[Contribution from the Chemical Laboratory of Harvard University]

ISOXAZOLINE OXIDES

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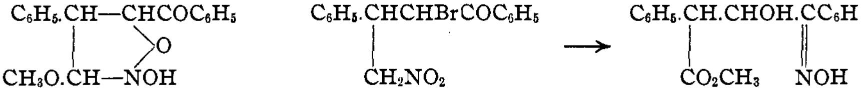
In the course of the investigation described in the preceding paper[1] it became evident that a substance which had been obtained by the action of methyl alcoholic potassium acetate on two stereo-isomeric α-bromo-γ-nitro ketones, and which had been regarded as an isoxazolidine derivative,[2] might have quite a different structure. We have, therefore, reëxamined this substance and most of its derivatives. It is not the isoxazolidine derivative (I) but an isomeric, open-chained oximido-hydroxy ester (II) and the formulas of its derivatives need to be revised accordingly.

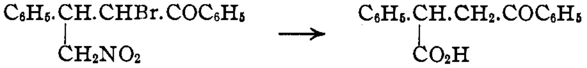


I (XIII)[3] II (XIII)

$$C_6H_5.CHCHBrCOC_6H_5(CH_2NO_2) \longrightarrow C_6H_5.CH(CO_2CH_3).CHOH.C(=NOH)C_6H$$

The new formula bears little resemblance to that of the bromo compound and no satisfactory mechanism for the formation of a substance with such a structure is as yet available, but the proof of the formula seems conclusive. Thus, a methoxyl determination by the method of Zeisel shows the presence of one such group. This can be removed by cautious treatment with alkali and the product is an acid that, on esterification through the silver salt, regenerates the methoxyl compound; this is, therefore, a methyl ester. The evidence of the presence of an oximido group is equally convincing. Cold methyl alcoholic hydrochloric acid removes the nitrogen as hydroxylamine hydrochloride and forms a ketonic ester from which the original product can be regenerated by treatment with free hydroxylamine. The one oxygen atom not accounted for thus far must be present in an hydroxyl group because the substance forms both a diacetate and a dibenzoate which still contain the methoxyl group. The substance, therefore, contains a carbomethoxyl, an oximido and an hydroxyl group.

The nature of the carbon chain was established by hydrolyzing the substance first to the oximido acid, then to the ketonic acid and finally reducing this with hydrogen iodide. The product was α-phenyl-β-benzoylpropionic acid; the carbon chain must, therefore, be the same as that in the bromo compound.



$$C_6H_5.CH(CH_2NO_2).CHBr.COC_6H_5 \longrightarrow C_6H_5.CH(CO_2H).CH_2.COC_6H_5$$

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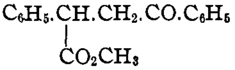
[1] Kohler and Shohan, This Journal, **48**, 2425 (1926).

[2] Kohler, *ibid.*, **46**, 503 (1924).

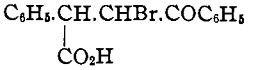
[3] Whenever a substance was described but incorrectly formulated in the earlier paper (Ref. 2) it will have two numbers in this paper; the number in parentheses is the same as that used in the earlier paper.

This relation between substance and reduction product left little doubt as to the location of the functional groups but this was nevertheless established directly by preparing the substance from α-phenyl-β-benzoylpropionic acid through the following steps.



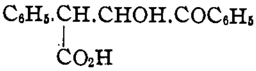
$\mathrm{C_6H_5.\underset{CO_2CH_3}{CH}.CH_2.CO.C_6H_5}$

III



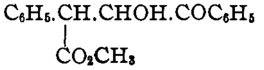
$\mathrm{C_6H_5.\underset{CO_2H}{CH}.CHBr.COC_6H_5}$

IV



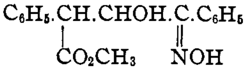
$\mathrm{C_6H_5.\underset{CO_2H}{CH}.CHOH.COC_6H_5}$

