[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

SOME ORTHOXAZINE DERIVATIVES

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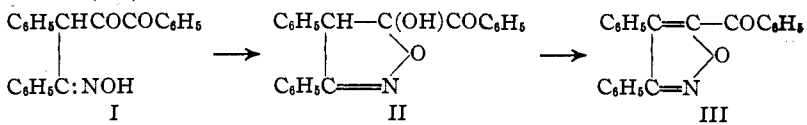
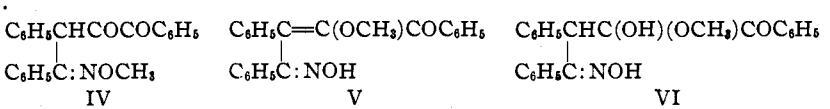
The starting point in the investigation herein described was a substance that appeared during a study of the transformations of the oxime of triphenylbutanetrione (I). In a preceding paper[1] it was shown that in neutral or faintly alkaline solution this yellow oxime spontaneously rearranges to an unstable hydroxy isoxazoline (II) which eventually changes into an isoxazole (III) that is stable.



If this same yellow oxime is boiled with methyl alcohol without previously neutralizing the trace of acid that it occludes it forms, along with the hydroxy-isoxazoline, an almost equal amount of another colorless compound. This new substance is even more labile than the hydroxy-isoxazoline. When its solution in acetone is diluted first with ether, then with much water, it reverts almost completely into the yellow oxime, and when it is allowed to remain in contact with the methyl alcoholic mother liquors it changes, more slowly but equally completely, into the hydroxy-isoxazoline. Owing to this great instability it is difficult to secure satisfactory evidence as to the structure of the new substance, only those reactions being serviceable which take place more rapidly than the rearrangement.

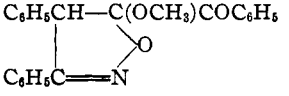
The composition of the substance is represented by the formula $C_{23}H_{19}O_3N$ which differs from that of the yellow oxime by CH_2. It forms sodium, monobenzoyl and mono-alkyl derivatives, and therefore has an active hydrogen atom; and a methoxyl determination by the Zeisel method shows the presence of one such group. The methoxyl derivatives of the oxime which meet these requirements are represented by Formulas IV and V.



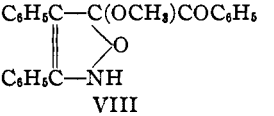
The first of these formulas scarcely deserves serious consideration, not only because it seems incredible that an oxime ether like this should form so easily, but also because there seems to be no reason why the color of such an ether should be materially different from that of the parent substance.

[1] THIS JOURNAL, 47, 3030 (1925).

The second formula is not open to either of these objections. Since the diketone has an exceedingly active carbonyl group, the formation of a hemi-acetal like VI would not be surprising, and loss of water from this in the manner required for the formation of the methoxyl compound would not be impossible. This formula, however, seems hardly consistent either with the great instability of the substance or the manner of its rearrangement. Reversion to the diketone would in this case involve rapid addition of water in such a manner as to regenerate the hemi-acetal; as this is the reverse of the common mode of addition to such a conjugated system, this formula must be regarded as highly improbable.

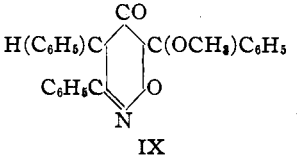


VII

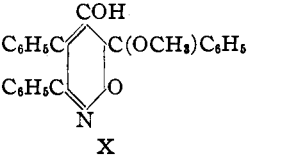


VIII

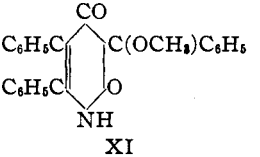
It seems clear from the foregoing discussion, that the methoxyl derivative is in all probability not an open-chained compound. Cyclic formulas like VII and VIII which contain an isoxazole ring are equally improbable. The substance represented by VII would not have an active hydrogen atom while that represented by VIII, if acidic at all, would not be expected to form a sodium compound in the presence of water; neither formula can be reconciled with the behavior of the substance towards phenylmagnesium bromide. There remains the possibility that the methoxyl compound contains a six-membered ring, such as is shown in IX, X and XI, which represent the tautomeric forms of an orthoxazine derivative.



