[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HOWARD UNIVERSITY]

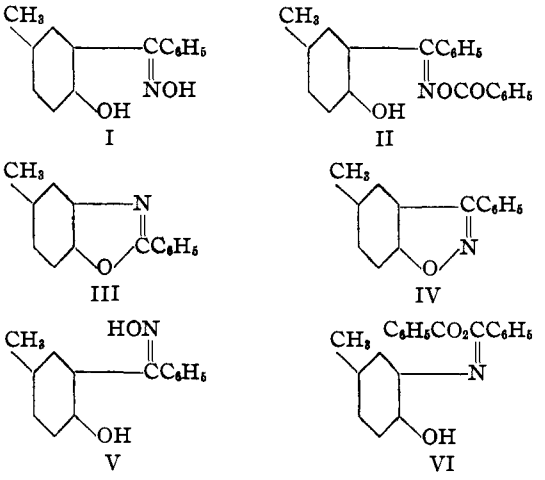
The Chemical Effects Accompanying Hydrogen Bonding. I. Acyl Derivatives of the 2-Hydroxy-5-methylbenzophenone Oximes

BY A. H. BLATT

The concept of hydrogen bonding was introduced into organic chemistry as early as 1913 by Pfeiffer[1] and since has been used extensively in order to account for deviations from the expected physical and chemical properties shown by certain organic compounds. This procedure of ascribing to the hydrogen bond the responsibility for deviations from the expected properties has for some time been recognized as unsatisfactory and numerous studies have been made, particularly by Sidgwick and his collaborators,[2] in order to learn by the comparison of bonded and non-bonded isomers precisely what effects do accompany bonding. The most significant results so far obtained have come from the examination of the physical properties of isomeric disubstituted benzene derivatives such as, for example, the *ortho* and *meta* nitrophenols, but it is obvious from steric considerations that, in so far as *chemical* interaction between the substituents is concerned, *ortho* and *meta* disubstituted benzene derivatives are not strictly comparable. Within the past year it has been shown[3] that the isomeric oximes of ketones such as *o*-hydroxybenzophenone differ from each other in that one isomer contains a hydrogen bond between oxygen and nitrogen while the other does not. Isomeric oximes of this type offer the most favorable opportunity for learning by direct comparison the chemical effects due to bonding and we have undertaken a study of such isomers in order to secure this information. In the present paper we report on the acyl derivatives of the 2-hydroxy-5-methylbenzophenone oximes.

Pyridine benzoylation of these oximes furnishes the oximino benzoates. The bonded benzoate (II) is hydrolyzed by sodium hydroxide to the parent oxime (I). It is rearranged by sodium carbonate to the benzoxazole (III) and is converted smoothly to the benzisoxazole (IV) on pyrolysis. The non-bonded benzoate (VI) is hydrolyzed to the parent oxime (V) with sodium hydroxide or sodium carbonate and on pyrolysis, which is not a clean-cut process, furnishes small amounts of the benzoxazole (III).

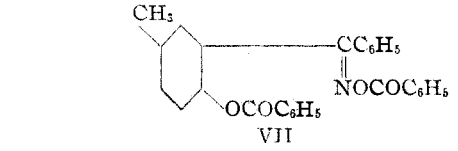
(1) Pfeiffer, *Ann.*, **398**, 137 (1913).
(2) Sidgwick and Callow, *J. Chem. Soc.*, 527 (1924).
(3) Blatt and Russell, THIS JOURNAL, **58**, 1903 (1936).

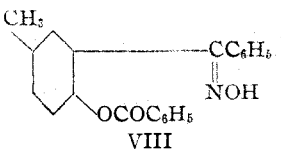


These results with the oximino benzoates confirm and parallel with one exception those reported earlier for the corresponding acetates.[3] That exception is the behavior on pyrolysis of the non-bonded acetate and benzoate. The latter gives benzoxazole; the former was reported to furnish a mixture of products, presumably benzoxazole and benzisoxazole, from which only the benzisoxazole was isolated. We have repeated the pyrolysis of the non-bonded acetate using larger quantities of material and have found that it does yield a mixture of benzisoxazole and benzoxazole. Because the proportions in which the two heterocycles are formed vary in different runs and because more of the isoxazole is formed from the acetate than from the benzoate, we believe that the primary pyrolysis product of the non-bonded acylated oximes is the benzoxazole; the benzisoxazole is presumably the result of a shift of configuration which takes place on heating and which precedes pyrolysis.

