Precautions.

Potassium persulfate often contains ammonium salts and retains traces even after 3 recrystallizations. In such a case it is necessary to run a blank and apply a correction for the amount used. In running this blank sugar free from nitrogen must be added to reduce most of the persulfate; otherwise some of the ammonium will be oxidized.

It is very essential that there be no water present during the oxidation of the organic matter by the persulfate, since in the presence of water some of the ammonia is oxidized.

A very large excess of persulfate gives low results.

A number of organic substances were analyzed by this method, the results being shown in the preceding table. Some of these substances which were not absolutely pure or of uncertain composition, were analyzed by the official Kjeldahl method (Expts. 12 to 37), and the results are compared. Ten cc. of approximately 0.6 N hypobromite solution was added in nearly every case. The thiosulfate was standardized against pure potassium dichromate.

Some preliminary experiments indicate that this method can be used for the determination of nitrogen in coal, but this work has not been completed.

Summary.

1. The Kjeldahl method of determining nitrogen can be made more rapid by adding potassium persulfate to the charred solution of organic matter in conc. sulfuric acid, complete oxidation occurring very quickly.

2. The ammonium salt formed may be accurately and quickly determined by adding to the neutralized solution an excess of standard hypobromite, and determining the excess by adding iodide and acid, and titrating the liberated iodine with thiosulfate.

ANN ARBOR, MICHIGAN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA.]

OXIDATION OF THE GRIGNARD REAGENT.

BY C. W. PORTER AND CAROLYN STEEL.

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If the general reactions of organic chemistry were named in the order of their importance from the standpoint of synthetic work the Grignard reaction would be one of the first on the list. Although only 20 years have passed since Grignard's discovery was announced there have appeared more than 500 papers devoted to applications of this reaction.

The formation of phenols through the oxidation of aryl magnesium halides mentioned by Bodroux[1] and Wuyts[2] seemed to us worthy of fur-

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[1] Bodroux, *Bull. soc. chim.*, [3] **31**, 33 (1904).

[2] Wuyts, *Compt. rend.*, **148**, 930 (1909).

ther investigation, and the work reported in this paper was undertaken to determine the influence of temperature upon the yield of phenol and the yields of the principal by-products, and to secure some evidence concerning the mechanism of the reaction. Bodroux obtained yields of phenol varying between 10 and 20%, and Wuyts identified diphenyl, diphenyl ether, and diphenyl-benzene among the oxidation products of phenylmagnesium bromide.

Ether solutions of the Grignard reagent prepared from magnesium turnings, phenyl bromide and ether were divided into several equal portions; and these aliquot parts of the same preparation were oxidized at different temperatures simultaneously. Each portion of the ether solution was placed in a tall glass cylinder in a thermostat and oxygen freed from carbon dioxide, dried, and finally saturated with ether vapor at the temperature of the thermostat, was bubbled through the solution for 5 hours. Preliminary experiments indicated that no increase in yield of phenol was secured by continuing the process longer than 5 hours. A thermometer was placed in the solution and a partial control of the temperature was accomplished by regulating the rate of flow of the oxygen. During the first 30 minutes of each run it was necessary to keep the liquid surrounding the cylinder below the temperature required in the reaction mixture. As the heat of reaction diminished the temperature of the bath was gradually increased. By this means the temperature of the solution was kept fairly constant, never deviating more than one degree from the mean. The solution acquired a greenish-yellow color as soon as the reaction began, and at the lower temperatures it gradually changed to an orange-red.

After hydrolyzing the oxidation product with ice and dil. hydrochloric acid the mixture was analyzed. Diphenyl was isolated by distillation with steam, then dried and weighed. The yield of diphenyl was practically constant and was as high in solutions that were not oxidized at all as in solutions through which oxygen had been passed for several hours. This indicates clearly that the formation of diphenyl is due exclusively to the direct action of magnesium on the phenyl bromide (Fittig's synthesis).

