Anal. Calcd. for $C_9H_6O_6$: C, 51.42; H, 2.88. Found: C, 51.36; H, 2.97.

Retene Ketone (1-Methyl-7-isopropylfluorenone).—Two grams of retenediphenic anhydride was dry distilled, yellow vapors coming over accompanied by carbon dioxide. The distillate soon solidified to a yellow, crystalline material; yield, 1.5 g. (90%). From 95% ethanol it appeared as long, flat, sulfur-yellow prisms, m. p. 89–90° (corr.). Mixed with an equal amount of an authentic specimen of retene ketone of m. p. 88–89° (corr.), it melted at 89–90° (corr.).

When retenediphenic acid or an intimate mixture of its anhydride and soda lime were distilled, retene ketone also resulted. In these cases, however, losses due to charring were appreciable and the yields lower.

Summary

1. Dehydration of retenediphenic acid with 95% sulfuric acid yields two isomeric methylisopropylfluorenone carboxylic acids.

2. These keto acids have been characterized by their oximes, methyl esters and the oximes of the latter.

3. Ammonia converts methyl acid retenediphenate into the previously reported retenediphenamic acid which undergoes the Hofmann degradation to yield 8-methyl-2-isopropylphenanthridone. Oxidation of the latter yields hemimellitic acid, thus establishing the structures of the compounds concerned.

4. Treatment of methyl acid retenediphenate with 95% sulfuric acid at room temperature yields the methyl ester of 1-methyl-7-isopropylfluorenone-5-carboxylic acid. The isomeric acid is therefore 6-methyl-2-isopropylfluorenone-5-carboxylic acid.

5. Pyrolysis of retenediphenic anhydride gives retene ketone in 90% yield.

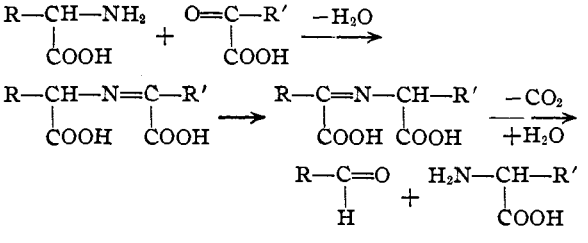
NEW YORK, N. Y. RECEIVED AUGUST 4, 1936

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

The Reaction between α-Ketonic Acids and α-Amino Acids

BY ROBERT M. HERBST

In a previous paper[1] it was shown that a reaction takes place between certain α-ketonic acids and α-amino acids. The first step in the reaction was assumed to be interaction between the carbonyl and the amino groups to form a Schiff base. The second step involved the migration of a hydrogen atom from the α-carbon of the amino acid to the α-carbon of the ketonic acid residue, and the third the elimination of carbon dioxide from the intermediate and the addition of water resulting in fission with the formation of an aldehyde and an amino acid.

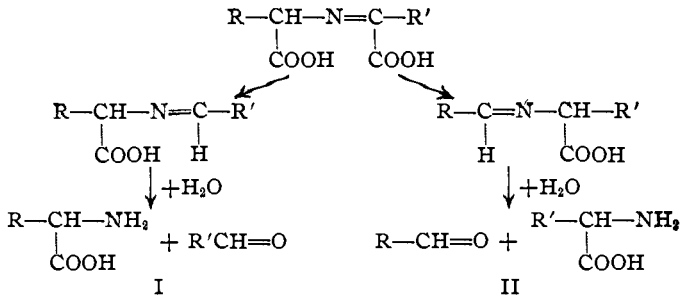


Certain results reported in the previous paper made it desirable to continue the investigation in order to gain greater insight into the mechanism of the reaction. In experiments with *l*-cystine and pyruvic acid acetaldehyde was always obtained in appreciable amounts. In the absence of any product, other than carbon dioxide, which could be attributed to the cystine molecule, it was concluded that the acetaldehyde was derived in some way from this source. This view has now been shown to be incorrect, for in the reaction between cystine and both phenylpyruvic and benzoylformic acids *no* acetaldehyde could be found. Moreover, small amounts of acetaldehyde have been isolated from the products of the reaction of pyruvic acid with *p*-methoxyphenylalanine, α-amino-*p*-methoxyphenylacetic acid, ethylcysteine, benzylcysteine and phenylcysteine, while with α-aminophenylacetic acid *no* acetaldehyde could be detected. These results point to pyruvic acid or some intermediate formed with the above amino acids as the principal source of acetaldehyde. The possibility remains that acetaldehyde could be formed by the secondary interaction of pyruvic acid with alanine formed during the primary reaction. In a separate experiment with this pair acetaldehyde was formed, but too slowly to account for its rapid formation in the reaction of pyruvic acid with cystine, S-phenylcysteine and S-benzylcysteine. The conclusion is therefore unescapable that the formation of acetaldehyde is largely a direct result of the primary reaction of pyruvic acid with the above amino acids.

