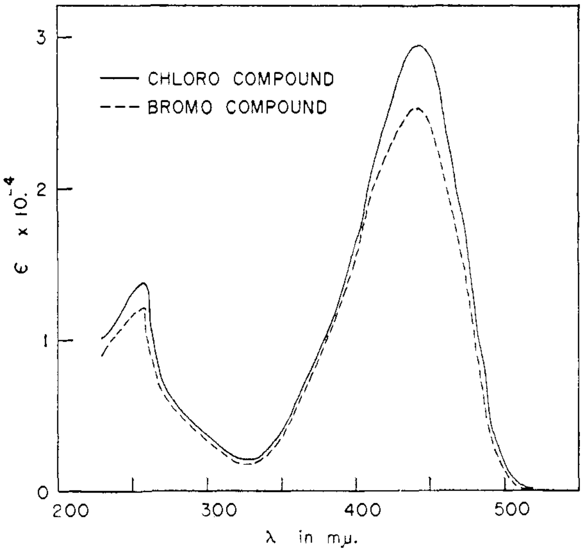


Fig. 1.—Absorption spectra of 2-bis-(phenylamino)-methyl-5-halofurans equimolar concentrations in absolute alcohol, recorded every 5 mμ at 20° with a Beckman model DU spectrophotometer.

Preparation of 2-Bis-(phenylamino)-methyl-5-chlorofuran (Structure VII where X = Cl).—5-Chloro-2-furaldehyde (1.31 g., 0.01 mole) was dissolved in a solution of aniline (0.93 g., 0.01 mole) and aniline hydrochloride (1.30 g., 0.01 mole) in absolute alcohol (25 ml.). After stirring and scratching there crystallized an orange solid (1.93 g.), m.p. 149–150° after two recrystallizations from ethyl alcohol. The same product was obtained when 5-chloro-2-furaldehyde was treated with aniline alone. A combustion analysis and drying to constant weight at 111° showed that this material contained one mole of water of hydration.

Anal. Calcd. for $C_{17}H_{15}ON_2Cl\cdot H_2O$: C, 64.45; H, 5.41; N, 8.85; Cl, 11.19. Found: C, 64.39; H, 5.58; N, 8.68; Cl, 11.31.

Acid Hydrolysis of Chloro Compound.—A sample of the chloro product was digested by boiling with 50% sulfuric acid for 15 minutes. The solution was then made alkaline with a 30% sodium hydroxide solution and steam distilled in a micro-kjeldahl apparatus. Titration of the distillate with 0.02 *N* hydrochloric acid indicated that 1.6 moles of the amine had been distilled for each mole of product originally present in the hydrolysis mixture. The neutralized solution was evaporated to 2 ml. and then treated with acetic anhydride and sodium hydroxide with shaking. The white solid which crystallized melted at 112–113°, as did a mixed sample with authentic acetanilide when placed on a Fisher block.

Preparation of 2-Bis-(phenylamino)-methyl-5-bromofuran (Structure VII where X = Br).—5-Bromo-2-furaldehyde (0.875 g., 0.005 mole) was dissolved in a solution of aniline (0.465 g., 0.005 mole) in absolute alcohol (25 ml.). The solution was gently heated. Upon cooling in ice there crystallized an orange solid (0.32 g.), m.p. 123–125.5° after two recrystallizations from ethyl alcohol. The same product was obtained when 5-bromo-2-furaldehyde was treated with aniline alone.

Anal. Calcd. for $C_{17}H_{15}ON_2Br\cdot H_2O$: C, 56.52; H, 4.74; N, 7.76; Br, 22.12. Found: C, 56.59; H, 4.60; N, 7.53; Br, 22.28.

Reaction of 5-Nitro-2-furaldehyde with Aniline and Aniline Hydrochloride.—5-Nitro-2-furaldehyde (1.41 g., 0.01 mole) was dissolved in a solution of aniline (0.93 g., 0.01 mole) and aniline hydrochloride (1.30 g., 0.01 mole) in absolute alcohol (25 ml.). Gentle heating of the solution and scratching of the flask resulted in the crystallization of a yellow solid (1.48 g.), m.p. 127.5° after three recrystallizations from ethyl alcohol. The same product was obtained when 5-nitro-2-furaldehyde was treated with aniline alone.

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[16] R. C. Elderfield and J. R. McCarthy, THIS JOURNAL, 73, 975 (1951), have by careful investigation accumulated evidence to show that *o*-phenylenediamine reacts with carbonyl compounds to yield benzimidazolines in many cases. This general reaction is not without analogy to those here described with the 5-halo-2-furaldehydes.

Anal. Calcd. for $C_{11}H_8O_3N_2$: C, 61.11; H, 3.73; N, 13.0. Found: C, 61.29; H, 3.83; N, 13.2.

