

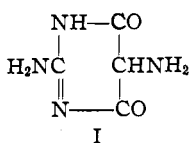
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]  
**RESEARCHES ON PYRIMIDINES. CXXVII.<sup>1</sup> THE STRUCTURE  
 OF CONVICINE<sup>2</sup>**

BY HARRY J. FISHER AND TREAT B. JOHNSON

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The fact that the nucleic acids on hydrolysis yield purine and pyrimidine nucleosides lends great interest to the occurrence in nature of simple pyrimidine glucosides. The only known compounds of this type are the two substances vicine and convicine, isolated by Ritthausen<sup>3</sup> from vetch some sixty years ago.



After various investigations by Ritthausen and others<sup>4</sup> it was finally established by Levene<sup>5</sup> that vicine was a glucoside of 2,5-diamino-4,6-dioxypyrimidine I, with the sugar probably attached to the nitrogen of the pyrimidine ring. As Hérissé and Cheymol<sup>6</sup> have recently shown that vicine is hydrolyzed by emulsin, it must be classed as a  $\beta$ -glucoside.

Convicine, which occurs with vicine in vetch, has not, previous to the present study, been isolated by any other investigator than Ritthausen. He showed that it possessed the empirical formula  $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_8 \cdot \text{H}_2\text{O}$ , was distinguished from vicine by not being readily soluble in dilute acids, and on hydrolysis yielded alloxantin and ammonia and a mother liquor which was strongly dextrorotatory and "reacted like sugar solutions to known sugar reagents." Schulze and Trier<sup>4</sup> in 1910 proposed a formula corresponding to alloxantin diglucoside plus two molecules of ammonia attached in some unknown manner. Johnson<sup>4</sup> later pointed out that the formula  $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_8$  corresponded exactly to that of either an amino-glucoside of dialuric acid or a glucoside of uramil.

<sup>1</sup> A report of this research was presented at the Organic Chemistry Symposium held in the Sterling Chemistry Laboratory of Yale University, New Haven, Connecticut, on December 28, 29 and 30, 1931.

<sup>2</sup> This paper is from a portion of a dissertation presented by Harry Johnstone Fisher to the Graduate School of Yale University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1931.

<sup>3</sup> Ritthausen and Kreuzler, *J. prakt. Chem.*, **2**, 333 (1870); Ritthausen, "Die Eiweisskörper der Getreidearten," Bonn, 1872, pp. 168-169; *J. prakt. Chem.*, **7**, 374 (1873); *Ber.*, **9**, 301 (1876); *J. prakt. Chem.*, **24**, 202 (1881); *ibid.*, **29**, 359 (1884); Schulze, *Z. physiol. Chem.*, **15**, 140 (1891); *Ber.*, **22**, 1827 (1891); *Z. physiol. Chem.*, **17**, 193 (1893); Ritthausen, *Ber.*, **29**, 894, 2108 (1896); von Lippmann, *ibid.*, **29**, 2653 (1896); Ritthausen, *J. prakt. Chem.*, **59**, 480, 482 (1899); Ritthausen and Preuss, *ibid.*, **59**, 487 (1899); Winterstein, *Z. physiol. Chem.*, **105**, 258 (1919).

<sup>4</sup> Schulze and Trier, *Z. physiol. Chem.*, **70**, 150 (1910-11); Johnson, *THIS JOURNAL*, **36**, 337 (1914); Johnson and Johns, *ibid.*, **36**, 545 (1914); Fischer, *Ber.*, **47**, 2611 (1914).

<sup>5</sup> Levene, *J. Biol. Chem.*, **18**, 305 (1914); Levene and Senior, *ibid.*, **25**, 607 (1916).

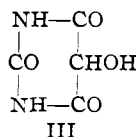
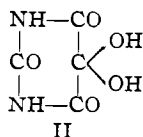
<sup>6</sup> Hérissé and Cheymol, *Compt. rend.*, **191**, 387 (1930); *Bull. soc. chim. biol.*, **13**, 29 (1931).

In an attempt to settle the question of the structure of convicine, the present authors have prepared a sample of this compound from broad beans (*vicia faba*), and have studied its products of hydrolysis and its behavior toward certain reagents.