(1) Herbst and Engel, *J. Biol. Chem.*, **107**, 505 (1934).

To determine the probable fate of the cystine structure, the reaction of several S-substituted cysteine derivatives with pyruvic acid was studied. Of these, ethylcysteine gave the clearest results since it was possible to demonstrate the formation of considerable quantities of ethylthioglycolic aldehyde as well as acetaldehyde. The analogous aldehydes from phenylcysteine and benzylcysteine, which were not found in the usual experiments, could be obtained only by continuous distillation of the volatile products from the reaction flask, and then only in small yield. In both cases considerable decomposition took place with the formation of thiophenol and diphenyl disulfide in the first, and of benzyl mercaptan in the second. No analogous aldehyde could be obtained from cystine even in distillation experiments.

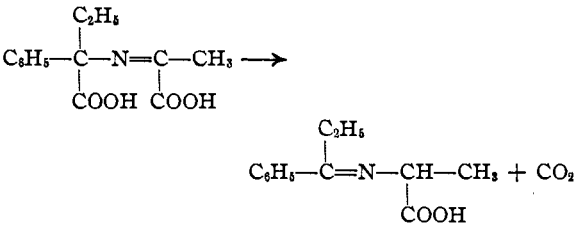
The results outlined above make it desirable to formulate a more labile mechanism for the reaction than the rather static mechanism given above.[2] It is now suggested that decarboxylation takes place while the molecule is momentarily in an unbalanced state during the shift of the double bond. The nature of the substituents R and R′ will influence the extent of decarboxylation in the direction of either Scheme I or II.



Such a mechanism would explain the observed formation of both aldehydes RCHO and R′CHO.

Schiff bases from α-phenyl-α-amino acids undoubtedly possess a strong tendency to form conjugated systems. Thus in the reaction of α-aminophenylacetic acid with pyruvic acid, the side chain double bond of the initial Schiff base is not conjugated with the benzene ring, and the tendency to form a conjugated system may explain the completeness of the reaction in the direction of Scheme II. When a methoxyl group is introduced in the para-position in aminophenylacetic acid, the tendency to form a conjugated system is possibly less strong, for the reaction proceeds partly in the direction of Scheme I.

Of particular interest are the results obtained in the reaction between α-amino-α-phenylbutyric acid and pyruvic acid, whereby propiophenone is produced in considerable quantity, together with mere traces of acetaldehyde. Here the tendency for the double bond to shift to the conjugated position is great, but this shift can occur only with simultaneous decarboxylation of the amino acid structure



Contrary to a previous statement[1] it has now been found that α-aminoisobutyric acid also reacts (though very slowly) with pyruvic acid. In this case a minute amount of acetaldehyde is formed, but *no* acetone could be detected. Clarke, Gillespie and Weisshaus[3] have observed a similar difference in the action of formaldehyde in formic acid on this pair of amino acids.

Further support for the view that decarboxylation occurs exclusively during tautomeric shift is the almost complete absence of decarboxylation on treating alanine with benzoylformic acid. The Schiff base formed by this pair is conjugated, hence there is no tendency for the double bond to shift; practically no carbon dioxide is eliminated, and only a trace of benzaldehyde is formed. That this result probably is not due to failure of benzoylformic acid to form a Schiff base is shown by the fact that it reacts with α-amino-*p*-methoxyphenylacetic acid, cystine and ethylcysteine.

Experimental

The experiments were carried on in the apparatus previously employed,[1] modified to permit gravimetric estimation of carbon dioxide. The gas coming from the reaction flask was passed through a series of wash bottles containing, respectively, 5% sodium bisulfite, 2% potassium per-

(2) Ingold and his co-workers [*J. Chem. Soc.*, 1778 (1935)] in a study of methyleneazomethine (C=N—C) systems from a kinetic point of view have found no evidence in support of an intermediate between tautomeric forms of simple Schiff bases. However, none of the systems considered involved carboxyl groups on the carbon atoms adjacent to the nitrogen [*cf.* also, Baker, Nathan and Shoppee, *ibid.*, 1847 (1935), and Turcan, *Bull. soc. chim.*, [5] **3**, 283 (1936)].