IX

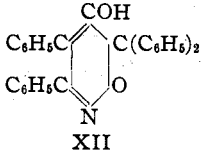


X

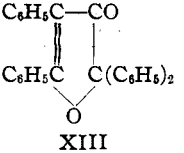


XI

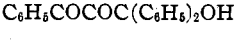
Very few orthoxazine derivatives are known, and as none of these has either carbonyl or hydroxyl in the 5 position all analogies on which the behavior of a substance like IX might be predicted are too remote to be of value. It is more instructive to compare the properties of the methoxyl compound with those of the product which is obtained by treating it with phenylmagnesium bromide, because this substance forms stable degradation products and consequently it is possible to prove that it is the orthoxazine derivative (XII).



XII



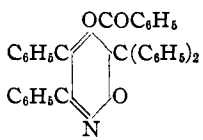
XIII

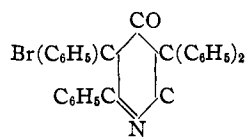


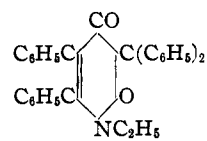
XIV

Thus the composition of the substance shows that the Grignard reaction results in the substitution of phenyl for methoxyl. When it is very cautiously oxidized with chromic acid it yields mainly benzil, benzilic acid and benzophenone and when it is digested with methyl alcoholic hydrogen chloride it passes very slowly into the cyclic ketone (XIII). These degradation products, which show the structure of the carbon chain and fix the position of the phenyl groups, would suffice to prove the structure of the substance even if no other evidence were available.

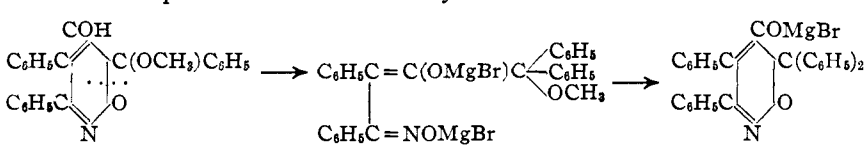
The relation between the substance and its derivatives supplies equally conclusive evidence. It is soluble in alcoholic alkali, is readily brominated and it also readily forms benzoyl and alkyl derivatives. The behavior of these derivatives towards ozone shows that they are not constituted alike. Thus the alkyl derivatives are very readily ozonized and give mainly the open-chained diketone (XIV). The benzoate is ozonized with great difficulty but is ultimately oxidized to benzil, benzilic acid and other products. The bromine substitution product is not attacked by ozone. This difference in behavior shows that the relation between these substances is that of derivatives of the three tautomeric forms of an orthoxazine:


XV

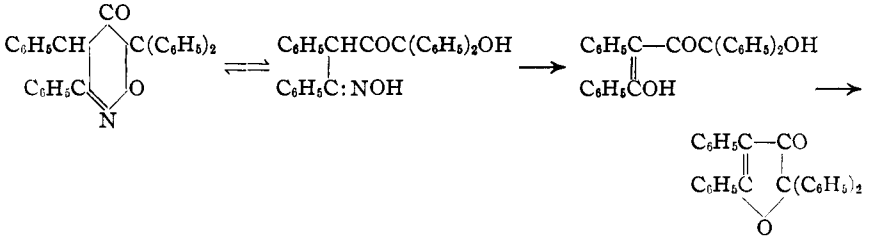

XVI


XVII

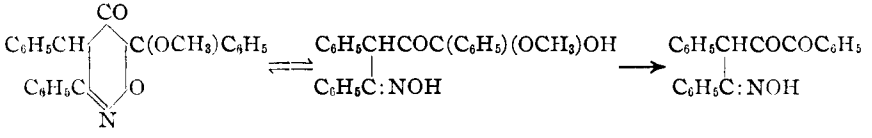
The methoxyl compound likewise forms a sodium derivative and is readily benzoylated and alkylated. The behavior of these derivatives toward ozone is exactly like that of the corresponding derivatives of the Grignard product. The conclusion seems justified, therefore, that the methoxyl compound also is an orthoxazine derivative. The course of the Grignard reaction which cannot be reconciled with any other formula can now be interpreted without difficulty.



The great difference in the relative ease with which the ring is opened in these various orthoxazine derivatives is probably due largely to differences in the character of the resulting open-chained compound. Thus the tetraphenyl derivative appears to be relatively insensitive because the only process by which the resulting open-chained compound is removed is the relatively slow hydrolysis of an oxime.



The triphenyl methoxyl derivative, on the other hand, is extremely unstable because the corresponding open-chained compound rapidly changes to the yellow diketone oxime.



The great difference between the behavior of the parent substances and that of their alkyl derivatives can be accounted for in a similar manner. General statements to the effect that the orthoxazine ring is easily opened, or that orthoxazine derivatives are unstable are not in harmony with the facts; the stability of orthoxazine derivatives like that of most other heterocyclic compounds depends largely upon the number, distribution and character of the substituents.

Experimental Part

I. 3,4,6-Triphenyl-5-hydroxy-6-methoxy-orthoxazine

Preparation.—A methyl alcoholic solution, made with pure, recrystallized triphenylbutanetrione oxime (I) and absolute methyl alcohol in the proportion of 15 g. of oxime to 100 cc. of the alcohol, is boiled for 20 minutes. If crystallization has not started by this time, it is induced either by inoculation or by rubbing the sides of the container with a glass rod. The boiling is continued for another 10 minutes, at the end of which the mixture usually bumps violently and is then set aside. During the boiling the solution, which at the outset should be pure yellow, turns pink or faintly purple owing to the formation of a small quantity of the purple anhydride referred to in a previous paper.[2] A deeper purple indicates that the solution is too acidic and that the oxime should be recrystallized before it is used for this purpose. The crystals are collected on a filter and washed, first with methyl alcohol until nearly colorless and finally with ether to remove a small quantity of adherent anhydride. The substance is then analytically pure; its weight should be a little more than half that of the oxime used.

The methyl alcohol washings and the filtrate are combined and boiled

[2] Ref. 1, p. 3033.

for another half hour during which the volume is allowed to decrease to nearly one-half. Cooling and inoculation lead to the deposition of a second crop and a repetition of the operation generally results in a little more, but this is usually contaminated with so much of the hydroxy-isoxazoline (II) and the purple anhydride that it is seldom feasible to purify it. The maximum yield is 75%, the average yield 65%. The substance can be recrystallized without serious loss by grinding it to a very fine powder, dissolving this rapidly in boiling absolute methyl alcohol, and cooling the solution in a freezing mixture.

Anal. Calcd. for $C_{23}H_{19}O_3N$: C, 77.3; H, 5.3; OCH_3, 8.7. Found: C, 77.1; H, 5.5; OCH_3, 8.5.

3,4,6-Triphenyl-5-hydroxy-6-methoxy-orthoxazine (X).—3,4,6-Triphenyl-5-hydroxy-6-methoxy-orthoxazine (X) crystallizes in colorless needles. When it is heated rapidly in a capillary tube it begins to turn red at about 160°, melts to a red liquid at about 173°, and then decomposes with effervescence. It is very sparingly soluble in ether, in methyl alcohol and in carbon tetrachloride, moderately soluble in chloroform and in acetone. All solutions are yellow. The color of the solution in chloroform turns more or less rapidly to pink and purple because traces of acid convert the substance into the purple anhydride. In other solutions the color usually remains constant but in these also the substance is unstable. Thus when it is suspended in a relatively small quantity of ether, the solution gradually deposits the yellow triketone oxime (I), and a similar suspension in methyl alcohol becomes filled with crystals of the hydroxy-isoxazoline (II). In the dark the pure, dry solid appears to be stable, but when it is exposed to light it gradually turns yellow, probably owing to autoxidation, since the product is completely insoluble and contains much less carbon and hydrogen than the original substance.