V



$\mathrm{C_6H_5.\underset{CO_2CH_3}{CH}.CHOH.COC_6H_5}$

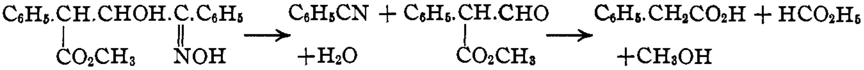
VI



$\mathrm{C_6H_5.\underset{CO_2CH_3}{CH}.CHOH.\underset{NOH}{C}.C_6H_5}$

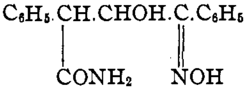
II (XIII)

The final product of this series was identical with that obtained from the bromonitro ketone and both the hydroxy acid V and the ketonic acid VI were the same as those previously obtained by hydrolysis. In this sequence the only step that does not invariably lead to a substance of known structure is the conversion of the bromo to the hydroxy acid. In the case of γ-ketonic acids this operation is sometimes accompanied by a transposition of the hydroxyl and carbonyl groups. That this rearrangement does not occur in the present instance is shown not only by the formation of a γ-ketonic acid on reduction but also by the behavior of the substance towards strong bases; as was shown in the earlier paper[4] concentrated alcoholic potassium hydroxide cleaves it quantitatively into benzonitrile, and formic and acetic acids.



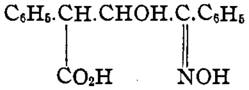
$$\mathrm{C_6H_5.\underset{CO_2CH_3}{CH}.CHOH.\underset{NOH}{C}.C_6H_5} \longrightarrow \mathrm{C_6H_5CN + C_6H_5.\underset{CO_2CH_3}{CH}.CHO + H_2O} \longrightarrow \mathrm{C_6H_5.CH_2CO_2H + HCO_2H_5 + CH_3OH}$$

The formulas of most of the derivatives described in the earlier paper are easily revised to accord with the new formula of the parent substance. Thus, the product obtained by the action of concd. aqueous ammonia on the ester is the amide VII, and the result of boiling the ester or its acetates with aqueous acetic acid is the oximido acid VIII. The ability to form a copper compound is, as recently announced by Feigl and his collaborators,[5] a general property of substances which have the group —CHOHC(=NOH)—. In accordance with their conclusions the ester shares this property with the amide and the acid but not with any derivative in which either the hydroxyl or the oximido group has been modified.



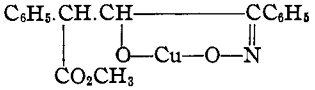
$\mathrm{C_6H_5.\underset{CONH_2}{CH}.CHOH.\underset{NOH}{C}.C_6H_5}$

VII (XIV)



$\mathrm{C_6H_5.\underset{CO_2H}{CH}.CHOH.\underset{NOH}{C}.C_6H_5}$

VIII (XII)



$\mathrm{C_6H_5.\underset{CO_2CH_3}{CH}.\underset{O-Cu-O-N}{CH{-}{-}{-}{-}CC_6H_5}}$

IX

As the ester has two different kinds of hydroxyl groups, it would be expected to form isomeric mono-acetates which would differ considerably

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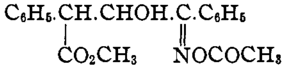
[4] Ref. 2, p. 516.

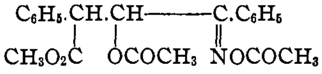
[5] Feigl, Sicher and Singer, *Ber.*, **58**, 2294 (1925).

with respect to ease of hydrolysis. Two such acetates have been obtained, one directly from the ester VII, the other by partial hydrolysis of the diacetate with sodium carbonate.



