[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Preparation and Reactions of Acetopyruvic Acid (α,γ-Diketo-*n*-valeric Acid)*

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In 1937 Krebs and Johnson[1] proposed that acetopyruvic acid is an intermediate in the biochemical synthesis of acetoacetic acid from acetic and pyruvic acids. This compound has been previously described by Mumm and Bergell,[2] who also prepared several derivatives. However, the results reported by these workers seemed to indicate that this compound spontaneously undergoes unknown reactions in aqueous solution. Since knowledge of such reactions would be of great importance in a critical evaluation of its possible biochemical role, further study of its preparation and properties seemed required. In routine practice it was also found that the synthesis, as described by Mumm and Bergell, gave irregular results and that the procedure could be improved materially.

In this report some details concerning the synthesis, two unreported derivatives, stability, acid behavior, and some data on the oxidation mechanisms of acetopyruvic acid are described.

I. Synthesis and Properties.—Difficulties encountered with the Mumm and Bergell procedure for this synthesis led to the trial of sodium methylate in the synthesis of the sodio-ester compound. Freri[3] has shown that the methyl ester is formed in this condensation when sodium methylate is used as condensing agent, and the methyl ester was thought to have the advantage of being more rapidly hydrolyzed than the ethyl ester. However, it was soon realized that the difficulties in the preparation of the free acid from the sodio-ester arose from the fact that the sodium salt of the acid produced was almost as rapidly hydrolyzed as was the ester itself under the conditions of the Mumm and Bergell synthesis. The reactions involved are schematized in reactions (1) and (2).

$$CH_3COCH{=}C(ONa)COOC_2H_5 + NaOH \longrightarrow CH_3COCH{=}C(ONa)COONa + C_2H_5OH \quad (1)$$

$$CH_3COCH{=}C(ONa)COONa + NaOH \longrightarrow CH_3COCH_3 + (COONa)_2 \quad (2)$$

In the Mumm and Bergell synthesis reaction 2 proceeds almost as fast as reaction 1, accounting for the low yields of acetopyruvic acid. However, if the sodio-ester is first dissolved in water (rather than suspended in 4 *N* sodium hydroxide as in the Mumm and Bergell procedure), and then treated with the calculated amount of sodium hydroxide, reaction 1 is complete in less than two minutes and reaction 2 does not take place to any great extent because there is no extra base present to allow this reaction to proceed.

The data upon which this clarification of procedure was based are reported below.

Finally it was observed that the synthesis of Mumm and Bergell using sodium ethylate gives even better results than the methyl alcohol variation, if the sodio-ester is also first dissolved, since it was found that the methyl ester tended to split into oxalate before saponification took place.

The free acid is quite soluble in water, and is stable in approximately neutral aqueous solutions at incubator temperatures for at least twenty-four hours. The crystalline acid may be preserved without change if pure.

Two derivatives, probably analogous to the substituted pyrazoles described by Mumm and Bergell, were prepared. Unlike known chelate compounds of copper and β-diketones, the copper salt here described was not volatile or soluble in organic solvents.

The free acid had been noted previously to undergo hydrolysis to oxalic acid and another fragment, presumably acetone. Both products were identified and determined quantitatively. The velocity of this hydrolysis in dilute alkali solutions was followed by determining the oxalic acid precipitated as cerous oxalate. The reaction is of the first order, and the velocity increases with increase in base concentration. At least two equivalents of base (or enough for dibasic neutralization) are required before hydrolysis can be detected. No chemical change could be detected in acid solution up to 38°.

The potentiometric titration curve shows the inflections of a typical dibasic acid, and the dissociation constants are those to be expected from a consideration of the structure of the compound.

The results of the hydrolysis and titration

* Supported in part by the Wisconsin Alumni Research Foundation.

(1) Krebs and Johnson, *Biochem. J.*, **31**, 772 (1937).

(2) Mumm and Bergell, *Ber.*, **45**, 3040 (1912).

(3) Freri, *Gazz. chim. ital.*, **68**, 612 (1938).

studies indicate that the compound is perfectly stable under physiological conditions of temperature and *p*H. The gas formation noted by Krebs and Johnson on mixing neutralized acetopyruvic acid with bicarbonate buffer has been confirmed under more definite conditions. This effect is not due to a chemical change in the acid, but may be explained by an increase in dissociation of the enol hydrogen with dilution. The apparent catalytic action of magnesium sulfate or glutamate in dilute solution remains unexplained, unless it is due to changes in solubility of carbon dioxide. All attempts to isolate a reaction product of acetopyruvic acid and glutamic acid in dilute aqueous solution failed. Schiff base formation is quite possible, but in this case it is difficult to see why an increase in acid groups should take place.