Anil of Thiophene-2-aldehyde.—Aniline (4.65 g., 0.05 mole) and thiophene-2-aldehyde (5.60 g., 0.05 mole) were placed in a Claisen flask (10 ml.) and heated until a second phase separated. The water thus formed was removed by distillation under slightly reduced pressure, and the residue was distilled to give a pale yellow oil (8.85 g.), b.p. 122–125° (2 mm.). This oil crystallized with difficulty in the cold, m.p. 16°.

Anal. Calcd. for $C_{11}H_9NS$: C, 70.55; H, 4.85; N, 7.48; S, 17.12. Found: C, 70.82; H, 4.76; N, 7.22; S, 16.90.

Acknowledgment.—The authors are indebted to Dr. Carl T. Redemann of this Laboratory and Dr. Adalbert Elek of the Elek Micro Analytical Laboratories for analysis of the compounds obtained and to Mr. Elmer R. Streed of this Laboratory for the absorption spectra.

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[CONTRIBUTION FROM THE SOUTHERN REGIONAL RESEARCH LABORATORY[1]]

Molecular Compound Formation between Acetamide and Long-chain Saturated Fatty Acids

BY FRANK C. MAGNE AND EVALD L. SKAU

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The binary freezing point diagrams for each of the polymorphic forms of acetamide with lauric, myristic, palmitic and stearic acids have been constructed. These give conclusive evidence of molecular compound formation between acetamide and each of these acids. The general formula for these compounds is $RCOOH \cdot NH_2COCH_3$. Cryoscopic molecular weight determinations show that in dilute 1,4-dioxane solutions acetamide is 40.4% associated, myristic acid is not appreciably associated, and the equimolecular compounds of acetamide and the fatty acids are from 93.5 to about 97% dissociated depending upon the chain length of the fatty acid.

A review of all the published binary freezing point diagrams, in which one constituent is a long-chain saturated fatty acid, reveals that the only ones which showed molecular compound formation were those in which the second constituent is either a homologous fatty acid or one of the cholic acids. It has now been found that molecular compounds are also formed with acetamide. The nature and composition of these compounds are shown by binary freezing point diagrams which have been constructed for acetamide with lauric, myristic, palmitic and stearic acids, respectively.

Experimental

The pure fatty acids were recrystallized samples obtained through the usual fractional distillation of their methyl esters. Their freezing points by the Francis and Collins cooling curve method,[2] using a calibrated thermocouple instead of a thermometer, were as follows: lauric acid, 43.77°; myristic acid, 53.85°; palmitic acid, 62.45°; and stearic acid, 69.29°. The acetamide was the best Eastman Kodak Co. product[3] dried in vacuum over phosphorus pentoxide.

The freezing points were determined by the static method. For each composition weighed amounts of acetamide and the fatty acid were sealed in a glass tube. A glass bead was included to ensure efficient stirring as the sample tubes were turned end-over-end in a constant temperature bath. Two temperatures a few tenths of a degree apart were found, one at which the last crystals just disappeared and the other at which a few crystals remained undissolved after prolonged agitation. The freezing point was taken as the mean of these two temperatures corrected for both thermometer calibration and emergent stem. For compositions between 45 and 65% acetamide in the stearic acid system the difference in these two temperatures amounted to as much as 0.4°.

Results and Discussion

The data for the complete binary freezing point diagrams are given in Table I and are represented graphically in Fig. 1. In each system a molecular compound forms which has the general formula $RCOOH \cdot NH_2COCH_3$. On the acetamide side of the diagram two freezing points were obtainable. The full lines represent the temperatures at which the various liquid compositions are at equilibrium with the stable polymorphic form of acetamide; the broken lines represent the corresponding metastable equilibria between the liquid and either the equimolecular compound or the low-melting modification of acetamide, as the case may be. The higher freezing point was always obtained on the initial melting of the samples, as would be expected since they contained the stable modification. After the samples had been heated some degrees above this temperature, however, the freezing points invariably fell on the lower broken curve, and in order to obtain the higher freezing point again it was necessary to shock-chill the molten sample in a Dry Ice–alcohol mixture. Subsequent heating of the solid resulted in momentary local melting near the surface followed by rapid transformation to the higher melting form.

It is apparent from the figure that, in addition to the 50–50 composition, each system exhibits three invariant points at which the crystalline equimolecular compound is one of the phases. These points appear at lower and lower acetamide concentrations as the chain length of the fatty acid becomes shorter. For both the stearic and the palmitic acid systems all three of these invariant points represent eutectics, two being stable and one metastable. In the myristic and lauric acid systems they represent a stable eutectic, an incongruent melting point and a metastable eutectic. That is, the invariant point at which the solid phases are the equimolecular compound and the higher-melting form of acetamide is a eutectic point in the stearic acid system but, because of the shift to lower acetamide concentrations with shorter fatty acid chain length, it becomes an in-

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[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

[2] F. Francis and F. J. E. Collins, *J. Chem. Soc.*, 137 (1936).

[3] The mention of names of firms or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.