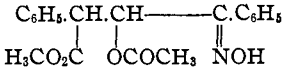
$C_6H_5.\underset{\underset{CO_2CH_3}{|}}{C}H.CHOH.\underset{\underset{NOCOCH_3}{\|}}{C}.C_6H_5$

X (VII)



$C_6H_5.\underset{\underset{CH_3O_2C}{|}}{C}H.\underset{\underset{OCOCH_3}{|}}{C}H\text{———}\underset{\underset{NOCOCH_3}{\|}}{C}.C_6H_5$

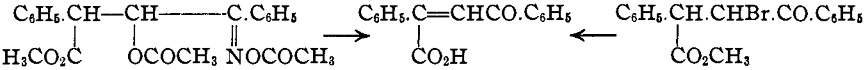
(XVI)



$C_6H_5.\underset{\underset{H_3CO_2C}{|}}{C}H.\underset{\underset{OCOCH_3}{|}}{C}H\text{———}\underset{\underset{NOH}{\|}}{C}.C_6H_5$

XII (XVIII)

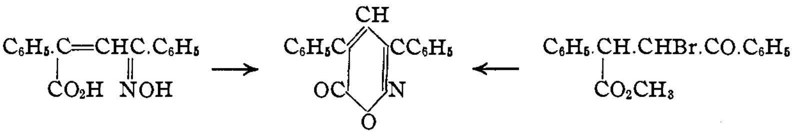
The two mono-acetates are cleaved by alkalies, like the oximido ester, into benzonitrile, formic and phenylacetic acids; the behavior of the diacetate is quite different. It dissolves in alcoholic potassium hydroxide with very little development of color and when the solution is acidified it deposits an acid which has the composition $C_{16}H_{11}O_3$, reduces permanganate readily and, when ozonized, gives as the only product benzoylformic acid. This substance is α-phenyl-β-benzoylacrylic acid. It is more easily obtained by the action of alkalies on α-phenyl-β-bromo-β-benzoylpropionic acid.



$C_6H_5.\underset{\underset{H_3CO_2C}{|}}{C}H\text{—}\underset{\underset{OCOCH_3}{|}}{C}H\text{———}\underset{\underset{NOCOCH_3}{\|}}{C}.C_6H_5 \longrightarrow C_6H_5.\underset{\underset{CO_2H}{|}}{C}{=}CHCO.C_6H_5 \longleftarrow C_6H_5.\underset{\underset{CO_2CH_3}{|}}{C}H.CHBr.CO.C_6H_5$

(XVI) XIII IV

This unsaturated acid served as a means of establishing the nature of an interesting substance that was frequently obtained in small quantities as a by-product in the preparation of the oximido ester. This well crystallized compound can be made at will by treating either the unsaturated acid or the bromo ester with hydroxylamine. Its composition and molecular weight are represented by the formula $C_{16}H_{11}O_2N$. It reduces permanganate in the cold and forms an ozonide which, when decomposed with water, gives benzoylformic acid; it is, therefore, the orthoxazine derivative XV.

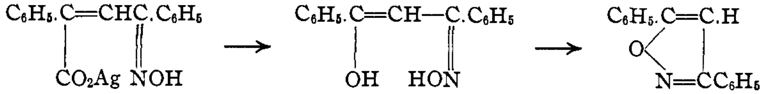


$C_6H_5.\underset{\underset{CO_2H}{|}}{C}{=}CH\underset{\underset{NOH}{\|}}{C}.C_6H_5 \longrightarrow$ XV $\longleftarrow C_6H_5.\underset{\underset{CO_2CH_3}{|}}{C}H.CHBr.CO.C_6H_5$

IV XV III

The orthoxazine derivative is remarkably insensitive both to acids and to bases; the ring is not opened either by concd. aqueous or hot concd. methyl alcoholic hydrochloric acid. Dilute aqueous alkalies are likewise without effect but concd. potassium hydroxide dissolves it and forms a salt of oximido-phenylbenzoylacrylic acid from which acids regenerate the cyclic compound.

By a process that is easy to comprehend, the orthoxazine derivative can be converted into a more stable isoxazole. It is sufficient for this purpose to dissolve the substance in alcoholic alkali and to boil this solution with excess of silver nitrate.