Benzoylation of the 2-hydroxy-5-methylbenzophenone oximes by the Schotten–Baumann procedure furnishes the dibenzoyl derivatives. Their behavior shows that, with replacement of the phenolic hydrogen atom, hydrogen bonding and its accompanying chemical effects disappear completely. The dibenzoate (VII) is hydrolyzed without rearrangement by sodium hydroxide or sodium carbonate. Sodium carbonate removes the oximino benzoyl group preferentially to fur-

nish the phenolic benzoate (VIII). Sodium hydroxide, depending upon the concentration and length of treatment, hydrolyzes the dibenzoate (VII) either to the same monobenzoate or to the parent oxime (I). The isomeric dibenzoate (IX) is not hydrolyzed by sodium carbonate but is hydrolyzed readily by sodium hydroxide, first to the oximino monobenzoate (VI) and then to the non-bonded oxime (V). The difference in the point of hydrolytic attack in the dibenzoates (VII) and (IX) is particularly noteworthy; in the former the oximino benzoyl group is removed while in the latter the phenolic benzoyl group is removed. This difference confirms on chemical grounds the suggestion made by Dr. S. B. Hendricks from symmetry considerations that in the non-bonded oxime and its derivatives, where there is no restriction upon free rotation about the bond between the substituted phenyl group and the extra-nuclear carbon atom, the configuration shown in formulas V and VI would be favored. For, while on steric grounds one would expect the oximino benzoyl group to be attacked first in VII, only an open configuration such as that in IX, and, not the conventional configuration shown in X is consistent with the preferential hydrolysis of the phenolic benzoyl group in the dibenzoyl derivative of the non-bonded oxime.


VII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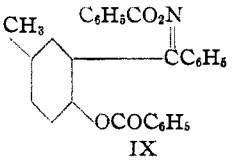
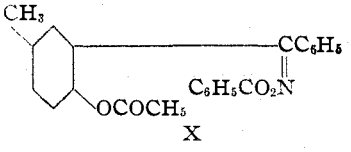
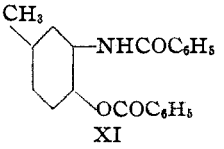
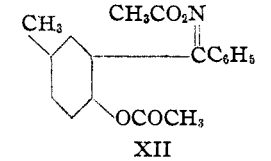

IX

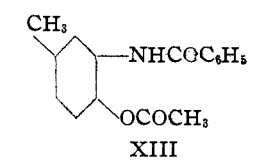

X


XI

The phenolic monobenzoate (VIII) contains a free oximino group and its behavior on a Beckmann rearrangement is significant. The oximes of *o*-hydroxy aromatic ketones containing a hydrogen bond between oxygen and nitrogen form benzoxazoles as Beckmann rearrangement products; the reaction is one variant of the second order Beckmann rearrangement. That this reaction is a consequence of hydrogen bonding is shown by the fact that the oxime (VIII), whose configuration is such as to lead to the formation of a benzoxazole but in which the hydrogen necessary for bonding has been replaced, undergoes a normal rearrangement to yield the amide (XI).

Our attempts to secure diacetates and a phenolic monoacetate corresponding to the benzoyl derivatives just described were only partially successful but they led to information of considerable interest in connection with shifts of configuration in the oximes and their derivatives. On treatment with acetyl chloride at room temperature the bonded oxime (I) underwent a Beckmann rearrangement to yield the benzoxazole (III). Acetyl chloride at its boiling point furnished the acetate of the bonded oxime. Prolonged action of boiling acetyl chloride on this acetate brought about a shift of configuration and gave the non-bonded oxime diacetate (XII). The same diacetate (XII) was obtained from the non-bonded oxime (V) or its acetate by the action of acetyl chloride either cold or at its boiling point. The non-bonded diacetate is hydrolyzed to the non-bonded oxime by alkali hydroxide or carbonate and by acids. Preferential hydrolysis of one acetyl group could not be effected. Although the change from the bonded to the non-bonded configuration on treatment with acetyl chloride made our attempts to secure a bonded diacetate unsuccessful, we secured indirect evidence of the existence of such a diacetate and of the reverse change from the non-bonded to the bonded configuration. When the non-bonded oxime or its acetate, the bonded oxime or its acetate, or the non-bonded oxime diacetate was treated with acetic anhydride and sulfuric acid, the product was O-acetyl-N-benzoyl-aminocresol (XIII). In our opinion this material —the Beckmann rearrangement product of the, as yet, not isolated phenolic acetate (XIV)—results from the partial hydrolysis and rearrangement of the diacetate of the bonded oxime.