About nine grams of convicine and a small amount of vicine were isolated from the beans. Our convicine was obtained in the form of perfectly colorless glistening, thin flat plates, decomposing without melting at 287°, and analyzing for the formula  $C_{10}H_{15}N_3O_8 \cdot H_2O$  found by Ritthausen. It gave a positive murexide test, and a positive Molisch test for carbohydrate. The Wheeler-Johnson test<sup>7</sup> and the color test with ammonia and phosphotungstic acid proposed by Johnson and Johns<sup>8</sup> as a test for the presence of a free amino group in the 5-positions of the pyrimidine cycle, were both negative. Diazobenzene sulfonic acid gave a deep orange-red color with both convicine and vicine. This red color was not found by Johnson and Clapp<sup>9</sup> to be given by pyrimidines which were substituted in the 3-position of the ring. The reaction of convicine with nitrous acid in the Van Slyke apparatus indicated the presence of a free amino group.

The convicine on hydrolysis with sulfuric acid yielded alloxantin (equal to 21.82% of the sample) and one molecule of ammonia. The presence of dextrose in the hydrolysis product was indicated by the formation of glucosazone. Tests for ketoses and pentoses were negative.

If convicine is a monoglucoside, the formula of the nitrogenous base must be  $C_4H_5N_3O_3$ . The base cannot be alloxantin itself, but must yield alloxantin and ammonia on acid hydrolysis. It is well known<sup>10</sup> that alloxantin in water solution is largely dissociated into alloxan II and dialuric acid III. Any base of the formula  $C_4H_5N_3O_3$  which would readily lose one nitrogen atom as ammonia to give either alloxan or dialuric acid would satisfy the structural requirements of the convicine base, as, since there is an oxidation-reduction equilibrium between alloxan and dialuric acid, and since alloxantin is more insoluble than either of these compounds, if either alloxan or dialuric acid were formed by acid hydrolysis of convicine, alloxantin would crystallize from the solution.



<sup>7</sup> Wheeler and Johnson, *J. Biol. Chem.*, **3**, 183 (1907).

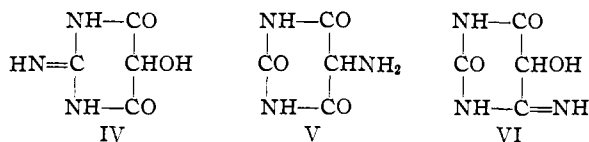
<sup>8</sup> Johnson and Johns, *THIS JOURNAL*, **36**, 972 (1914).

<sup>9</sup> Johnson and Clapp, *J. Biol. Chem.*, **5**, 163 (1908-9).

<sup>10</sup> Biilmann and Bentzon, *Ber.*, **51**, 522 (1918); Biilmann and Lund, *Ann. chim.*, **19**, 137 (1923); Biilmann and Mygind, *Bull. soc. chim.*, **47**, 532 (1930).

Derivatives of alloxan are excluded from consideration by the fact that they would contain one too many oxygen atoms.

Three possible derivatives of dialuric acid suggest themselves:

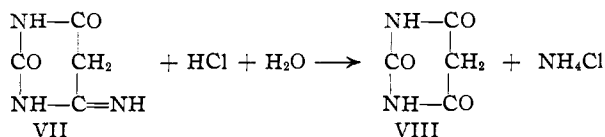


Formula IV is that of 2-iminodialuric acid, V is uramil and VI is 4-iminodialuric acid.

Levene<sup>5</sup> showed that divicine I was oxidized by potassium chlorate and hydrochloric acid with rupture of the pyrimidine ring to yield guanidine. We oxidized some of our convicine in the same manner, and from the reaction product urea was isolated, but no guanidine. This indicates that the convicine base does not contain a 2-imino group, and consequently does not possess the structure IV. There is further no evidence in the literature that a 2-imino group is split off from pyrimidines by dilute acids as ammonia.

If the convicine base possessed the structure of uramil, the 5-amino group should condense with cyanic acid to form a pseudouric acid derivative. Baeyer<sup>11</sup> showed long ago that this reaction did take place with uramil, and Levene<sup>5</sup> in like manner prepared 2-iminopseudouric acid from divicine. We found it impossible to cause any reaction to take place between convicine and potassium cyanate. From this positive evidence, and for the negative reason that there is no evidence in the literature that a 5-amino group is easily lost from a pyrimidine,<sup>12</sup> we believe that the convicine base cannot be uramil, V.