The Sodium Derivative.—When the finely powdered methoxy compound is shaken with excess of 10% aqueous sodium hydroxide it rapidly changes to a crystalline sodium compound. For the purpose of analysis this was washed with methyl alcohol and ether.

Anal. Calcd. for $C_{23}H_{18}O_3NNa$: Na. 6.1. Found: 6.3.

Both the solid sodium compound and its aqueous solution are colorless but the latter soon becomes yellow. When it is acidified it deposits a mixture of the methoxyl compound and the yellow oxime.

The Benzoate, $C_{30}H_{23}O_4N$.—The methoxyl compound was benzoylated both by means of the Schotten-Baumann reaction and by using pyridine as condensing agent. The benzoate was also made by shaking the pure solid sodium compound with an ethereal solution of benzoyl chloride. All procedures gave the same benzoate, but the pyridine method was found to be much the most satisfactory. A solution of 6 g. of the methoxyl compound in 30 g. of pyridine was treated with 6 g. of benzoyl chloride which was added in three equal portions. The temperature rose rapidly and the yellow color of the solution turned to pink and finally purple. The solution was set aside for an hour, then diluted with water and ether, and acidified with dilute acid. The ethereal layer was extracted with saturated sodium carbonate which removed some unchanged methoxyl compound,

dried and allowed to evaporate. It deposited 4.2 g. of a crystalline product which was purified by recrystallization from ether.

Anal. Calcd. for $C_{30}H_{23}O_4N$: C, 78.1; H, 5.0; OCH_3, 6.7. Found: C, 78.0; H, 5.2; OCH_3, 6.2.

The benzoate crystallizes in fine, colorless needles that melt without decomposition at 163°. It cannot be hydrolyzed to the methoxy compound. It dissolves slowly in cold methyl alcoholic sodium hydroxide but this converts it quantitatively into sodium benzoate and the isoxazole (III). It is also hydrolyzed by cold methyl alcoholic hydrogen chloride, but the final product is composed largely of the purple anhydride.

OZONIZATION OF THE BENZOATE.—Although the benzoate was ozonized repeatedly, the only definite products that could be isolated were small quantities of benzil and phenylglyoxylic acid. These products indicate that the substance is an *o*-benzoate derived from the hydroxyl modification X. The evidence is not conclusive because the very slow absorption of ozone, the appearance of an orange-colored precipitate during the ozonization, and the small quantities of definite products—about 0.3 g. from 3.5 g. of benzoate—show that the process is not confined to the usual addition of ozone to a double linkage.

Alkylation.—After an unsuccessful attempt to alkylate the methoxyl compound with pyridine and methyl iodide, an almost quantitative methylation was obtained as follows. The finely powdered methoxyl compound was added in small quantities to a cold solution of two equivalents of methyl iodide in an excess of 10% sodium methylate solution. Reaction was immediate and the methyl derivative began to separate before all of the methoxyl compound had been added. The mixture was diluted with ether and acidified with dilute acid. Most of the product was deposited at this stage; the remainder was obtained by evaporating the ether solution. The substance was purified by recrystallization from methyl alcohol. The yield of pure product was 5.8 g. from 6 g. of methoxyl compound.

Anal. Calcd. for $C_{24}H_{21}O_3N$: C, 77.6; H, 5.7. Found: C, 77.3; H, 5.1.