(3) Clarke, Gillespie and Weisshaus, THIS JOURNAL, **55**, 4571 (1933).

manganate in 2.5% sulfuric acid, two bottles with concentrated sulfuric acid and a Geissler absorption bulb charged with 40% potassium hydroxide. Suitable blank runs showed that only carbon dioxide was absorbed by the potash bulbs. The gas capacity of the whole train when charged was about 100 cc. Carbon dioxide was estimated periodically by changing the Geissler bulbs and weighing them. This method of estimating carbon dioxide was quicker and more convenient than that previously employed, and seemed to give more accurate results.

Pyruvic acid alone in aqueous solution slowly gives off carbon dioxide under the conditions of the experiment. The values obtained during actual experiments have been corrected, therefore, by subtraction of a suitable blank value, on the assumption that the amino acid has no effect on the rate of carbon dioxide production from pyruvic acid as such.

The reactions of pyruvic acid with α-aminophenylacetic acid and with cystine were repeated in the modified apparatus for purpose of comparison. Both experiments were in all other respects duplicates of those previously reported.

The isolation of reaction products followed in general the methods previously employed. After completion of the reaction aldehydic products were isolated as derivatives with dimedon (dimethyldihydroresorcinol) or 2,4-dinitrophenylhydrazine. Derivatives already described in the literature were identified by their melting points and mixed melting points with authentic specimens; others were identified by elementary analysis.[4] Aldehydes of low molecular weight were found in the sodium bisulfite wash bottle; less volatile aldehydes were isolated from the reaction mixture. To estimate quantitatively the amount of volatile carbonyl derivatives taken up in the bisulfite trap, the excess and bound bisulfite were titrated with standard iodine solution, as in the estimation of acetaldehyde in lactic acid determinations.[5] No acetaldehyde or other volatile carbonyl derivative is formed from pyruvic acid alone under the experimental conditions, as determined by suitable blank experiments.

Amino acids were isolated from the reaction mixture as previously described (except as noted in specific instances), either as such or in the form of derivatives with phenyl isocyanate or benzenesulfonyl chloride. Identity was established by elementary analysis, or by melting point and mixed melting point, respectively.

Mercaptans were isolated from the ether soluble portion of the reaction mixtures by vacuum distillation after removal of the solvent. Identification as 2,4-dinitrophenyl derivatives[6] rested upon melting points controlled by admixture of authentic specimens.

Reactions with Pyruvic Acid

In general 10 mml. of amino acid and 40 mml. of pyruvic acid were employed, exceptions being the reaction with α-aminophenylacetic acid where 30 mml. of pyruvic acid was employed, and the reaction with cystine where 5 mml. of the amino acid was employed. The yields of carbon dioxide and duration of the reactions are indicated by the curves in Figs. 1 and 2.

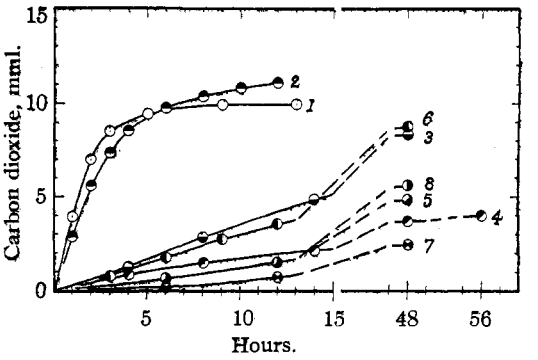


Fig. 1.—Rate of carbon dioxide evolution during reaction of pyruvic acid with: 1, α-aminophenylacetic acid; 2, α-amino-*p*-methoxyphenylacetic acid; 3, *p*-methoxyphenylalanine; 4, glutamic acid; 5, alanine; 6, leucine; 7, α-aminoisobutyric acid; 8, α-amino-α-phenylbutyric acid.

α-Aminophenylacetic Acid.—No acetaldehyde could be found. The yields of alanine (7.5 mml.) and benzaldehyde (7 mml.) were about the same as in earlier experiments.