For the third possibility for the structure of the convicine base, that of 4-iminodialuric acid VI, there is strong evidence. While this compound has never been synthesized, its reduced form VII has been prepared by Traube<sup>13</sup> and by Conrad.<sup>14</sup> It is very significant that Conrad found that VII on warming with dilute acids was converted quantitatively to barbituric acid VIII and ammonia



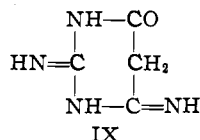
<sup>11</sup> Baeyer, *Ann.*, **127**, 3 (1863).

<sup>12</sup> The statement of Beilstein [3d ed., *Ergänzungsband I*, p. 767] that 1,3-dimethyluramil is converted into ammonia and tetramethylalloxantin on boiling with hydrochloric acid is evidently due to a misreading of a paper by Fischer and Ach, *Ber.*, **28**, 2475 (1895).

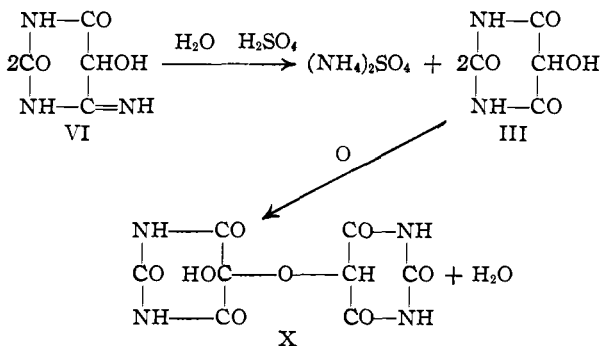
<sup>13</sup> Traube, *Ber.*, **33**, 1381, 3043 (1900).

<sup>14</sup> Conrad, *Ann.*, **340**, 310 (1905).

Of particular interest in connection with the relative stability of amino groups in the 2- and 4-positions of the pyrimidine cycle is Conrad's finding that the compound IX was hydrolyzed by dilute acids to 2-iminobarbituric acid and ammonia.

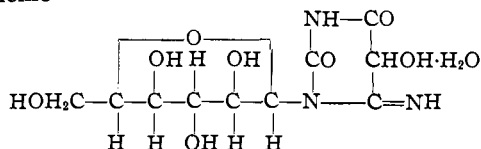


If the imino group in position 4 is so unstable in the barbituric acid derivatives, it is not to be anticipated that it would be any more stable in a dialuric acid derivative such as VI. The quantitative formation of one molecule of ammonia on hydrolysis of convicine with acids, and the presence of alloxantin in the hydrolysis product, are then very simply explained as being due to the hydrolysis of VI to dialuric acid III, which is readily oxidized to alloxantin X



For these reasons the authors believe that the properties of convicine can best be explained by assigning to the pyrimidine nucleus in convicine the structure VI. While the position of attachment of the sugar to this pyrimidine is in doubt, from analogy with vicine and the nucleosides of nucleic acid, and because the Wheeler-Johnson test is negative, the attachment of the sugar to a nitrogen of the pyrimidine ring seems most probable. It is true that convicine is not so highly resistant to acid hydrolysis as are the natural pyrimidine ribosides, which are nitrogen-linked, but the resistance to hydrolysis of the latter is apparently associated with the presence of a 4,5-double bond, and disappears on reduction of this double bond.

The following structure is therefore proposed for the naturally occurring glucoside convicine



Work is now in progress in this Laboratory on methods of synthesizing both vicine and convicine.

The authors wish to express their thanks to Doctors Bailey and Vickery of the Connecticut Agricultural Experiment Station for extending the facilities of their laboratories for part of this investigation, and to Professor W. E. Ford of the Department of Mineralogy, Yale University, for the crystallographic examination of alloxantin.