The methyl derivative crystallizes in thick, colorless plates and melts, with decomposition, at 147°. It is sparingly soluble in ether and in cold methyl alcohol, moderately soluble in boiling methyl alcohol, readily soluble in acetone and in chloroform. The results of a methoxyl determination by the Zeisel method indicate that the substance is a methylation product of the tautomeric modification represented by XI. Thus when the substance was heated with hydrogen iodide in the usual manner it gave, in place of 8.4% of methoxyl calculated for one such group, 9.5% after heating for an hour, 11.6% after heating for two hours, and 13.1% on prolonged heating. The Zeisel method here as with other substances which contain both OCH_3 and NCH_3 does not distinguish sharply between these two groups but the behavior is quite different from that of substances containing two methoxyl groups.

The behavior towards ozone also indicates that this is a derivative of the modification represented by XI. Unlike the benzoate the substance combines with ozone readily, the solution remains colorless and it

deposits a colorless, slightly explosive ozonide. It proved to be a difficult matter to get any significant products from this ozonide. In various experiments it was decomposed by water, dilute acid, sodium carbonate and sodium bisulfite, but in each case the principal product was an uncrystallizable paste containing all of the nitrogen and most of the rest of the molecule. The only definite products isolated were benzoic and phenylglyoxylic acids and these were obtained only in very small quantities.

Reaction with Organic Magnesium Compounds.—This reaction occurs in at least two distinct steps. At low temperatures it merely results in the liberation of an equivalent quantity of hydrocarbon and the replacement of the active hydrogen by magnesium. All of the methoxyl compound is recovered when the solution is acidified. At the boiling point of ether, a second molecule of the organic magnesium compound participates, forming a yellow magnesium derivative which on acidification gives a product that has a hydrocarbon residue in place of the methoxyl group. Solid products were obtained with methyl-, ethyl- and phenylmagnesium bromides but only the one from phenylmagnesium bromide was studied, because this seemed most likely to give transformation products that could be identified. The preparation was as follows.

Ten g. of the finely powdered methoxyl compound was added in two or three portions to an ethereal solution of phenylmagnesium bromide made from 6 g. of magnesium. The temperature of the mixture rose rapidly to the boiling point as the solid dissolved. The mixture was stirred for an hour during which the new magnesium compound separated either as a yellow paste or as a crystalline solid. This, when decomposed with ice and acid in the usual manner, turned into an impalpable, pale yellow powder difficult to filter and wash. A partial purification was accomplished by dissolving the powder in 5% methyl alcoholic sodium hydroxide and reprecipitating by cautious addition of acetic acid. The substance was thus obtained in a form in which it was readily washed and dried, and which was much more slowly oxidized by contact with air. For complete purification it was dissolved in pyridine and reprecipitated with ether. It separated in fine, lemon-yellow needles which are sparingly soluble in all common solvents and melt with decomposition at about 220°.

Anal. Calcd. for $C_{28}H_{21}O_2N$: C, 83.4; H, 5.2. Found: C, 83.3; H, 5.4.

II. 3,4,6,6-Tetraphenyl-5-hydroxy-orthoxazine

The proof that this Grignard product is an orthoxazine derivative was presented in the introduction. As it is too sparingly soluble for successful ozonization, the choice between the three possible tautomeric modifications is necessarily more or less arbitrary. For the purpose of naming, it seems best to assume the hydroxyl form because the color of the substance is exactly the same as that of its magnesium derivative and that of its solution in alkalies. Towards substituting agents it behaves precisely like a phenol, the one hydrogen atom connected with the ring being replaced, with the greatest ease, by halogens and by nitro and sulfonic acid groups. Like the phenols, also, it readily undergoes autoxidation

and forms complex products which are insoluble in alkalies. These substitution products are, doubtless, due to addition and elimination because when bromine is added to a cold, concentrated solution of the substance in glacial acetic acid it precipitates a sparingly soluble, colorless product which almost immediately redissolves, and then the bromine substitution products begin to appear.