Alanine.—Titration of the excess and bound sodium bisulfite in the trap indicated the presence of 4.4 mml. of an aldehyde, subsequently identified as acetaldehyde. Practically all of the alanine could be recovered in pure form.

Leucine.—Most of the isovaleric aldehyde was carried over into the bisulfite trap. Titration of an aliquot of the bisulfite solution indicated 12.2 mml. of aldehydes, however, since a permanent end-point in the titration was reached only after the solution began to smell strongly of isovaleric acid, much of the iodine was probably used to oxidize the aldehyde. Acetaldehyde could not be found. About 4 mml. of alanine was isolated.

α-Amino-*p*-methoxyphenylacetic Acid.—The high yield of carbon dioxide (11 mml.) may be explained by the formation of acetaldehyde according to Scheme I. Alanine was isolated in good yield, while anisaldehyde was obtained in 70–75% yield.

***p*-Methoxyphenylalanine.**—Acetaldehyde was found in appreciable amounts. Alanine was isolated in small yield. *p*-Methoxyphenylacetaldehyde was obtained in the form of a condensation product with pyruvic acid, the lactone of α-keto-γ-hydroxy-δ-(*p*-methoxyphenyl)-valeric acid, which crystallized from the reaction mixture as it cooled, giving needles from hot water, m. p. 160°.[7]

Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.50; OCH_3, 14.1; neut. eq., 220. Found: C, 65.66; H, 5.24; OCH_3, 13.83; neut. eq. (by back titration), 233.

With phenylhydrazine in 95% alcohol, slightly acidified by the addition of a few drops of glacial acetic acid, the phenylhydrazone is formed, m. p. 163° (dec.).

Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.65; H, 5.85; N, 9.03; OCH_3, 10.0. Found: C, 69.74; H, 5.82; N, 9.03; OCH_3, 10.29.

An attempt to prepare the 2,4-dinitrophenylhydrazone by boiling a solution of the ketolactone in 50% acetic acid with an equivalent amount of 2,4-dinitrophenylhydrazine resulted in the decomposition of the ketolactone; only

(4) The author wishes to thank Mr. William Saschek of this department for the micro-analyses included in this report.

(5) Clausen, *J. Biol. Chem.*, **52**, 263 (1922).

(6) Bost, Turner and Norton, THIS JOURNAL, **54**, 1985 (1932).

(7) All melting points are corrected.

pyruvic acid 2,4-dinitrophenylhydrazone, m. p. 222°, could be isolated.

Glutamic Acid.—The amino acid mixture obtained by the usual procedure was treated with zinc oxide to remove glutamic acid, after which alanine could be isolated as the benzenesulfonyl derivative. No aldehydic products could be isolated.

α-Aminoisobutyric Acid.—The yield of carbon dioxide was 2.5 mml., as compared with only 0.3 mml. of aldehyde. The bisulfite solution was fractionated carefully after alkalinization with sodium bicarbonate, and the distillate caught in a trap immersed in a solid carbon dioxide–alcohol bath. Although the distillate smelled of acetaldehyde, not sufficient 2,4-dinitrophenylhydrazone for purification and identification could be obtained. As in earlier experiments the aminoisobutyric acid was recovered almost quantitatively.

α-Amino-α-phenylbutyric Acid.—From the reaction mixture 1.4 mml. of propiophenone and a small amount of alanine were isolated. The bisulfite solution contained 0.6 mml. of carbonyl compounds, of which 0.05 mml. could be identified as acetaldehyde.

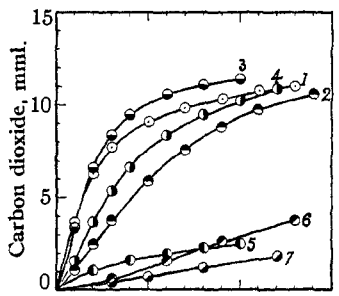


Fig. 2.—Rate of carbon dioxide evolution during the reaction of: 1, cystine with pyruvic acid; 2, S-ethylcysteine with pyruvic acid; 3, S-phenylcysteine with pyruvic acid; 4, S-benzylcysteine with pyruvic acid; 5, cystine with phenylpyruvic acid; 6, cystine with benzoylformic acid; 7, α-amino-*p*-methoxyphenylacetic acid with benzoylformic acid.