XVI

The orthoxazine derivative can also be converted into the isoxazole directly by merely digesting it with acetyl chloride—a novel transformation that merits further investigation.

Experimental Part

I. Elimination of Hydrogen Bromide from the Bromonitro Ketone

In the earlier work, the bromine compound was shaken at the ordinary temperature with excess of methyl alcoholic potassium acetate. This gave a mixture of nitrocyclopropane derivative and oximido ester in variable proportions depending upon the stereo-isomeric modification of the bromine compound that was used. After the true character of the oximido ester had been established this no longer seemed the most advantageous procedure when the preparation of the ester was the sole purpose of the operation, because it appeared probable that whatever might be the detailed mechanism of the process, the transposition of the oximido group was due to the acidity of the solution.

In accordance with this conclusion it was found that, by adding sufficient acetic acid at the outset, it is possible to suppress the formation of cyclopropane derivative completely. In a mixture of equal weights of methyl alcohol and glacial acetic acid both bromine compounds gave exclusively the oximido ester, the yield of pure ester being over 95%. Owing to the difficulty of separating the mixture of bromine compounds it is, however, more economical to start with the crude, washed and dried mixture. The procedure follows.

A suspension of 87 g. of the bromine compound and 75 g. of potassium acetate in a mixture of 75 g. each of methyl alcohol and glacial acetic acid is boiled for three hours, then cooled and poured into 750 cc. of water. The resulting oily suspension is thoroughly extracted with ether, the ethereal solution washed first with water, then with sodium carbonate until it is free from acetic acid, dried, concentrated and finally evaporated. It deposits about 70 g. of a crystalline solid, which gives 65 g. of pure oximido ester; yield, 86%.

Hydrolysis to the Oximido Acid.—The finely powdered ester was shaken with thrice the calculated quantity of normal aqueous potassium hydroxide and a little ether. The mixture immediately assumed a bright orange color which remained until all of the solid ester had disappeared, then soon faded to a pale yellow which was permanent. The solution was filtered to remove a small quantity of amorphous solid, then poured into 50 cc. of ether and cautiously acidified. In order to avoid hydrolysis of the oximido group excess of acid was avoided and the mixture was frequently shaken during acidification. The ethereal layer was removed, washed with water, dried over sodium sulfate, evaporated to 20 cc. and diluted with petroleum ether to incipient cloudiness. The solution deposited the acid in fine, white needles which after recrystallization from petroleum ether melted at 168°, with decomposition. The yield was 7 g. from 10 g. of ester, or 75%.

Anal. Calcd. for $C_{16}H_{15}O_4N$: C, 67.3; H, 5.4. Found: C, 67.1; H, 5.3.

α,γ-Diphenyl-β-hydroxy-γ-oximinobutyric acid is readily soluble in all common organic solvents except petroleum ether. It forms a bright green copper compound when its ethereal solution is shaken with aqueous copper acetate. In order to establish its relation to the oximido ester its silver salt was made by shaking a solution of silver nitrate with a concentrated solution of the acid in sodium carbonate. The dry silver salt reacted readily with methyl iodide and gave a solid that was identical with the one obtained from the bromine compound.

Hydrolysis to the Ketonic Ester.—The oximido ester was dissolved in the minimum quantity of a mixture made from equal volumes of methyl alcohol and concd. hydrochloric acid. The resulting solution was boiled for an hour, then allowed to cool slowly. It deposited a solid that on recrystallization from a mixture of ether and petroleum ether separated in long, flat needles melting at 78°.

Anal. Calcd. for $C_{17}H_{16}O_4$: C, 71.8; H, 5.6. Found: C, 71.6; H, 5.6.

Methyl-α,γ-diphenyl-β-hydroxy-γ-oxobutyrate, VI, is soluble in common organic solvents except petroleum ether. It does not form a copper compound when its ethereal solution is shaken with copper acetate. Its relation to the oximido ester was established by treatment with hydroxylamine as follows. To a solution of 2 g. of the ester in 20 cc. of methyl alcohol were added in succession 0.5 g. of precipitated calcium carbonate and 1.0 g. of finely powdered hydroxylamine hydrochloride. This mixture was warmed on a steam-bath for 30 minutes and then allowed to stand overnight. As it failed to deposit a solid product it was diluted with water and extracted with ether. The ethereal layer after the usual washing and drying yielded 0.5 g. of the oximido ester.

