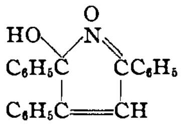
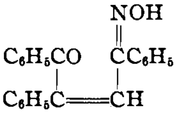
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HOWARD UNIVERSITY]

# Hydroxy Pyrrole Nitrones. II

BY A. H. BLATT

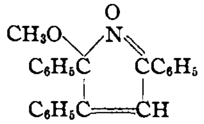
In a recent article[1] we presented the evidence which established the structure of the hydroxy pyrrole nitrone (I) formed by the interaction of hydroxylamine hydrochloride and *cis*-phenyldibenzoylethylene. In this article we present some new facts concerning the ring–chain isomerism exhibited by the nitrone and the oxime (II) together with a more complete description of the chemical behavior of these two substances.

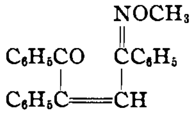

I


II

In acid media the stable isomer is the hydroxy nitrone; in alkaline solutions the oxime is stable. However, we have found that rapid conversion of the oxime to the nitrone is effected only by the halogen acids. As a consequence it is now possible to prepare either isomer at will by appropriate acidification of the sodium derivative of the oxime: the use of acetic acid furnishes the oxime while hydrochloric acid furnishes the nitrone. The principal physical differences between the isomers are color and solubility, the nitrone being more deeply colored and less soluble than the oxime. The only chemical reaction we have found which will differentiate between the isomers is that with acetic anhydride—a reaction described in detail later.

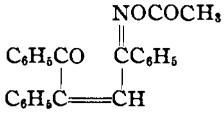
Along with the conversion of the oxime to the nitrone by acids should be considered the formation of derivatives of the nitrone by alkylation of the oxime in alkaline solution. The alkylation of the nitrone itself in acid solution was described in the first paper.[1] When the oxime is treated with methyl iodide in sodium methylate solution, the only product which can be isolated is the nitrone ether (III). If the oxime in sodium hydroxide is treated with methyl sulfate, there is formed, in addition to the cyclic ether (III), varying amounts of the oxime ether (IV). The relative proportions in which these ethers are formed are determined by factors which are not completely established but we have found that high concentration of alkali and low temperature favor the formation of the oxime ether.

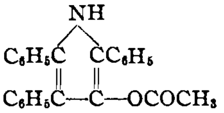

III

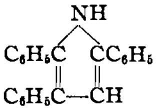

IV

Although several interpretations are possible for the facts just presented about the conversion of the oxime to the nitrone and the formation of the nitrone ether on alkylation of the oxime, none of the interpretations has anything decisive in its favor and we prefer to treat these facts, for the present at least, purely descriptively. A different situation exists with respect to the much more complex reactions which take place between the oxime and nitrone and acetic anhydride and acetyl chloride, for here a satisfactory interpretation is available. Of these two reactions that with acetic anhydride is the more important for it provides a chemical method for differentiating between the isomers. Consequently we shall consider it first.

When the oxime is treated with acetic anhydride, it furnishes the acetate (V) which is not affected by further treatment with the reagent. The nitrone on treatment with acetic anhydride gives the diacetoxypyrrole (VI). The structure of this material is established by its stepwise reduction through the acetoxypyrrole (VII) to the known triphenylpyrrole (VIII)


V


VII


VIII

The transformation of the hydroxy nitrone to the diacetoxypyrrole (VI) is best accounted for by esterification of the hydroxyl group and addition of acetic anhydride to the nitrone system, followed by a 1,3 shift of the acetoxy group from the 2 to the 4 carbon atom of the ring, with a subsequent loss of acetic acid—the reaction series A.