Experimental

1. Synthesis.—Sodium ethyl acetopyruvate was prepared by the condensation of ethyl oxalate with acetone, using sodium ethylate as condensing agent, according to Marvel and Dreger.[4] Instead of filtering the compound from the reaction mixture it was separated by centrifugation, discarding the supernatant liquid. The compound was then dried *in vacuo*, powdered, and stored in a vacuum desiccator.

To obtain the free acid,[4a] 2.00 g. of the powdered sodium ethyl acetopyruvate was dissolved in a minimum volume of water (about 30 ml.) and exactly one equivalent of 4.00 *N* sodium hydroxide (2.78 ml.) was added from a buret with swirling. Exactly two minutes after the addition of the base was completed the solution was made acid by the addition of 3 *N* sulfuric acid. The solution was then extracted with ethyl ether in a Kutscher–Steudel type continuous extractor[5] for several hours or shaken out with 6–7 portions of ether in a separatory funnel. The solution was then dried over anhydrous sodium sulfate and the ether removed *in vacuo* at 30°. The crude crystals which formed slowly after the ether was removed were recrystallized from carbon tetrachloride. A slight yellow tinge remained even on repeated recrystallization. For absolute purity, the compound was then sublimed *in vacuo* (20 mm., 60°), the sublimate being caught on the outer surface of a cold finger type condenser. The sublimate, which was colorless, was dried *in vacuo* and recrystallized from carbon tetrachloride, giving colorless prisms of analytical purity. The usual yield was ≐1.0 g. of the pure compound, or 70% of the theoretical; m. p. (cor.) 98°.

Anal. Calcd.: C, 46.16; H, 4.65. Found: C, 45.97; H, 4.80.

The free acid when absolutely colorless may be kept for months in a vacuum desiccator at 0°. In the presence of any colored impurity, however, a slow decomposition into a brown resinous mass takes place.

2. Derivatives.—Two previously unreported derivatives of the compound were prepared.

1-(*p*-Nitrophenyl)-5-methylpyrazole-3-carboxylic Acid.—One gram of acetopyruvic acid dissolved in a minimum volume of ethanol was mixed with an equivalent weight of *p*-nitrophenylhydrazine hydrochloride dissolved in warm ethanol. The mixture was kept warm for thirty minutes and then slowly diluted with water. Orange needles separated and were filtered off. The crude compound was dissolved in a minimum volume of hot ethanol, toluene was added (equal volume) and the mixture boiled down to the volume of the added toluene. On cooling yellow-orange needles crystallized out; m. p. (cor.) 223–224°.

Anal. Calcd.: C, 53.44; H, 3.67. Found: C, 53.23; H, 3.82.

1-(2,4-Dinitrophenyl)-5-methylpyrazole-3-carboxylic Acid.—One gram of acetopyruvic acid in ethanol solution was mixed with an equivalent weight of 2,4-dinitrophenylhydrazine hydrochloride in warm ethanol solution. The mixture was kept warm for thirty minutes and diluted with a large volume of water. A voluminous mass of yellow crystals separated. The compound was recrystallized from toluene as bright yellow needles; m. p. (cor.) 239–241°.

Anal. Calcd.: C, 45.21; H, 2.76. Found: C, 45.45; H, 3.06.

Copper Salt.—A green, slightly soluble, copper salt was obtained by treating a solution of acetopyruvic acid with copper sulfate. This salt could be recrystallized from boiling water without decomposition.

Anal. Calcd. for $Cu(AP)_2 \cdot 2H_2O$: Cu, 17.9. Found: Cu, 17.8.

Insoluble salts with calcium, barium, bismuth and mercurous ions were also obtained, but were not investigated further.

3. Identification of Hydrolysis Products.—Mumm and Bergell had previously identified oxalic acid as one of the hydrolysis products when acetopyruvic acid is hydrolyzed by strong bases. Acetone was presumed to be the other fragment. To establish the identity of the acetone fragment, 2 g. of the acid was treated with an excess of 6 *N* sodium hydroxide. After two hours, the mixture was neutralized and distilled. The distillate was treated with alcoholic 2,4-dinitrophenylhydrazine hydrochloride. The crystals formed melted at 126°. An authentic acetone derivative melted at 126°; mixed melting point 126°.