Cystine.—The bisulfite trap yielded 1 mol. of acetaldehyde. The formation of 11 mml. of carbon dioxide, an excess of 1 mml. over that expected in accordance with Scheme II, together with the formation of acetaldehyde, indicates partial reaction in the sense of Scheme I. No hydrogen sulfide was formed during the reaction; the only sulfur-containing material found was a black resin from which no definite compound could be isolated.

S-Ethylcysteine.—Acetaldehyde was found in the bisulfite trap together with a small amount of ethylthioglycolic aldehyde. Quantitative estimation of the aldehyde content of the trap was impossible because of secondary oxidations. Steam distillation of the reaction mixture yielded considerable quantities of an oil, the dimedon derivative (m. p. 93–94°) of which analyzed correctly as that of ethylthioglycolic aldehyde.

Anal. Calcd. for $C_{20}H_{30}O_4S$: C, 65.52; H, 8.26; S, 8.75. Found: C, 65.63; H, 8.17; S, 8.61.

Alanine was isolated in good yield. The odor of ethyl mercaptan was evident in the reaction mixture.

S-Phenylcysteine.—No aldehydic product, except acetaldehyde, of which there was 2.3 mml. in the bisulfite trap, could be found among the reaction products. Alanine was isolated in good yield. A large amount of brown oil, from which thiophenol and diphenyl disulfide were isolated, was formed during the reaction.

When the reaction was run with continuous distillation of the volatile products, instead of under reflux as was usually the case, phenylthioglycolic aldehyde was found in small amount in the distillate, and isolated as the dimedon derivative, m. p. 127–128°.

Anal. Calcd. for $C_{24}H_{30}O_4S$: C, 69.51; H, 7.30; S, 7.74. Found: C, 69.43; H, 7.34; S, 7.73.

In this case the reaction was run over a period of two to three hours, and the volume in the reaction flask was kept constant by the slow addition of water through a dropping funnel. A small amount of phenylthioglycolic aldehyde was prepared for purpose of comparison by boiling a solution of 5.5 g. of thiophenol with 7.6 g. of chloroacetal in absolute alcohol containing 1.15 g. of sodium for fifty hours, followed by hydrolysis with hydrochloric acid. The product, obtained in poor yields, was isolated as the dimedon derivative and proved to be identical with the substance obtained in the above reaction.

S-Benzylcysteine.—Acetaldehyde, 1.6 mml. by titration, was the only aldehydic product which could be isolated. Alanine was formed in good yield. A large amount of brown oil separated during the reaction, and upon careful examination yielded only a small quantity of benzyl mercaptan. By applying the technique of continuous distillation to this reaction it was possible to obtain a small amount of benzylthioglycolic aldehyde, dimedon derivative, m. p. 88–89°; and 2,4-dinitrophenylhydrazone, m. p. 156–157°.

Anal. Calcd. for $C_{26}H_{32}O_4S$: C, 70.04; H, 7.53; S, 7.49. Found: C, 70.23; H, 7.51; S, 7.69.

Anal. Calcd. for $C_{15}H_{14}N_4O_4S$: C, 51.99; H, 4.08; S, 9.26. Found: C, 51.98; H, 4.12; S, 9.28.[8]

Reaction with Phenylpyruvic Acid

Cystine.—During the reaction of cystine (2.5 mml.) with phenylpyruvic acid (10 mml.), 2.5 mml. of carbon dioxide was evolved in the course of ten hours (Fig. 2, Curve 5). The reaction was not continued over a longer period since phenylpyruvic acid gradually condenses with itself under the conditions of the experiment. No acetaldehyde nor other aldehydic products could be found among the products of the reaction. Some difficulty was encountered in separating the small amount of phenylalanine formed from traces of cystine. Analytically pure phenylalanine was obtained after decomposition of the cystine by boiling the amino acid mixture with a suspension of calcium hydroxide to which a few crystals of lead acetate had been added. After removal of the calcium and lead, phenylalanine separated upon concentrating the solution.

Reactions with Benzoylformic Acid

Usually 10 mml. of amino acid and 10 mml. of benzoylformic acid were employed in the reactions; in the case of cystine 5 mml. of the amino acid was employed. Except where specifically stated the duration of the reactions and yields of carbon dioxide are shown in Fig. 2.

Alanine.—After nine hours, only a trace of benzaldehyde (about 0.05 mml.) could be isolated, and the carbon

(8) Micro Dumas determinations on 2,4-dinitrophenylhydrazine or derivatives thereof are not reliable.

dioxide evolved was less than could be estimated (0.1–0.2 mml.).