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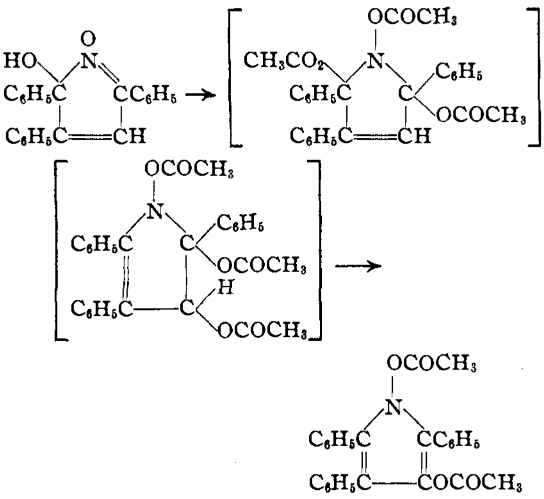
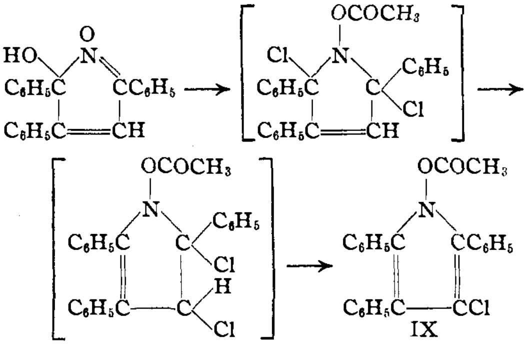
(1) Blatt, THIS JOURNAL, 56, 2774 (1934).



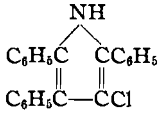
VI (A)

The various steps in this reaction are entirely consistent with the chemical behavior of the systems involved. Cyclic tertiary hydroxyl groups are esterified by acetic anhydride;[2] acetic anhydride adds to the nitrone system in the fashion indicated;[3] esters rearrange more readily than the alcohols from which they are derived;[4] while the allylic shift and elimination of two hydrogens or their equivalent from dihydroaromatic systems are too well known to require comment. In addition, however, to its consistency with known facts, the interpretation which we have given for the acetic anhydride reaction has the advantage that a perfectly parallel process enables one to account for the principal product formed in the reaction between the nitrone and acetyl chloride.

When the nitrone is treated with acetyl chloride three products are formed. Two of these products crystallize together, forming a constant melting mixture which is extremely difficult to separate and which was for some time erroneously regarded as a single substance. By suitable treatment, however, a separation can be effected and the principal reaction product thus isolated is 1-acetoxy-2,3,5-triphenyl-4-chloropyrrole (IX). The structure of this material is based upon its reduction, through the intermediate triphenylchloropyrrole (X), to triphenylpyrrole (VIII). The chloroacetoxypyrrole (IX) is the product to be expected if the reaction with acetyl chloride parallels the course outlined for the reaction with acetic anhydride: replacement of the hydroxyl group by chlorine and addition of acetyl chloride to the nitrone system, followed by a 1,3 shift of the chlorine atom from the 2 to the 4 carbon atom of the ring and, finally, loss of hydrogen chloride—the reaction series B.



IX (B)



X

The comments made about the various steps in the reaction series A apply equally well to those in series B so that it is necessary here only to remark that Ruggli[3] has shown that acetyl chloride adds to nitrones.

With the principal product of the acetyl chloride reaction disposed of we may consider the two secondary products. The first of these, which crystallizes with the pyrrole, is nitrogen free but contains oxygen and chlorine. It is triphenylchlorofuran (XI). The second, which contains the nitrogen eliminated in the formation of the furan, is diacethydroxamic acid (XII). We are not in a position to offer a satisfactory accounting for the formation of these two products. Certain facts, however, are indicative. The furan and hydroxamic acid are not formed from the pyrrole (IX) for that substance is not affected by treatment with acetyl chloride. They are formed when the open-chain oxime or its acetate is treated with acetyl chloride. In these last two reactions about the same proportions of pyrrole, furan and hydroxamic acid result as do from the nitrone itself. The most reasonable interpretation of these facts is that the pyrrole is formed from the nitrone while the furan and hydroxamic acid are formed from the open-chain acetate. This, however, involves the assumption of an equilibrium between the nitrone and an open-chain derivative on treatment with acetyl chloride and still leaves obscure the process whereby the furan and hydroxamic acid are formed from the open-chain acetate.

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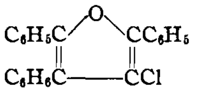
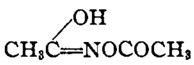
(2) Allen and Spanagel, THIS JOURNAL, **54**, 4338 (1932).

(3) Ruggli, Bolliger and Leonhardt, *Helv. Chim. Acta*, **6**, 594 (1923).

(4) Meerwein, *Ann.*, **453**, 16 (1927).