Hydrolysis to the Hydroxy Ketonic Acid.—Both the oximido acid and the ketonic ester can be hydrolyzed further, the former by treatment with acid, the latter by digestion with bases. The oximido acid is hydrolyzed with the utmost ease. It is sufficient to dissolve it in a large volume of dil. sodium carbonate solution, add a little ether and then an excess of hydrochloric acid. The solution gradually deposits the pure, nitrogen-free acid.

Anal. Calcd. for $C_{16}H_{14}O_4$: C, 71.1; H, 5.2. Found: C, 71.0; H, 5.2.

The acid obtained in this manner is readily soluble in alcohol and in ether and is sparingly soluble in water. When heated in a capillary tube it melts at 115°, resolidifies at 117–120° and remelts at 142°. When the ketonic ester VI is digested with aqueous alkalies it gradually dissolves. If the resulting solution is diluted with much water and then very cautiously acidified it likewise deposits the acid melting at 115°, but when it is acidified without previous dilution it deposits an acid which melts at 142° and is identical with the product formed by heating the lower-melting acid. The transition from the higher- to the lower-melting acid involves the loss of half a molecule of water which is doubtless present as water of crystallization because when a dilute solution of the sodium salt of the higher-melting acid is acidified it deposits the acid melting at 115°.

Anal. Calcd. for $C_{16}H_{14}O_4{}^1/_2H_2O$: H_2O, 3.2. Found: 3.1. Calcd. for $C_{16}H_{14}O_4$: C, 71.1; H, 5.2. Found: C, 71.0; H, 5.2.

α-Phenyl-β-hydroxy-β-benzoylpropionic acid, V, is readily soluble in all common organic solvents except petroleum ether. It is very sparingly soluble in water. Its structure was established both by synthesis and by reduction.

For the purpose of synthesizing an hydroxyketonic acid of known structure, hydrogen cyanide was added to the benzalacetophenone by the procedure of Kohler and Allen,[6] and the resulting nitrile of phenylbenzoylpropionic acid hydrolyzed to the corresponding acid by the method of Lapworth and Wechsler.[7] The resulting acid was then brominated by slowly adding one equivalent of bromine to a solution of 50 g. of the pure, dry acid in 250 cc. of chloroform. Bromination started promptly and proceeded rapidly at the ordinary temperature but the solution was boiled toward the end of the operation in order to prevent premature deposition of the sparingly soluble bromo acid. The solution solidified when allowed to cool. The solid was freed from chloroform by suction, washed with water and recrystallized from glacial acetic acid. It separated in colorless needles melting at 207–208°; yield, about 50 g.

Inasmuch as the bromo acid is both an α-bromo ketone and a β-bromo acid it gives a variety of products when it is treated with various reagents capable of replacing bromine with hydroxyl. The following experiment illustrates a satisfactory method for securing the hydroxy acid. Twenty g. of the bromo acid was dissolved in two equivalents of N aqueous sodium hydroxide. The orange-yellow solution gradually deposited a small quantity of flocculent material. After two hours this was removed by filtration. Ether was poured on top of the clear solution, which was then acidified with dil. hydrochloric acid. The ethereal layer was washed, dried, concentrated and diluted with petroleum ether. It gradually deposited 11 g. of acid which melted at 142°, and this melting point was the same after admixture of the acid which was obtained by hydrolyzing the oximido ester.