Benzoylation.—The benzoate was obtained in the calculated quantity by treating the substance with benzoyl chloride in the presence of pyridine and recrystallizing the product from acetone. It is sparingly soluble in acetone and in chloroform, practically insoluble in other common solvents. It crystallizes in colorless needles and melts at 217°.

Anal. Calcd. for $C_{35}H_{25}O_3N$: C, 82.8; H, 5.0. Found: C, 82.5; H, 5.3.

The substance is doubtless an *o*-benzoate, because when its dilute solution in methyl alcohol is treated with either base or acid, the benzoate is very rapidly hydrolyzed to benzoic acid and the hydroxyl compound. The behavior of the substance on ozonization also indicates that it is an *o*-benzoate. The absorption of ozone is very sluggish. It leads to the deposition of a mixture composed of a complex oxidation product similar to that formed in the autoxidation of the parent substance and an ozonide. This mixture was decomposed with water and the resulting oil distilled with steam. The distillate contained benzophenone. The gummy residue in the flask was partially soluble in ether. From this solution sodium carbonate extracted a small quantity of benzilic acid (0.3 g. from 2 g. of benzoate).

3,4,6,6-Tetraphenyl-4-bromo-5-oxo-orthoxazine (XVI).—The bromine substitution product is very readily made by adding bromine to a solution of the hydroxyl compound in glacial acetic acid and precipitating the product with water. It is most easily purified by recrystallization from benzene from which it separates in colorless tables that contain a molecule of benzene and melt at 160°.

Anal. Calcd. for $C_{28}H_{20}O_2NBr.C_6H_6$: C_6H_6, 13.8. Found: C_6H_6, 13.6. Calcd. for $C_{28}H_{20}O_2NBr$: C, 69.7; H, 4.1. Found: C, 69.7; H, 4.2.

3,4,6,6-Tetraphenyl-4-chloro-5-oxo-orthoxazine.—The chlorine compound may be made like the bromine compound but it is most easily obtained by the action of phosphorus pentachloride on the hydroxyl compound.

Thus, a suspension of 2 g. of this substance and 4 g. of the pentachloride in 50 cc. of carbon tetrachloride was shaken until all of the substance had dissolved. The solution was washed with ice water, then dried, concentrated and diluted with petroleum ether. It gave 2 g. of the chlorine compound which crystallized in small cubes and melted at 165°.

Anal. Calcd. for $C_{28}H_{20}O_2NCl$: C, 76.8; H, 4.6; Cl, 8.1. Found: C, 76.4; H, 4.7; Cl, 8.1.

Neither the bromine nor the chlorine compound is attacked by ozone; both are reduced to the hydroxyl compound by prolonged boiling in methyl alcohol.

3,4,6,6-Tetraphenyl-4-nitro-5-oxo-orthoxazine.—A suspension of 3 g.

of the hydroxyl compound in 10 cc. of glacial acetic acid was treated with 10 cc. of concd. nitric acid.

The solid dissolved at once. The clear solution, on dilution with water, deposited colorless crystals which were washed, dried and recrystallized from acetone. This gave 1.6 g. of a product that separated in needles and melted with decomposition at 150°. The nitro compound is not attacked by ozone, and like the halogen substitution products it can be hydrolyzed to the hydroxyl compound by prolonged boiling with methyl alcohol.

Anal. Calcd. for $C_{28}H_{20}O_4N_2$: C, 75.0; H, 4.5. Found: C, 74.8; H, 4.7.

2-Ethyl-3,4,6,6-tetraphenyl-5-oxo-orthoxazine (XVII).—Two g. of the hydroxyl compound was suspended in 40 cc. of absolute alcohol, the suspension was cleared by the addition of enough sodium ethylate to dissolve the solid, 6 g. of ethyl iodide was added to the solution, which was then boiled for 15 minutes when it was found to be neutral. The resulting clear liquid on cooling and evaporation deposited colorless diamond-shaped plates. After washing, drying and recrystallization from alcohol the yield was 1.7 g. of a product which melted at 167–168°.