A quantitative determination of both hydrolysis products by titration of the acetone in the distillate with iodine and permanganate titration of the oxalic acid in the residue showed that 98.9% of the acetopyruvic acid used was accounted for: 1.30 g. of acetopyruvic acid yielded 0.578 g. of acetone (calcd. 0.58 g.) and 0.890 g. of oxalic acid (calcd. 0.90 g.).

(4) Marvel and Dreger, "Org. Syn.," VI, 40 (1926).

(4a) It was also possible to obtain the free acid by hydrolysis of the free ester with concentrated hydrochloric acid at room temperature for a week. The yields, however, did not warrant the use of this method.

In the determination of the time required for basic hydrolysis described here in detail, the reaction was followed by measuring the *p*H, which drops from that due to the added base to *p*H 9.5 (secondary neutralization point) in approximately two minutes. The time has been found to vary with different preparations of the sodio-ester and for best results should be determined on each fresh batch of the crude material.

(5) Kutscher and Steudel, *Z. physiol. Chem.*, **39**, 473 (1903).

4. **Determination of Velocity Constant and Order of Reaction of the Alkaline Hydrolysis.**—Twenty-five ml. of 0.020 *M* acetopyruvic acid was mixed with 25 ml. of 4 *N* sodium hydroxide and kept at room temperature (23.6°). At intervals, 2 ml. of the reaction mixture was pipetted out into test-tubes containing 1 ml. of 5 *N* hydrochloric acid to stop the reaction. A drop of 0.1% brom cresol purple indicator was added to each tube and the contents titrated to the transition shade of the indicator with 2 *N* sodium hydroxide (*p*H 5.2–6.8). Finer adjustment was made with 0.1 *N* acid or base. One ml. of cerous chloride solution (0.6%) was added and the tube allowed to stand for two hours. The contents of the tubes were filtered through sintered glass funnels (Pyrex 3G3) and the cerous oxalate precipitate in the tube and funnel washed with cold water. The precipitated cerous oxalate in the test-tube and filter was then dissolved with hot 10% sulfuric acid and the filtrate caught in a clean suction flask. The test-tube and filter were then washed with cold water and the washings also collected. The contents of the flask were heated to 60–70° and titrated with 0.01 *N* potassium permanganate. The data were found to represent an experimental first order reaction.

TABLE I

TEMPERATURE 23.6°

Time, min.	Oxalic formed, ml. $KMnO_4$	K (min.$^{-1}$) $K = \frac{1}{t}\log_e \frac{C_0}{C_t}$
60	2.28	0.0126
120	3.35	.0126
140	3.52	.0122
180	3.85	.0125
200	4.00	.0132
	Average	.0126

5. **The Effect of Acid and Base Concentration on the Velocity of Hydrolysis.**—A series of experiments similar to the above using hydrochloric and sulfuric acids in concentrations ranging from 0.01 *N* to 5 *N* were carried out. No measurable formation of oxalic acid was observed under these conditions.

The results of another series using variable concentrations of sodium hydroxide are reported in Fig. 1. The acetopyruvic acid concentration was uniformly 0.01 *M*. It will be observed that measurable hydrolysis occurred only when more than two equivalents of base are present in the solution.

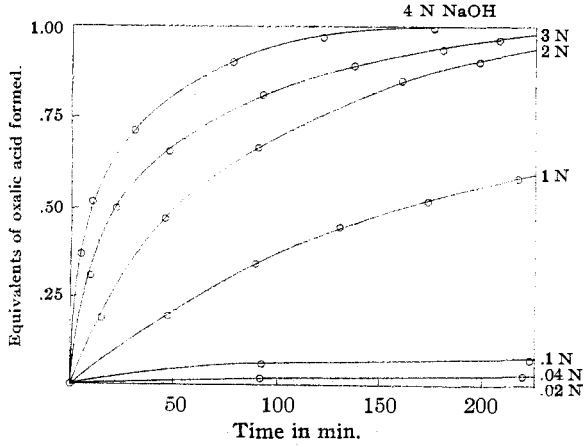

Fig. 1.—Hydrolysis of acetopyruvic acid by sodium hydroxide of varying concentration.