### Experimental Part

**The Isolation of Convicine.**—Five kilograms of finely powdered broad beans was mixed with 10 liters of 3% sulfuric acid and allowed to stand overnight. The next day the mixture was neutralized with warm 20% barium hydroxide solution and made into a thick paste with filter paper clippings. This paste was squeezed under a hydraulic press, the turbid filtrate made barely acid with sulfuric acid and filtered again on a Buchner funnel through paper pulp. The clear yellow filtrate was treated with an excess of a 10% solution of mercuric sulfate in dilute sulfuric acid, and made alkaline with barium hydroxide solution. The heavy precipitate which came down was allowed to settle, and most of the supernatant liquid was siphoned off. The precipitate was separated from the balance of the liquid and washed with water in a centrifuge. It was then suspended in water with some barium carbonate and the mixture saturated with hydrogen sulfide. The mercuric sulfide was filtered off, and the filtrate concentrated at atmospheric pressure to a volume of 400 cc. The dark red solution was cooled, made just acid to litmus with sulfuric acid, and mixed with 1600 cc. of 95% alcohol. The precipitate which came down was filtered off and the light yellow filtrate was further concentrated *in vacuo* to incipient crystallization. The residue was transferred to a crystallizing dish and placed in a desiccator over sulfuric acid. On standing overnight it usually solidified. It was treated with a little water and the nearly white convicine filtered off. Recrystallization from about 100 parts of boiling water with the addition of a little norite yielded glistening, perfectly white leaflets, decomposing without melting at 287°.

Re-concentration of the mother liquor yielded further amounts of less pure convicine; total yield from 5 kg. of broad beans, 2.32–2.35 g. of crude convicine.

For analysis, the convicine was crystallized four times from boiling water and dried to constant weight at 100°.

*Anal.* Calcd. for  $C_{10}H_{13}N_3O_8 \cdot H_2O$ : C, 37.14; H, 5.27; N, 13.01. Found: C, 38.20; H, 5.37; N, 13.08.

Amino nitrogen determinations were run in the micro Van Slyke apparatus on samples of convicine dissolved in half-normal sodium hydroxide: convicine, 0.0100 g., 0.0100 g.:  $N_2$ , 0.83, 0.89 cc. at temperature, 30°. Amino N found: 4.5, 4.8.

Convicine is soluble to the extent of about one per cent. in boiling water. It is readily soluble in normal sodium hydroxide and insoluble in chloroform and glacial acetic acid. Its cold saturated aqueous solution does not give a precipitate with potassium mercuric iodide, aqueous iodine–potassium iodide or saturated mercuric chloride, but does give a white precipitate with acid mercuric nitrate solution. Its aqueous solution boiled for two minutes with Fehling's solution shows no copper reduction.

A small amount of vicine was isolated by fractional crystallization of the material obtained by concentration of the mother liquor of the first crop of convicine. It was identified by its melting point (241–242°)<sup>15</sup> and by analysis.

*Anal.* Calcd. for  $C_{10}H_{16}N_4O_7 \cdot H_2O$ : N, 17.39. Found: N, 17.72.

**Hydrolysis with Sulfuric Acid.**—2.000 g. of well-crystallized convicine and 40 cc. of

<sup>15</sup> Levene found 242°; Winterstein, 239–242°.

six normal sulfuric acid were heated for six minutes in a boiling water-bath in a closed flask connected with a hydrogen generator. The convicine dissolved. The mixture was allowed to stand under hydrogen for four days. On one day's standing, a few crystals could be seen around the edges of the liquid, and at the end of the fourth day a considerable quantity of quite large and well-formed alloxantin crystals had separated. The flask was cooled in ice water, opened and the contents filtered in a current of carbon dioxide through a filter paper into a 200-cc. volumetric flask. The residue on the paper was washed with ice water, the washings being collected in the 200-cc. flask. The crystals were then dried in a vacuum desiccator over sulfuric acid at room temperature. They weighed 0.4363 g., or 21.82% of the convicine taken.

**Identification of Alloxantin.**—The crystals turned violet-pink with ammonium hydroxide solution, and this solution turned deep blue on adding a little ferric chloride.

A crystallographic comparison with an authentic sample of alloxantin by Prof. W. E. Ford showed that the two were identical in crystal habit and refractive indices, and gave the same interference figure.

The crystals were recrystallized from boiling water in an atmosphere of carbon dioxide, dried and analyzed.

*Anal.* Calcd. for  $C_8H_6N_4O_8 \cdot 2H_2O$ : N, 17.40. Found: N, 17.62.