Anal. Calcd. for $C_{30}H_{25}O_2N$: C, 83.5; H, 5.8. Found: C, 83.3; H, 5.9.

The substance is sparingly soluble in ether, moderately soluble in boiling alcohol, readily soluble in acetone and chloroform. The corresponding methyl derivative was obtained in a similar manner. It crystallized in square tables and melted at 189–190°.

Anal. Calcd. for $C_{29}H_{23}O_2N$: C, 83.1; H, 6.0. Found: C, 83.1; H, 5.9.

Structure of Alkyl Derivatives.—The methylation was carried out with 10 g., the yield was almost quantitative, and a careful examination of the mother liquors failed to disclose any other methylation product. The structure of the methyl derivative was established by a Zeisel determination which proved the absence of a methoxyl group and by ozonization which showed that there is no alkyl group in the 4 position. The ozonization was carried out in chloroform with ozonized oxygen that contained about 6% of ozone. Ozonization was complete in three hours. The clear yellow chloroform solution was evaporated, the residue boiled with water and the resulting paste distilled with steam. This removed small quantities of benzophenone and benzoic acid. The paste left in the distilling flask was extracted with ether, which formed a pale yellow solution and left a brown residue that contained nitrogen and had an odor similar to methylamine when it was digested with concentrated alkali. The dried ethereal solution, on evaporation, gave the diketone $C_6H_5COCO\text{-}C(C_6H_5)_2OH$ in almost the calculated quantity. The alkyl group must, therefore, be in combination with nitrogen.

Oxidation.—The pure, dry hydroxyl compound appears to be perfectly stable in the air, but its solutions in acetone and chloroform gradually lose their color and deposit a colorless solid that is insoluble in alkalies

and decomposes at about 165°. The same or a similar change takes place much more rapidly when the crude product is left exposed to the air, more than half of it becoming insoluble in alkalies in the course of a few days. Acetone solutions rapidly reduce permanganate and here also the product is a white powder which is insoluble in alkalies and which decomposes at about 165°. The amount of permanganate that was reduced varied slightly in different experiments but always indicated that two atoms of oxygen were involved in the process. All attempts to get the exceedingly insoluble white product in a form that would give consistent analytical results were unsuccessful.

Ring-opening.—The foregoing account deals with transformations of the orthoxazine derivative in which the ring is not involved. In all these operations there are indications that the ring does not completely escape. Direct experiments showed that the ring can be opened by digesting the substances with alcoholic solutions of acids and bases, and even by protracted boiling with Grignard reagents. The product in all of these cases is the deep yellow furan ketone (XIII).

Summary

1. The paper gives an account of the transformations of two orthoxazine derivatives which have an hydroxyl or an oxo group in the 5 position.

2. One of these is relatively stable, the other extremely unstable. It is shown that the stability of orthoxazine derivatives, like that of other heterocyclic compounds depends upon the number, character and distribution of the groups in combination with the atoms constituting the ring.

CAMBRIDGE 38, MASSACHUSETTS

[CONTRIBUTION FROM THE PROTEIN INVESTIGATION LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE ISO-ELECTRIC POINTS OF VARIOUS PROTEINS

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Having samples of many vegetable proteins,[1] most of which had been prepared in this Laboratory in connection with other studies, it was thought that an excellent opportunity was offered for making a comparative study of these proteins with respect to one of their most interesting physico-chemical properties, namely, their iso-electric points. Aside from obtaining information regarding the iso-electric points of the individual proteins, such a study, in which the work was done by the same workers using the same method, and under constant experimental conditions, would

[1] For the sources and preparation of the proteins used in this study see Jones, Gersdorff and Moeller, *J. Biol. Chem.*, **62**, 183 (1924).