6. **Potentiometric Titration.**—Exactly 62 ml. of 0.0162 *M* solution of acetopyruvic acid (carbon dioxide-free) was titrated with 0.101 *N* sodium hydroxide using a standard Beckmann glass electrode and potentiometer. The curve obtained shows inflections at *p*H 5.0 and *p*H 9.5 which are well-defined and show that the acid is dibasic. At *p*H 7.4 1.27 equivalents of sodium hydroxide are bound.

7. **Acid Dissociation Constants.**—The two dissociation constants of acetopyruvic acid at 25° were calculated from accurate measurements of the *p*H of known solutions of acetopyruvic acid (made up by weight from the pure compound in carbon dioxide-free water) by means of the glass electrode. The method was checked with several organic acids of known dissociation constants, with which it gave the expected results.

TABLE II

DISSOCIATION CONSTANTS OF ACETOPYRUVIC ACID, TEMP. 25.0°

	Concn. of acid, *M*	*p*H	α	K_a
K_I	0.0485	2.00	0.207	2.6×10^{-3}
K_{II}	0.0325	5.01	0.00031	3.2×10^{-9}

8. **Behavior with Buffers.**—Ten ml. of 0.050 *M* phosphate buffer (*p*H 7.09) was mixed with 10 ml. of 0.040 *M* acetopyruvic acid neutralized to *p*H 7.08. The resultant *p*H (7.16) was found to be identical with the *p*H of the buffer alone when diluted with water. No gas formation could be detected (tested in standard Warburg respirometers) at 38°.

Carbon dioxide exchange in a bicarbonate buffer system was measured in the Warburg apparatus at 38°; 0.0250 *M* sodium bicarbonate was in equilibrium with 5% carbon dioxide in nitrogen. The calculated *p*H (from the Henderson–Hasselbalch equation $pK = 6.14$) was 7.50. Acetopyruvic acid, neutralized to *p*H 7.50, was added from the side arm. The data are shown in Table III.

TABLE III

FORMATION OF CARBON DIOXIDE ON MIXING ACETOPYRUVATE SOLUTIONS WITH BICARBONATE BUFFER OF SAME *p*H

In main compartment 2.8 ml. of 0.025 *M* sodium bicarbonate in equilibrium with 5% carbon dioxide in nitrogen.

Side arm, 0.3 ml.	Microliters of CO_2 evolved in 2 hours
1 Acetopyruvate (0.25 *M*)	14
2 Acetopyruvate, 0.05 *M* $MgSO_4$ (*p*H 7.50)	58
3 Acetopyruvate, 0.01 *M* glutamate (*p*H (7.50)	26
4 Water	0

The data show that gas is evolved only in the bicarbonate buffer and not in the phosphate buffer. The gas formation seems to be promoted by magnesium sulfate and glutamate, or may be due to a reduced solubility of carbon dioxide in these solutions.

II. The Oxidation of Acetopyruvic Acid.—In this preliminary study of the oxidation of this compound it was desired to answer one question clearly. Is acetopyruvic acid oxidized in the manner visualized by Krebs and Johnson thus

$$CH_3COCH_2COCOOH \longrightarrow CH_3COCH_2COOH + CO_2$$
$$\longrightarrow CH_3COCH_3 + 2CO_2 \quad (3)$$

or, is it also oxidized at the methylene carbon atom

$$CH_3COCH_2COCOOH \longrightarrow [CH_3COCOCOCOOH] \longrightarrow$$
$$[CH_3COCOOH] + 2CO_2$$
$$\longrightarrow CH_3COOH + 3\,CO_2\,? \quad (4)$$

For this purpose potassium permanganate was used as the oxidizing agent, because much work with this reagent has given a secure background for the interpretation of the reaction mechanism in terms of the reaction products.[6] The characteristic end-products—acetic acid and acetone—are not difficult to determine, and are such as would provide the answer to the above question.

The character of the oxidation products isolated indicates that at least these two mechanisms are involved. When a limited amount of permanganate (0.4 mole) was used in the presence of sulfuric acid, the products isolated were acetone, oxalic acid, carbon dioxide, and unknown volatile reducing compounds (probably acetol and pyruvic acid). In faintly alkaline solutions acetic acid and carbon dioxide were the only products obtained. In more strongly alkaline solution, acetic acid, oxalic acid, and carbon dioxide were obtained.