Phenol was estimated in the distillate and in the residue by titration. Measured volumes of standard potassium bromate and bromide were added to the acidified phenol solutions to precipitate tribromphenol. After adding an excess of potassium iodide the mixture was refluxed at 50° for 15 minutes, then titrated with sodium thiosulfate. As indicated in the following tables the optimum temperature for the formation of phenol is 0°. The calculations are based upon the yields that would have been obtained if the Grignard reagent used had been quantitatively converted into phenol or into diphenyl.

TABLE I.—OXIDATION RESULTS.

	Temp. 7° to 9°.		Temp. 0°.	
	C_6H_5OH. %	$C_6H_5C_6H_5$. %	C_6H_5OH. %	$C_6H_5C_6H_5$. %
	19.7	9.5	24.0	....
	18.9	11.4	24.3	11.7
	19.8	7.5	....	11.3
	20.8	10.1	23.0	11.1
	20.2	9.9	21.4	9.5
	19.4	9.3	22.9	10.9
	....	....	21.8	11.3
Average,	19.8	9.6	22.9	10.9

	Temp. 20°.		Temp. 32°.	
	C_6H_5OH. %	$C_6H_5C_6H_5$. %	C_6H_5OH. %	$C_6H_5C_6H_5$. %
	16.6	12.7	16.6	10.5
	19.1	10.7	17.1	12.2
	20.3	8.6	16.8	10.6
	19.0	10.5	18.0	9.4
	20.1	10.6	17.0	10.9
Average,	19.1	10.6	16.8	10.7

As ordinarily prepared the Grignard reagent is protected from atmospheric oxygen only by ether vapor in the reflux condenser. That such protection is inadequate is shown by the presence of phenol in freshly prepared samples of the reagent. Five preparations of phenyl-magnesium bromide through which oxygen had not been passed gave the following yields:

C_6H_5OH. %	$C_6H_5C_6H_5$ %
3.0	8.4
2.4	10.1
3.2	10.9
2.0	9.3
3.4	10.7
2.8	9.9

When evaporated or distilled with steam the yellow ether extract of the hydrolyzed product yields a tar. This was isolated and distilled under reduced pressure. The major part of the distillate that boiled between 230° and 258°, at 3 mm. pressure crystallized in colorless needles consisting of *p*-diphenyl-benzene, $C_6H_5C_6H_4C_6H_5$ (m. p. 205°). The quantity of the tar varied between $^1/_{10}$ and $^4/_{10}$ of the weight of the phenyl groups in the reagent. From this tar was isolated also, quinone and *p*-*p'*-dihydroxy-diphenyl in quantities sufficient to enable us to identify them by melting points and qualitative tests.

It is a generally accepted principle that in the process of oxidation by air or gaseous oxygen at ordinary temperatures a peroxide is the first product. A molecule rather than a single atom of oxygen takes part in the reaction. The peroxide may be stable or it may have only a transitory existence. If it constitutes only an unstable intermediate product half of the oxygen is liberated in the so-called active or nascent state. Now, the formation of quinone in the oxidation of phenyl-magnesium bromide indicated the presence of active oxygen, for neither phenol nor phenyl bromide is oxidized to quinone by the air alone. Furthermore, when triphenyl-methyl-magnesium bromide is oxidized by air the stable, insoluble triphenyl-methyl peroxide is precipitated.[1] We assume therefore, that phenol is produced as indicated in the following equations:

$$C_6H_5MgBr + O_2 \longrightarrow C_6H_5OOMgBr. \qquad (1)$$

$$C_6H_5OOMgBr + C_6H_5MgBr \longrightarrow 2C_6H_5OMgBr. \qquad (2)$$

$$C_6H_5OMgBr + H_2O \longrightarrow C_6H_5OH + MgBrOH \qquad (3)$$

p-Dihydroxy-diphenyl and diphenyl ether are formed by a reaction between the products of Equations 1 and 2 and subsequent rearrangement or decomposition of the unstable peroxide.

$$C_6H_5OOMgBr + C_6H_5OMgBr \longrightarrow C_6H_5OOC_6H_5 + (MgBr)_2O \quad (4)$$

$$C_6H_5OOC_6H_5 \longrightarrow HOC_6H_4C_6H_4OH \qquad (5)$$

$$C_6H_5OOC_6H_5 \longrightarrow C_6H_5OC_6H_5 + O \qquad (6)$$

Quinone appears as an oxidation product of the peroxide by the atomic oxygen released as indicated in Equation 6.