α-Amino-*p*-methoxyphenylacetic Acid.—From the reaction mixture an aldehyde fraction consisting of benzaldehyde and anisaldehyde was isolated as a mixture of the 2,4-dinitrophenylhydrazones, m. p. 231–233°. The corresponding derivatives of benzaldehyde and anisaldehyde melt at 242–243° and 255°, respectively. The yield of hydrazones calculated as benzaldehyde was 0.5 mml. A methoxyl determination showed 3.5% OCH_3, indicating a mixture of 64% benzaldehyde and 36% anisaldehyde. The amino acid fraction (8.0 mml.) consisted chiefly of aminomethoxyphenylacetic acid (OCH_3, 15.64; N, 7.43).

Cystine.—No benzaldehyde nor hydrogen sulfide could be detected. From the reaction mixture about 0.5 mml. of α-aminophenylacetic acid was isolated after removal of the last traces of cystine by treatment with lime and lead acetate.

S-Ethylcysteine.—During twelve hours only 0.7 mml. of carbon dioxide was formed. From the bisulfite trap a small quantity of ethylthioglycolic aldehyde was isolated. Neither benzaldehyde nor acetaldehyde could be identified as reaction products.

Summary

The investigation of the reaction between α-amino acids and α-ketonic acids has been extended in such a manner as to demonstrate more clearly the mechanism involved. The first steps in the reaction appear to lead to the formation of a Schiff base with a carboxyl group on each of the carbon atoms adjacent to the central nitrogen atom. The double bond of the methyleneazomethine system has a tendency to shift (C—N=C ⟶ C=N—C) with the simultaneous elimination of carbon dioxide from either carboxyl group, both processes being dependent upon the nature of the other carbon substituents. In the case of α-amino-α-phenylbutyric acid this tendency is so pronounced that it takes place in spite of the necessity of forcing the displacement of the carboxyl group. Two new Schiff bases are assumed to result. Of these, one hydrolyzes to give the original amino acid and the aldehyde formed by decarboxylation of the ketonic acid; the other decomposes to form a new amino acid, derived from the ketonic acid by amination and reduction, and the aldehyde resulting from the oxidative deamination and decarboxylation of the original amino acid.

NEW YORK, N. Y. RECEIVED JULY 25, 1936

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

Further Observations Concerning the Crystallization of Undercooled Liquids

BY WILLIAM T. RICHARDS, EDWARD C. KIRKPATRICK AND CARL E. HUTZ

Introduction

The belief that homogeneous crystallization[1] of metastable liquids could be described successfully in statistical terms originated with the Kinetic Theory of Heat. With the writings of de Coppet, Ostwald and Tammann, which are too well known to require summary here, this belief became increasingly articulate. Recently Volmer[2] and Stranski[3] have expressed it in terms of Fluctuation Theory, and theoretical aspects of homogeneous crystallization have now advanced as far as the imperfect state of the Kinetic Theory of Liquids permits. Experiment has failed to keep pace with this development. A few cases of homogeneous crystallization, notably those described by Tammann[4] in very viscous melts, where the linear crystallization velocity is extremely small, have been reported. In general, however, a considerable body of scattered experimental work has served only to confuse the issue by the introduction of heterogeneous effects. The mechanism of heterogeneous crystal nucleus formation presents, however, certain points of interest in its own right, although the language for its description is at present less unequivocal and aesthetic than that for homogeneous crystallization. When the immense practical importance of the problem of efficiently inducing crystallization in metastable liquids, affecting, as it does, most organic preparations and many technical processes, is recalled it will be clear that any investigation concerning crystallization which contributes to the present knowledge, however imperfectly its results may be formulated, deserves attention. The object of the present communication was initially to determine the homogeneous crystallization rates of a number of liquids. It was found necessary, however, to interpret the re-

(1) The term "homogeneous crystallization" will be used throughout to designate crystallization occurring in the free liquid, as opposed to "heterogeneous crystallization" which occurs at an interface.

(2) M. Volmer, *Z. Electrochem.*, **35**, 55 (1929).

(3) I. N. Stranski and R. Kaischew, *Z. physik. Chem.*, **B26**, 100, 317 (1934).

(4) G. Tammann, "Kristallisieren and Schmelzen," 1903.