It was anticipated that the reactivity of the methylene hydrogen toward permanganate would be so great that the reaction (3) would be obtained only in quite acid solutions. This was found to be true. When less acid solutions were used the methylene group was attacked and the acetic acid obtained in place of acetone indicated that reaction (4) was taking place. Here the triketo acid, $CH_3COCOCOCOOH$, may be visualized as the intermediate step and the products formed other than acetic acid would be determined by the circumstances of the further breakdown of this labile compound.

Experimental

1. The Titrimetric Oxidation of Acetopyruvic Acid.—When acetopyruvic acid in 4 *N* sulfuric acid solution at 60° is titrated with permanganate an end-point is approached marked by a sharp decrease in time of decolorization. It requires one mole of permanganate for each mole of acetopyruvic acid to reach this point: 25.00 ml. of 0.0277 *M* acetopyruvic acid required 25.00 ml. of 0.141 *N* potassium permanganate; one mole of potassium permanganate = one mole of acetopyruvic acid.

To determine the nature of the products of the oxidation obtained at this stage 1.00 g. of acetopyruvic acid dissolved in 4 *N* sulfuric acid was oxidized with 1.22 g. of potassium permanganate (*i. e.*, 2.5 atoms O or 1 mole potassium permanganate) using the closed system technique described by Witzemann.[7] In this way 0.846 g. of carbon dioxide was recovered (as barium carbonate), corresponding to approximately 2.5 moles of carbon dioxide from each mole of the acid. The reaction mixture was distilled and the distillate was found to contain substances reactive with alkaline hypoiodite, thought to be acetone in part, at least. The 2,4-dinitrophenylhydrazone of acetone was isolated from the distillate (m. p. 126°, authentic sample 126°, mixed m. p. 126°). The distillate was also found to reduce Benedict and Tollens reagents. The Schiff test for aldehydes was negative. A trace of volatile acid could be detected but was not identified. By further oxidation of the distillate with acid permanganate (which does not oxidize acetone under these conditions) a decrease in iodine titration and a formation of volatile acid were noted. The acid formed gave a positive lanthanum reaction[8] indicating the presence of acetic acid. It was concluded that possibly acetol or pyruvic acid was the reducing compound in the distillate. No stoichiometric relationships were developed for the products obtained, but the results show that the oxidation was proceeding in part according to reaction (3) above.

2. Oxidation of Acetopyruvic Acid by a Limited Amount of Potassium Permanganate.—0.800 g. of acetopyruvic acid in a minimum volume of 4 *N* sulfuric acid was oxidized by 0.388 g. of potassium permanganate (1.0 atom O = 0.4 mole $KMnO_4$) in a flask cooled by ice water. Carbon dioxide was produced. After neutralization of the reaction mixture, crystals of manganous oxalate separated (identified by potassium permanganate titration of the oxalic acid liberated from a dried and weighed sample and content of manganese). The mixture gave a negative test for aldehydes with Schiff reagent. Acetone was identified in the distillate of the reaction mixture by isolation of its semicarbazone (m. p. 191°, authentic sample 190°, mixed m. p. 190°). An unknown reducing compound as described in oxidation no. 1 was also found in the distillate.

3. Oxidation of Acetopyruvic Acid with Alkaline Permanganate.—0.25 g. of acetopyruvic acid in 115 ml. of water mixed with 50 ml. of *M*/6 sodium monophosphate was oxidized in the closed system apparatus with 1.00 g. of potassium permanganate in 85 ml. of water: 0.253 g. of carbon dioxide was formed (as barium carbonate); 0.111 g. of acetic acid was formed (identified by Duclaux constants).[9]

The above is a typical result of such an oxidation, and it was concluded that the acetopyruvic acid was quantitatively oxidized in this instance

$$CH_3COCH_2COCOOH \longrightarrow CH_3COOH + 3CO_2$$

(6) Witzemann, *J. Biol. Chem.*, **95**, 219 (1932).

(7) Witzemann, *ibid.*, **107**, 475 (1934).

(8) Krüger and Tschirch, *Ber.*, **62**, 2776 (1929).

(9) Virtanen and Pulkki, THIS JOURNAL, **50**, 2138 (1928).