Assuming that some phenyl peroxide was formed as indicated above and that Equations 5 and 6 represent relatively slow reactions we hydrolyzed 3 samples in the presence of zinc filings and hydrochloric acid to reduce the peroxide to phenol. The average yield of phenol in the 3 experiments was 24.7%. The average for unreduced runs at the same temperature was 22.9%. When sulfur instead of oxygen is used phenyl disulfide appears as one of the principal products. Taboury,[2] however, concluded that the disulfide is formed chiefly through oxidization of thiophenol, for when the reaction was carried out in an atmosphere of hydrogen less of the disulfide and more thiophenol appeared among the products formed.

Summary.

The optimum temperature for the production of phenol by oxidation of the Grignard reagent is about 0°. The production of diphenyl is nearly constant and is due to a side reaction in the formation of the Grignard reagent.

The presence of quinone and *p-p'*-dihydroxy-diphenyl among the oxida-

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[1] Schmidlin, *Ber.*, 39, 631, 4184 (1906).

[2] Taboury, *Bull. soc. chim.*, [3] 29, 761 (1903); *ibid.*, 31, 1183 (1904).

tion products, the increase in yield of phenol when hydrolyzed in the presence of a reducing agent and the precipitation of triphenyl-methyl peroxide when triphenyl-methyl-magnesium bromide is oxidized, suggests the formation of phenyl peroxide as an intermediate product.

BERKELEY, CALIF.

[CONTRIBUTION OF THE MULFORD BIOLOGICAL LABORATORIES.]

ANTIBODY STUDIES—PART 3. A PRELIMINARY REPORT ON THE CHEMICAL NATURE OF BACTERIAL ANTIBODIES.[1]

BY F. M. HUNTOON, P. MASUCCI, AND EDITH HANNUM.

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I. Introductory.

This paper deals merely with certain chemical facts concerning bacterial antibodies. A complete discussion of the antigen-antibody reaction will be published at a later date.

Immediately following the signing of the Armistice, a large supply of anti-pneumococcic serum became available. Experiments were undertaken to devise methods for extracting or isolating the protective antibodies from such serums as free from other serum constituents as possible.

The application of the principle of bacterial sensitization was utilized. Pneumococcus antigen variously treated was immersed in its specific serum and after allowing the antibodies to combine with the antigen or better, perhaps, after the antibodies were adsorbed by the antigen, the mixture was centrifuged. The sediment consisting of the highly sensitized bacteria was then treated with various reagents in order to split off the protective antibodies from the antigen. The resultant solution was candle filtered and its antibody content determined by protection tests on mice according to the U. S. Hygienic Lab. method for testing anti-pneumococcic serum.[2]

The filtered solution was almost as clear as water, very rarely showing any opal-escense. A typical proximate chemical analysis of such extracts as well as their content of antibodies shown by protection tests is given in Tables I and II, respectively.

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[1] Presented by P. Masucci before the Division of Biological Chemistry at the Chicago Meeting of the American Chemical Society, September 6–10, 1920.

[2] The protection test is carried out as follows. For each serum or extract tested, 3 white mice, approximately 18 g. in weight are injected with 0.2 cc. of serum, followed within less than 5 minutes by 0.1 cc. or less of culture. Both injections are made intraperitoneally. Similar injections are made at the same time with the Hygienic Laboratory Control Serum on 6 mice, 3 of which are injected at the beginning of the test, and 3 at the end.

The mice are observed for 96 hours, and mice living over 96 hours are taken as survivals (S). Those dying before 96 hours are marked with the number of hours they lived. (See Table II.)