Reduction of the Hydroxyketonic Acid.—Since other experiments showed that in the presence of bases some hydroxyketones undergo a rearrangement that involves transposition of the hydroxyl and carbonyl groups, it was necessary to prove that this had not occurred during the replacement of bromine by hydroxyl. For this purpose 0.4 g. of red phosphorus and 6 g. of 45% hydriodic acid were added to a solution of 2 g. of the hydroxy acid in 20 g. of glacial acetic acid. This mixture was boiled for an hour, then filtered. The filtrate was diluted with water and shaken with ether. This extracted 1.2 g. of β-phenyl-γ-benzoylpropionic acid—proof that the carbonyl group had not been shifted in the operations involved in the preparation of the hydroxy acid.

Preparation of the Oximido Ester from the Hydroxyketonic Acid.—From the ketonic acid the oximido ester may be made either by first esterifying or by first introducing the oximido group. The latter course is the more satisfactory. Thus, 2 g. of hydroxylamine hydrochloride was added to a solution of 5 g. of the acid in thrice the equivalent quantity of N sodium hydroxide solution. The solution was warmed for 15 minutes, then cooled and very cautiously acidified in the presence of ether. The ether layer yielded 3.5 g. of the same oximido acid which had been obtained originally by hydrolyzing the oximido ester and from which the ester is readily obtained by esterification either through the silver salt or with methyl alcohol and hydrogen chloride. The proof of the structure of the ester is, therefore, complete.

II. Acylation of the Oximido Ester

The oximido ester has two hydroxyl groups which are sufficiently different in character to permit acylation in steps. Thus acetic anhydride in a few hours converts the ester completely into a mono-acetate and requires weeks to form a diacetate. The ester is too sensitive to alkalies for acylation by the Schotten-Baumann reaction but it can be benzoylated by al-

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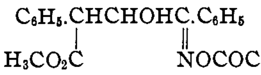
[6] Kohler and Allen, This Journal, **46**, 1526 (1924).

[7] Lapworth and Wechsler, *J. Chem. Soc.*, **97**, 41 (1910).

lowing it to react with benzoyl chloride in the presence of ether and sodium carbonate. Under these conditions only one benzoyl group is introduced, but the dibenzoate can be obtained without difficulty by operating in pyridine.

The structure of the diacyl derivatives follows directly from that of the ester. For the mono-acyl derivation two formulas are possible; in view of the great ease with which oximes are acylated, it seems altogether probable that the first acyl enters the oximido group. For the same reason the second mono-acetate—obtained by hydrolyzing the diacetate—doubtless is to be represented by XII.

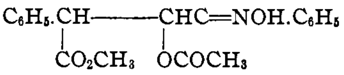


$$\begin{array}{l}\mathrm{C_6H_5.CHCHOHC.C_6H_5}\\ \qquad\;|\qquad\qquad\quad\|\\ \mathrm{H_3CO_2C\qquad\quad NOCOCH_3}\end{array}$$

X (VII)

$$\begin{array}{l}\mathrm{C_6H_5.CHCHOHC.C_6H_5}\\ \qquad\;|\qquad\qquad\quad\|\\ \mathrm{H_3CO_2C\qquad\quad NOCOC_6H_5}\end{array}$$

XI



$$\begin{array}{l}\mathrm{C_6H_5.CH\text{———}CHC{=}NOH.C_6H_5}\\ \qquad\quad|\qquad\quad|\\ \qquad\mathrm{CO_2CH_3\;\;OCOCH_3}\end{array}$$

XII (XVII)

THE MONO-ACETATE.—The finely powdered ester was shaken with twice its weight of acetic anhydride for eight hours. The liquid was then allowed to evaporate in a vacuum desiccator containing solid sodium hydroxide, the residue was washed with cold ether and recrystallized from a mixture of ether and petroleum ether. One recrystallization gave a pure product melting at 126°.

Anal. Calcd. for $C_{19}H_{19}O_6N$: C, 66.9; H, 5.6; OCH_3, 9.1. Found: C, 66.9; H, 5.4; OCH_3, 8.8.