XI



$CH_3C(OH){=}NOCOCH_3$

XII

## Experimental

**Interconversion of the Nitrone (I) and the Oxime (II).**—The crude nitrone is dissolved in dilute sodium hydroxide solution (6.0 g. nitrone in 100 cc. of 5% sodium hydroxide) and filtered from insoluble material.[5] The filtrate on acidification with hydrochloric acid furnishes a pale yellow precipitate which rapidly turns deep yellow. This material is the hydroxy nitrone (I). Analyses of a sample washed with water and dried gave: C, 80.9; H, 5.35. Calcd. for $C_{22}H_{17}O_2N$: C, 80.7; H, 5.2. It melts with decomposition at about 180°, is very sparingly soluble in the common solvents and on treatment with acetic anhydride furnishes the diacetoxypyrrole (VI). The nitrone is also obtained when the alkaline solution of the oxime is acidified with hydrobromic acid. If, however, the sodium hydroxide solution is made just acid with acetic acid the precipitate formed is pale yellow and does not change color on standing. This pale yellow material is the oxime (II). It melts at about 180°—approximately the same temperature as the nitrone—and decomposes after it has melted. It is very soluble in the common solvents—so much so that if any considerable excess of acetic acid is used in the acidification of the sodium salt the oxime will redissolve—and on treatment with acetic anhydride forms the oxime acetate (V). The conversion of oxime to nitrone is best effected and illustrated by adding a drop of hydrochloric acid to the acetone solution of the oxime. Precipitation of the deep yellow nitrone follows.

The surprising stability of the oxime in the absence of halogen acids described in the preceding paragraph has led us to reëxamine the analytical samples which we had previously reported[1] as the hydroxy nitrone. On checking the behavior of these samples toward acetic anhydride we find that all of the recrystallized material is open-chain oxime; of the purified samples only those are hydroxy nitrone which have either been precipitated from alkaline solution by a halogen acid or which have been crystallized by the addition of hydrochloric acid to the solvent.

**Methylation of the Sodium Salt of the Oxime.**—This methylation can be carried out using either sodium methylate and methyl iodide or sodium hydroxide and methyl sulfate. Using the iodide the only alkylation product which can be isolated is the nitrone methyl ether, melting at 172°, which has already been described.[1] Using methyl sulfate there is also formed the oxime ether (IV). Thus to 6.5 g. of the oxime dissolved in 150 cc. of 5% sodium hydroxide and cooled to 10° is added during an hour 7.5 g. of methyl sulfate. The solution is stirred during the addition and for two hours longer, then left overnight. Next day it is filtered from the sticky precipitate. The filtrate on acidification furnishes a small amount of nitrone. The precipitate is dissolved in 15 cc. of hot alcohol, filtered and allowed to crystallize, taking generally two crops of crystals. The procedure at this point depends upon the proportions in which the isomeric methyl derivative are formed. If the crystals melt above 155°, they are recrystallized from alcohol and yield pure nitrone ether melting at 172°. If the crystals melt between 125–155°, they are leached with small portions of boiling ether which extracts the oxime ether and leaves undissolved the nitrone ether. If the crystals melt below 125°, they are crystallized from methyl alcohol and furnish pure oxime methyl ether melting at 121°. Usually one crop of crystals is largely nitrone ether and the other crop is largely oxime ether.

In a series of methylations carried out as closly as possible according to the description above, the proportion of the two methyl derivatives varied between such wide limits that in some runs the 172° nitrone ether predominated, while in other runs the 121° oxime ether predominated. Although from these variations it was evident that undetermined factors were influencing the course of the methylation, we were able to show that increase of temperature and decrease of alkali concentration suppressed practically completely the formation of oxime ether.

The pure oxime methyl ether (IV) is a pale yellow solid which melts at 121°. (*Anal.* Calcd. for $C_{23}H_{19}O_2N$: C, 80.9; H, 5.6; $OCH_3$, 9.1. Found: C, 81.0; H, 5.6; $OCH_3$, 8.5.) It is moderately soluble in methyl and ethyl alcohols and very soluble in ether, acetone and chloroform. Its structure is established by hydrolysis with boiling aqueous hydrochloric acid to phenyldibenzoylethylene.

**Reaction with Acetic Anhydride.**—When 6.5 g. of the nitrone was heated at 80° for 45 minutes with 20 cc. of acetic anhydride, the solid dissolved and a dark red solution resulted. This solution, after cooling and decomposing with water, furnished about 6.0 g. of crude product which was purified for analysis by crystallization from alcohol and from acetone and petroleum ether.