**Determination of Ammonia Formed.**—The contents of the 200-cc. volumetric flask were made to volume, and a 10-cc. aliquot was diluted, an excess of magnesium oxide and a small piece of paraffin added, and distilled into standard tenth-normal hydrochloric acid. The distillate was titrated with standard tenth-normal sodium hydroxide. Ammonia N found from 0.1000 g. of convicine, 0.00398 g., or 3.98%; per cent. total N in convicine,  $C_{10}H_{18}N_8O_8 \cdot H_2O$ , 13.00. The ammonia N is then 30.6% of the total N.

**Identification of Dextrose.**—The balance of the solution, not used for the ammonia determination, was treated with an excess of lead carbonate, filtered, and the residue washed with cold water. The filtrate and washings were saturated with hydrogen sulfide and the lead sulfide filtered and washed with water. The filtrate and washings from the lead sulfide were concentrated *in vacuo* on the water-bath to a small volume, filtered again and the concentration continued at room temperature in a vacuum desiccator. The solution, which was straw-colored until almost dry, turned to a deep red thick sirup.

This sirup was extracted ten times with boiling 95% alcohol, and the alcoholic extracts evaporated to dryness at room temperature in a vacuum desiccator. The residue was a clear scarlet sirup.

This sirup was dissolved in a little water, 1 cc. of 10% mercuric sulfate solution was added and the orange-pink precipitate was filtered and washed with water. The light yellow filtrate was made alkaline with barium hydroxide solution, saturated with carbon dioxide, filtered and the precipitate washed.

The clear pale straw-colored filtrate was evaporated in a vacuum desiccator over sulfuric acid at room temperature to constant weight; wt. of brownish-yellow sirup, 1.1035 g.

The sirup was dissolved in 10 cc. of water and a 2-cc. portion was heated with 0.2 g. of phenylhydrazine hydrochloride and 0.3 g. of sodium acetate in a boiling water-bath. In a few minutes the osazone began to crystallize out. After heating for an hour and a half, the reaction mixture was cooled and the osazone filtered and washed with water. Recrystallized once from 1:1 alcohol-acetone and once from 50% alcohol, it melted at 207–208°, and had the characteristic crystalline appearance of glucosazone. A mixed melting point with a known sample of glucosazone showed no depression. The weight of twice recrystallized material was 0.0506 g.

Portions of the sirup gave no color when heated with hydrochloric acid and resorcin and no violet-red color with hydrochloric acid and phloroglucinol.

The balance of the sirup was treated with nitric acid by the technique of van der Haar<sup>16</sup> for the isolation of potassium acid saccharate. A few crystals separated, but on recrystallizing till colorless their weight was only 2.8 mg.

**Oxidation with Potassium Chlorate.**—0.2000 g. of pure recrystallized convicine was mixed with 1.5 cc. of 1:1 hydrochloric acid, and 0.04 g. of potassium chlorate added in portions. The mixture was agitated from time to time. The convicine gradually dissolved. On standing overnight, the solution still smelled of chlorine. It was concentrated *in vacuo* at room temperature over stick potassium hydroxide to dryness. The residue was taken up in a little alcohol, filtered and the filtrate evaporated to dryness at room temperature *in vacuo*. The residue was taken up in a little water, made alkaline with sodium hydroxide and sodium picrate solution added. There was an odor of ammonia, but no trace of a precipitate.

The solution was diluted, acidified with hydrochloric acid and extracted with ether until there was no more color in the ether layer. The aqueous solution was then evaporated to dryness at room temperature over stick potassium hydroxide, taken up in 1 cc. of water, 3.5 cc. glacial acetic acid and 0.5 cc. of a 10% methyl alcoholic solution of xanthidrol added. A precipitate came down which under the microscope consisted of small needles clustered together at the ends. It was filtered, washed with alcohol and dried *in vacuo*.

*Anal.* Calcd. for dixanthylurea,  $C_{27}H_{20}N_2O_8$ : N, 6.67. Found: N, 6.88.

**Reaction with Potassium Cyanate.**<sup>17</sup>—0.526 g. of convicine was warmed to about 70° with a mixture of 15 cc. of glacial acetic acid and 5 cc. of absolute alcohol. It did not appear to dissolve. One gram of potassium cyanate was added in portions from time to time. There was no odor of hydrocyanic acid. The mixture was digested on the water-bath for two and one-half hours, the temperature rising to 79°. It was then allowed to stand overnight. The next day the insoluble matter was filtered off, washed with absolute alcohol and dried in a vacuum desiccator. It weighed 0.450 g., or 85.6% of the convicine taken. An analysis showed that it was unchanged convicine.