The ester is very easily regenerated by boiling the acetate with methyl alcohol containing a trace of acid. The acetate dissolves in cold, *N* aqueous potassium hydroxide with little change in color but, like the ester itself, is cleaved to benzonitrile, phenylacetic acid and formic acid.

THE MONOBENZOATE, XI.—An ethereal solution containing 3 g. of the oximido ester and 10 g. of benzoyl chloride was shaken with excess of a saturated solution of sodium carbonate for ten hours. The ethereal layer was separated, dried and evaporated. It left a small quantity of a high-melting solid imbedded in a colorless oil. When a solution of this oil in absolute ether was diluted with an equal volume of petroleum ether, it deposited the pure benzoate in large, colorless plates; m. p., 114°.

Anal. Calcd. for $C_{24}H_{21}O_6N$: C, 71.4; H, 5.2. Found: C, 71.4; H, 5.3.

THE DIBENZOATE.—A solution of 2 g. of the ester and 10 g. of benzoyl chloride in 15 g. of pyridine was heated on a steam-bath for 30 minutes, then cooled and poured into water. The ethereal extract of the aqueous suspension was freed from pyridine by shaking with dil. hydrochloric acid, then dried, concentrated and diluted with petroleum ether. It deposited needles which melted at 113°. This is almost the same melting point as that of the monobenzoate, but a mixture of the two melted 25° lower.

Anal. Calcd. for $C_{31}H_{25}O_6N$: C, 73.4; H, 4.7. Found: C, 73.2; H, 4.7.

THE DIACETATE.—The diacetate may be made by keeping a solution of the ester in acetic anhydride for several weeks, but it is more easily obtained by dissolving either the ester or its mono-acetate in acetyl chloride and allowing this solution to evaporate slowly over solid sodium hydroxide in a vacuum desiccator. It was purified by crystallization from ether-petroleum ether from which it separates in tables; m. p., 98°.

Anal. Calcd. for $C_{21}H_{21}O_6N$: C, 65.8; H, 5.5; OCH_3, 8.1. Found: C, 65.7; H, 5.4; OCH_3, 7.9.

THE ISOMERIC MONO-ACETATE, XII.—A solution of 5 g. of the diacetate in 50 cc. of ether was shaken with 50 cc. of *N* aqueous ammonia for 24 hours. The ethereal layer, on evaporation, yielded 4 g. of a product which after purification by recrystallization from ether-petroleum ether melted at 142°. Like its isomer this acetate regenerated the oximido ester when its solution in methyl alcohol was saturated with hydrogen chloride.

Anal. Calcd. for $C_{19}H_{19}O_6N$: C, 66.9; H, 5.6; OCH_3, 9.1. Found: C, 67.0; H, 5.3; OCH_3, 9.0.

α-Phenyl-β-benzoylacrylic Acid, XIII.—Like the two isomeric mono-acetates, the diacetate of the oximido ester gradually dissolves in normal potassium hydroxide, but unlike these it is not degraded into simpler compounds by the alkali. When a solution that had been kept for an hour at the ordinary temperature was acidified and extracted with ether, it yielded a substance that crystallized from a mixture of ether and petroleum ether in fine needles; m. p., 124°.

Anal. Calcd. for $C_{16}H_{12}O_3$: C, 76.2; H, 4.7. Found: C, 76.1; H, 4.6.

The substance was soluble in sodium carbonate, gave a crystalline copper compound when its ethereal solution was shaken with aqueous copper acetate, and its solution in acetone reduced permanganate.

Ozonization.—A solution in chloroform was saturated with ozone, then evaporated in a current of air. It left a pasty ozonide which dissolved when shaken with ice water. The resulting solution was shaken with phenylhydrazine hydrochloride. It deposited a crystalline compound that was identified as the phenylhydrazone of benzoylformic acid by comparison with a sample made directly from the acid. As this was the sole product formed in the ozonization, the original substance must be phenylbenzoylacrylic acid.