*Anal.* Calcd. for $C_{26}H_{21}O_4N$: C, 75.9; H, 5.1. Found: C, 75.9; H, 5.1.

**1,4-Diacetoxy-2,3,5-triphenylpyrrole** (VI) is a colorless solid which melts at 151°. It is moderately soluble in the common solvents. On exposure to light it develops a bright orange-yellow color. The formation of this color is reversible, for when the orange material is protected from the light the color disappears. The change from colorless to colored and its reversal can be made a number of times without changing the melting point of the material. Acetoxy groups in the substance can be shown by warming its alcoholic solution with a drop of hydrochloric acid, whereupon the odor of ethyl acetate develops.

**Reduction of the Diacetoxypyrrole (VI) to the Acetoxypyrrole (VII).**—When 5.0 g. of the diacetoxypyrrole in 100 cc. of hot acetic acid was heated on a water-bath for five hours with 10.0 g. of zinc dust and a crystal of copper sulfate then filtered, cooled and poured into water the acetoxypyrrole precipitated. The product was crystallized from acetic acid and water and 3.7 g. of pure material melting at 188° was obtained.

*Anal.* Calcd. for $C_{24}H_{19}O_2N$: C, 81.6; H, 5.4. Found: C, 81.3; H, 5.5.

**4-Acetoxy-2,3,5-triphenylpyrrole** (VII) is quite soluble in acetone and acetic acid, moderately soluble in alcohol. It can be distilled without decomposition at about 250° in an oil pump vacuum. The presence of an acetoxy group

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[5] In the preparation of the nitrone there is formed a small amount of a colorless, nitrogen-free product whose melting point is 204° and which shows on analysis: C, 85.4, and H, 5.7. No study has been made of this material.

in the material can be shown by the formation of ethyl acetate on heating the alcoholic solution of the pyrrole with hydrochloric acid. The acetyl group can be hydrolyzed but the product is most intractable. Consequently, in order to establish the structure of the material, it was necessary to reduce it with a minimum of hydrolysis.

**Reduction of the Acetoxypyrrole (VII) to Triphenylpyrrole (VIII).**—To a reducing mixture of 0.5 g. of iodine, 1.6 g. of red phosphorus, 0.5 cc. of water and 20 cc. of glacial acetic acid there was added 0.8 g. of the acetoxypyrrole. The mixture was boiled for ten and one-half hours, filtered from the phosphorus and, after cooling, extracted with ether and the ether extract washed with thiosulfate. After evaporation of the ether, the residue was taken up in alcohol and filtered on successive days from small deposits of hydrolysis product. Finally the concentrated alcoholic solution was vacuum distilled. The product boiled at 245° at 2 mm. and after crystallization from alcohol was shown by a mixed melting point to be 2,3,5-triphenylpyrrole.

**Reaction with Acetyl Chloride.**—Ten grams of the hydroxynitrone is covered with 20 cc. of acetyl chloride and the reaction mixture warmed on the steam-bath for thirty minutes, during which time the solid dissolves and hydrogen chloride is evolved. The orange-yellow solution is cooled and placed in a desiccator over sodium hydroxide sticks until the acetyl chloride is removed. At this point the product is an off-white solid mixed with a small amount of oil. It is filtered, using a sintered glass funnel, washed with acetic acid and the filtrate and washings are returned to the desiccator (see below).

The solid, which weighs 8.5–9.0 g., melts sharply to an opaque liquid at about 108°. On exposure to light it turns superficially a bright orange color. It can be recrystallized from a variety of solvents but the recrystallized material, like the crude product, melts at about 108° to an opaque liquid which clears sometimes at 119° and other times at about 130°. This material is a mixture of the pyrrole (IX) and the furan (XI) whose separation is a tedious process but which can best be effected by dissolving the crude product in chloroform (2 cc. per gram) and adding petroleum ether (13 cc. per gram). From this solution on careful cooling the pyrrole usually separates first in opaque hemispheres. If the crystallization is followed closely, it is possible to decant the liquid from the crystals of the pyrrole as soon as the stout transparent prisms of the furan begin to appear. The succeeding crystal deposits are mixtures of furan and pyrrole and must be recrystallized from the same solvent mixture. If the crystallization is sufficiently slow so that large crystals are formed, it is possible to make a partial purification by mechanical separation of the two kinds of crystals. Finally both products are crystallized from alcohol.