4. Oxidation of Acetopyruvic Acid with Permanganate in the Presence of Excess Alkali.—To a solution of 0.500 g. of acetopyruvic acid in 20 ml. of water was added 2.04 g. of potassium permanganate (5 atoms O) dissolved in 80 ml. of 0.485 *N* sodium hydroxide (7 equivalents). The permanganate was decolorized completely but no further decolorization of permanganate could be obtained with this amount of acid. The solution was filtered and made up to 200 ml. Analyses were then made on aliquots for acetic acid, oxalic acid, and carbon dioxide, the expected products. Found: 0.102 g. of acetic acid; 0.476 g. of oxalic acid; and 0.220 g. of carbon dioxide. These figures represent 97% recovery of the carbon. One mole of acid yielded 1.38 moles of oxalic acid, 0.45 mole of acetic acid, and 1.32 moles of carbon dioxide.

These results indicate that about half of the acetopyruvic acid used was oxidized by each of the two reactions

$$CH_3COCH_2COCOONa \longrightarrow [CH_3COCOONa] + (COONa)_2 \longrightarrow CH_3COONa + Na_2CO_3 + (COONa)_2 \quad (5)$$

$$CH_3COCH_2COCOONa \longrightarrow [CH_3COCOONa] + (COONa)_2 \longrightarrow [CH_2{=}COHCOONa] + (COONa)_2 \longrightarrow Na_2CO_3 + 2(COONa)_2 \quad (6)$$

Of these (5) accounts for the acetic acid found and (6) accounts for the excess over one mole of oxalic acid found.

Summary

1. A reliable procedure for the synthesis of acetopyruvic acid is given, and two derivatives of the compound are described.

2. Fundamental properties of this compound were determined and related to its synthesis and stability.

3. Acetopyruvic acid is stable in neutral and acid solutions. In strongly basic solutions it is hydrolyzed to acetone and oxalic acid, by a reaction of the first order, the velocity of which depends on the concentration of the base.

4. This acid is dibasic and the dissociation constants are $K_I = 2.6 \times 10^{-3}$ and $K_{II} = 3.2 \times 10^{-9}$, respectively.

5. Oxidation with potassium permanganate showed that acetopyruvic acid is attacked in two ways depending on the reaction conditions.

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[CONTRIBUTION FROM THE MORLEY CHEMICAL LABORATORY OF WESTERN RESERVE UNIVERSITY]

The Study of the Action of an Aluminum–Aluminum Chloride Catalyst in Friedel–Crafts Reactions. Benzoylation

BY OLIVER GRUMMITT AND E. N. CASE[1]

It is now well known that highly purified aluminum chloride is catalytically inactive in the cracking and isomerizing of paraffin hydrocarbons.[2] Promoters for these reactions include a hydrogen halide or a substance such as water or an alkyl halide which is capable of forming the halide.[2] The powerful promoting action of a hydrogen halide in these reactions has suggested the possibility of moderating the catalytic activity of aluminum chloride by adding a substance which will react with the hydrogen halide. Hall and Nash[3] in studying the polymerization of ethylene by aluminum chloride found that added magnesium or aluminum inhibited cracking and retarded the formation of a catalyst–hydrocarbon complex without hindering the polymerization reactions. It was suggested that the metal reacted with any hydrogen chloride formed during the reaction and thus reduced the activity of the catalyst. Further evidence of the reduced activity of an aluminum–aluminum chloride catalyst was obtained by Smith,[4] who observed that the presence of aluminum inhibited the cracking of *n*-heptane by aluminum chloride at 98° almost completely.

In order to investigate more fully the nature of the action of aluminum in reactions catalyzed by aluminum chloride and possibly to improve certain of those reactions, a study of this catalyst combination in several typical Friedel–Crafts reactions has been undertaken. The present report covers the benzoylation of benzene by benzoyl chloride to form benzophenone.

For purposes of comparison the benzophenone synthesis was carried out in the usual way with a mole ratio of benzene: benzoyl chloride: aluminum chloride of 1.0/0.55/0.57. In carbon disulfide as a solvent, the average yield of benzophenone was 62%. Carefully duplicated experiments

(1) Sherwin-Williams Research Fellow in Organic Chemistry, Western Reserve University.

(2) Ipatieff and Grosse, *Ind. Eng. Chem.*, **28**, 461 (1936), and subsequent papers.

(3) Hall and Nash, *J. Inst. Petroleum Tech.*, **23**, 679 (1939).

(4) A. Smith, M.A. Thesis, Western Reserve University, 1939.