*Anal.* Calcd. for  $C_{10}H_{15}N_3O_8 \cdot H_2O$ : N, 13.00. N found in this sample of convicine, 12.31. N found in reaction product, 12.86.

### Summary

1. The presence of convicine in broad beans (*vicia faba*), reported by Ritthausen, has been confirmed.
2. On acid hydrolysis convicine yields alloxantin, one molecule of ammonia and glucose which gives its corresponding glucosazone by interaction with phenylhydrazine.
3. Potassium chlorate oxidizes convicine with the formation of urea. No guanidine is formed.
4. Convicine did not react with potassium cyanate to form a urea derivative.

<sup>16</sup> A. W. van der Haar, "Anleitung zum Nachweis, etc., der Monosaccharide und Aldehydsäuren," Gebrüder Borntraeger, Berlin, 1920, p. 100.

<sup>17</sup> Bailey and Snyder, *THIS JOURNAL*, **37**, 935 (1915); Bailey and Read, *ibid.*, **37**, 1884 (1915); Bailey and Mikeska, *ibid.*, **38**, 1771 (1916); Bailey and Moore, *ibid.*, **39**, 279 (1917).

5. A structure for convicine is proposed. It is represented as a hexoside of 4-iminodialuric acid.

6. This research is being continued.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS AND THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

## TRIPHENYLVINYLMAGNESIUM BROMIDE

By C. FREDERICK KOELSCH<sup>1</sup>

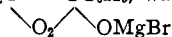
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During the course of an investigation on the unusual reactivity of the halogen in benzoyldiphenylmethyl bromide, a need was felt for a method by which substituted derivatives of the parent diphenylacetophenone could be prepared. It was thought that the enol forms of such ketones might be obtained by a reaction analogous to that sometimes used for the preparation of phenols, namely, by the oxidation of a properly chosen Grignard reagent. Such a Grignard reagent would have the  $\text{—MgX}$  group attached to an unsaturated carbon atom and would be of the type  $\text{RR}'\text{C}=\text{C}(\text{MgX})\text{C}_6\text{H}_5$ , where R and R' are aromatic radicals.

A compound of this type has not been prepared previously. The most closely related Grignard reagents are styrylmagnesium bromide<sup>2</sup> and  $\beta,\beta$ -diphenylvinylmagnesium bromide.<sup>3</sup> Whether or not the more highly substituted triphenylvinylmagnesium bromide could be obtained was a question which had to be settled by experiment. In the present paper it is shown that triphenylvinyl bromide forms a Grignard reagent, and some reactions of this compound are described.

A number of so-called negative results were obtained in the study of this Grignard reagent. With *dry air* or *oxygen*, a yellow ether-insoluble substance was formed, but decomposition of this with dilute acids gave a tarry oil from which no diphenylacetophenone could be obtained; on steam distillation of this tar no trace of benzophenone, which would be formed from a possible peroxide,  $(\text{C}_6\text{H}_5)_2\text{C}—\text{C}_6\text{H}_5$ , was obtained.



*Thionyl chloride* reacted vigorously to give an unworkable oil, while *benzyl chloride* was recovered nearly quantitatively after six hours' refluxing in ether; *triphenylchloromethane* was rapidly and completely converted into triphenylmethyl, isolated as the peroxide; the other product was a yellow ether-soluble glassy substance from which no crystalline material could be obtained. *Acetone* was apparently condensed by the reagent with the formation of mesityl oxide and triphenylethylene. *Benzophenone* gave a deep red solution, but carbonation followed by hydrolysis showed that the whole of the Grignard reagent was still present, and the ketone was recovered unchanged.

<sup>1</sup> National Research Fellow in Chemistry.

<sup>2</sup> Rupe and Proske, *Ber.*, **43**, 1231 (1910).

<sup>3</sup> Lipp, *ibid.*, **56**, 571 (1923); Ziegler, *ibid.*, **55**, 2257 (1922); Ziegler and co-workers, *Ann.*, **443**, 161 (1925); and previous papers.