The higher melting reaction product, **1-acetoxy-2,3,5-triphenyl-4-chloropyrrole (IX)**, melts at 152°. (*Anal.* Calcd. for $C_{24}H_{18}O_2NCl$: C, 74.3; H, 4.65; N, 3.6. Found: C, 74.5; H, 4.6; N, 3.86.) It is moderately soluble in methyl and ethyl alcohols and acetic acid, very soluble in chloroform and insoluble in petroleum ether. On exposure to light it develops a bright orange color. The presence of halogen in the molecule is shown by qualitative tests. The pyrrole is unaffected by treatment with acetyl chloride.

The lower melting reaction product, **2,3,5-triphenyl-4-chlorofuran (XI)**, melts at 119°. Its identity as the furan is established by analysis (*Anal.* Calcd. for $C_{22}H_{15}OCl$: C, 79.9; H, 4.5; Cl, 10.7. Found: C, 79.8; H, 4.6; Cl, 11.1) and by comparison with an authentic specimen of the furan prepared according to the procedure of Japp and Burton.[6] Mixtures of the furan and pyrrole melt sharply at 108° but the melt is opaque and does not clear until a higher temperature.

The third product of the reaction is obtained by vacuum distillation of the oil remaining after the original filtrate and acetic acid washings (see above) have been kept in a desiccator over solid sodium hydroxide until the acetic acid has been removed. The first drops of distillate are liquid and have the odor of benzonitrile. Then, when the temperature has risen to about 130° at 8 mm., the distillate solidifies to a mass of colorless needles. These needles contain nitrogen, melt at 89–90° and their solution in water is acid to litmus. This aqueous solution gives no color on addition of ferric chloride but after standing for about an hour a deep red color develops. These are the properties of diacethydroxamic acid (XII) and a comparison of our product with a specimen of that substance prepared according to Hantzsch[7] established their identity.

Because of the losses in purification it is difficult to make a more accurate estimate of the amounts of pyrrole, furan and diacethydroxamic acid formed in the reaction between the nitrone and acetyl chloride than to say that the pyrrole is the principal product. However, since the hydroxamic acid is water soluble while the pyrrole is destroyed by treatment with alkali, it is possible to make a fairly close estimate of the amount of furan formed. This is done by dissolving the crude solid reaction product after washing with acetic acid (see the first paragraph of this section) in alcohol, adding an excess of concd. aqueous sodium hydroxide, boiling for one hour and diluting with water. The pure furan results. Analyzing in this way the products formed from acetyl chloride and equivalent amounts of nitrone, open-chain oxime, and oxime acetate it is found that the yield of furan is about the same (30–40%) in all three cases.

**Reduction of the Acetoxypyrrole (IX) to Triphenylchloropyrrole (X).**—A solution of 0.5 g. of the acetoxypyrrole in 20 cc. of glacial acetic acid with 2.0 g. of zinc dust, a crystal of copper sulfate and a drop of hydrochloric acid was heated for six hours on the water-bath then filtered, cooled and poured into water. The precipitate was filtered, dried and crystallized from dilute alcohol; yield, 0.35 g.

*Anal.* Calcd. for $C_{26}H_{16}NCl$: C, 80.1; H, 4.9. Found: C, 80.1; H, 5.2.

**2,3,5-Triphenyl-4-chloropyrrole (X)** melts at 140–141°. It is quite soluble in acetic acid and alcohol. It distils at about 250° in an oil pump vacuum. Qualitative tests show the presence of halogen.

**Reduction of Triphenylchloropyrrole (XI) to Triphenylpyrrole (VIII).**—One gram of the chloropyrrole, with 4 cc. of freshly distilled hydriodic acid and a small amount of red phosphorus, was heated in a sealed tube at 150° for four hours. After opening, the contents of the tube were boiled

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(6) Japp and Burton, *J. Chem. Soc.*, **51**, 430 (1887).

(7) Hantzsch, *Ber.*, **25**, 700 (1892).

with acetic acid and filtered. The filtrate after taking up in ether, washing and drying was evaporated and the residue was distilled in an oil pump vacuum at 240°. The distillate, crystallized from dilute alcohol, furnished 0.4 g. of 2,3,5-triphenylpyrrole melting at 141–142°. Identification was made by comparison with an authentic specimen of triphenylpyrrole. Although triphenylpyrrole and triphenylchloropyrrole melt at almost the same temperature, mixtures of the two melt below 130°.