Preparation from α-Phenyl-β-benzoylpropionic Acid.—When the methyl ester of the ketonic acid is brominated in chloroform it forms two monobromo derivatives, both of which crystallize in needles and which melt, respectively, at 78° and 108°. These are manifestly stereo-isomers because boiling methyl alcohol converts the lower-melting into the higher-melting compound.

Anal. Calcd. for $C_{17}H_{15}O_3Br$: C, 58.8; H, 4.3; Br, 23.1. Found (108°): C, 58.6; H, 4.4; Br, 22.7; (78°) Br, 23.1.

A methyl alcoholic solution of these bromo compounds was boiled with excess of potassium hydroxide for 15 minutes, then poured into water and acidified. From the water solution ether extracted an acid that was purified by recrystallization from a mixture of ether and petroleum ether. It melted at 124° and proved to be the same acid that was obtained by the action of alkalies on the diacetate of the oximido ester. The yield was excellent.

III. 3,5-Diphenyl-6-oxo-orthoxazine

The orthoxazine derivative crystallizes very slowly from the oily by-products of the reaction between potassium acetate and the bromonitro ketone. It is easily made by treating phenylbenzoylacrylic acid with an equivalent quantity of hydroxylamine but it is more readily prepared in quantity directly from the methyl ester of β-bromophenylbenzoylpropionic acid. The procedure is as follows.

A solution of 50 g. of the bromo ester and 15 g. of hydroxylamine hydrochloride in 400 cc. of 5% methyl alcoholic potassium hydroxide is boiled for an hour, then distilled until the orthoxazine derivative begins to crystallize from the hot solution. It separates in flat needles and is easily purified by recrystallization from methyl alcohol; yield, 24 g.

Anal. Calcd. for $C_{16}H_{11}O_2N$: C, 77.1; H, 4.4. Found: C, 77.2; H, 4.5.

3,5-Diphenyl-6-oxo-orthoxazine, XV, is sparingly soluble in ether, moderately soluble in alcohol, readily soluble in acetone. It melts at 153°. It does not combine with bromine but its solution in acetone readily reduces permanganate.

Ozonization.—The oxazine was ozonized in chloroform and the ozonide decomposed with water in the customary manner. The water solution, when heated with phenylhydrazine hydrochloride, gave the phenylhydrazone of benzoylformic acid.

Change to **3,5-Diphenyl-isoxazole.**—Silver nitrate was added in excess to the strongly alkaline solution of the oxazine in potassium hydroxide. The resulting mixture, when warmed on a steam-bath, almost immediately began to deposit a new substance in glistening plates. After recrystallization this melted at 142°. It was identified as diphenyl-isoxazole by comparing its melting point with that of a mixture of the substance and the isoxazole. The same isoxazole was obtained when a solution of the oxazine in acetyl chloride was boiled for several hours.

Summary

1. The principal products of the reaction between methyl alcoholic potassium acetate and α-bromo-β-phenyl-γ-nitropropiophenone are a nitrocyclopropane derivative and an hydroxy-oximido ester and not, as stated in an earlier paper, a nitrocyclopropane derivative and an hydroxy-isoxazolidine.

2. One of the by-products of the reaction is an oxazine derivative. This, like the oximido ester, has been synthesized by methods that leave no doubt as to its structure.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF OREGON]

THE CONTROL OF "BIOS" TESTING AND THE CONCENTRATION OF A "BIOS"

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I. Introduction

The stimulating effect produced by a given extract upon the growth of yeast cannot, as originally suggested by one of us,[1,2] be taken as a satisfactory measure of the amount of vitamin B in the extract. This fact has been proved by our own experiments[3] as well as those of others. It remains an interesting question whether or not the substances which are responsible for the stimulation of yeast growth are among those which also affect the growth of animals. Little doubt remains that what has been for convenience called "vitamin B" is a group of substances. Evi-

[1] Williams, *J. Biol. Chem.*, **38**, 465 (1919).

[2] Williams, *ibid.*, **42**, 259 (1920).

[3] Williams, *ibid.*, **46**, 113 (1921).