## Summary

This article describes the ring–chain isomerism of 2-hydroxy-2,3,5-triphenylpyrrolenine oxide and the monoxime of phenyldibenzoylethylene, and the behavior of these isomers toward alkylating agents, acetic anhydride and acetyl chloride.

WASHINGTON, D. C. RECEIVED FEBRUARY 1, 1936

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[CONTRIBUTION NO. 1361 JOURNAL SERIES, MINNESOTA AGRICULTURAL EXPERIMENT STATION]

# Physico-Chemical Studies on Lecithin

BY HENRY B. BULL AND VERNON L. FRAMPTON[1]

In spite of the great amount of work on the lecithins, our knowledge concerning this class of compounds is still most unsatisfactory. The difficulty is no doubt due to the very labile character of lecithin. There appears to be no unanimity concerning such a simple property as the isoelectric point and each new communication brings new values differing from all the others. Table I gives some of the values reported in the literature.

TABLE I

| | |
|---|---|
| Price and Lewis[2] (1933) | 2.7 |
| Chain and Kemp[3] (1934) | 6.7 |
| Fujii[4] (1924) | 2.7 |
| Sueyoshi and Kawai[5] (1932) | 4.7 |
| Rona and Deutsch[6] (1926) | 1.75 |
| Remesow[7] (1930) | 2.0–2.8 |

We wish to report in this paper a study of the isoelectric point of mixtures of lecithin and cephalin and a value for the isoelectric point of lecithin obtained by extrapolating to zero concentration of cephalin.

As noted above, Sueyoshi and Kawai[5] found the isoelectric point of lecithin prepared by the method of Sueyoshi[4] to be at a *p*H of 4.7. The method of Sueyoshi depends upon the difference in solubility of lecithin and cephalin in ethyl alcohol as a means of separating these two compounds.

We have modified the method of Sueyoshi somewhat and since we believe the manner of preparation of lecithin is tremendously important we present our modification in detail. As will be shown this method will produce lecithin containing considerable quantities of cephalin.

### Method

All solvents employed were carefully purified. The peroxide normally found in laboratory ether was removed completely. All temperatures higher than 40° were avoided. The material was at no time exposed to air, being always covered with solvent. All filterations were conducted in the dark and in the cold. While not being used the preparation was stored at −15° in the dark and under acetone.

The yolks of the eggs were dehydrated with cold acetone, the acetone drained off and the dehydrated yolks allowed to remain in contact with warm 95% ethyl alcohol for thirty minutes. The residue was then placed in an extractor and extracted with ether until colorless. The alcohol and ether extracts were combined, a small amount of hydroquinone added as anti-oxidant, and the whole evaporated at 40° or below under low pressure to a thick oil. This was extracted with and finally kneaded with small portions of cold acetone in a mortar many times until it assumed a wax-like consistency and no further color was extracted. The preparation at this point should be only faintly yellow. It was dissolved in a minimum quantity of ether and the solution stored at −15° for twelve hours. The precipitate which consisted largely of cerebrosides and sphingomyelin was filtered off in the cold. The filtrate was evaporated greatly under diminished pressure to a viscous liquid which was then poured slowly into a large volume of cold acetone with constant stirring. The white precipitate was collected and the ether treatment repeated. The combined lecithin and cephalin were dissolved in a minimum quantity of warm alcohol and the solution allowed to stand overnight at −15° and filtered at that temperature. This treatment is supposed to remove the cephalin which is considered to be insoluble in alcohol. Actually, as will be shown, the cephalin is only partly removed. The alcohol solution was evaporated under low pressure and poured into cold acetone. This was repeated. About 70 g. of clear nearly colorless wax-like lecithin was obtained from 15 dozen eggs. This material seems to remain unchanged for a long time if stored at −15° in the dark under acetone.

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(1) This research was made possible by a generous grant from the Graduate School of the University of Minnesota.

(2) H. B. Bull, *J. Phys. Chem.*, **39**, 577 (1935).

(3) E. Chain and I. Kemp, *Biochem. J.*, **28**, 2052 (1934).

(4) Y. Sueyoshi, *J. Biochem.* (Japan), **13**, 145 (1931).

(5) Y. Sueyoshi and K. Kawai, *ibid.*, **15**, 277 (1932).

(6) H. Fishgold and E. Chain, *Biochem. J.*, **28**, 2044 (1934).

(7) I. Remesow, *Biochem. Z.*, **218**, 86 (1930).