XII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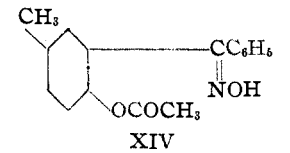
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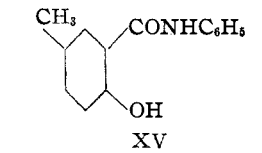
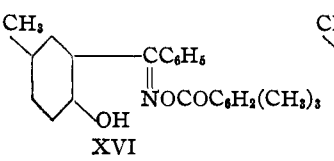

XV

Treatment of the non-bonded oxime (V) in pyridine or in 20% potassium hydroxide solution with benzenesulfonyl chloride furnished, as was to be expected, *p*-cresotinic anilide (XV), the normal rearrangement product. Similarly, the bonded oxime (I) in pyridine with benzenesulfonyl chloride yielded, normally, the benzoxazole (III). When, however, the bonded oxime in 20% potassium hydroxide reacted with benzenesulfonyl chloride, the product was the benzisoxazole (IV). This *trans* ring closure, which is, to the best of our information, the first successful direct conversion of an *o*-hydroxyaromatic ketoxime to a benzisoxazole, indicates that removal even by salt formation of the hydrogen atom necessary for bonding can destroy bonding. Incidentally, this observation confirms the assumption made by Meisenheimer[4] in 1924 that dehydration of an *o*-hydroxybenzophenone oxime to a benzisoxazole would be a *trans* ring closure and would take place with the isomer in which the oximino hydroxyl group is *anti* to the phenolic hydroxyl group.

Since we had found previously[5] that the introduction of highly hindered acyl groups was of value in distinguishing between those reactions of acylated oximes which involved addition and those which did not, we prepared and examined the 2,4,6-trimethylbenzoates of the 2-hydroxy-5-methylbenzophenone oximes. The bonded trimethylbenzoate (XVI), like the corresponding acetate and benzoate, yielded the benzoxazole (III) on treatment with sodium carbonate and on pyrolysis furnished the benzisoxazole (IV). This same trimethylbenzoate, in contrast with the acetate and benzoate, also furnished benzoxazole on treatment with sodium hydroxide. The non-bonded trimethylbenzoate (XVII) decomposed on pyrolysis and was not affected by the same treatment with carbonate or hydroxide which served to convert its isomer to benzoxazole. However, when the time of the reaction with sodium hydroxide was extended this trimethylbenzoate, since hydrolysis was prevented, also furnished benzoxazole. It is clear from these results, taken in conjunction with those obtained with the acetate and benzoate, that hydrolysis and benzoxazole formation proceed through different mechanisms and that an increase in the concentration of base has a greater effect upon the rate of hydrolysis than upon the rate of benzoxazole formation. Hydrolysis, which can be stopped by hindrance, involves an addition reaction while benzoxazole formation, which is unaffected by hindrance, does not involve addition. The effect of bonding is to facilitate the formation of benzoxazole but the same result can also be brought about by preventing the competing reaction of hydrolysis.



XVI XVII

The results which we presented earlier[3] together with those in this article establish the existence of a series of chemical reactions in which *o*-hydroxybenzophenone oximes and their acyl derivatives containing a hydrogen bond between oxygen and nitrogen differ from their non-bonded stereoisomers. That these differences in chemical behavior can legitimately be ascribed to bonding is evidenced by the fact that they disappear when the hydrogen atom necessary for bonding is replaced. It is still too early to speak with any confidence of the exact way in which hydrogen bonding operates to bring about these effects but it is clear that bonding acts primarily to facilitate certain reactions at the expense of other reactions; it does not make one reaction inevitable and another reaction impossible. This may be restated in somewhat different fashion as follows. Hydrogen bonding, like other types of valence in organic compounds, reveals itself through characteristic chemical behavior. However, the strength of hydrogen bonds, as measured by the energy required to break them,[6] is small by comparison with other types of valence and it is not surprising, therefore, that the chemical effects which accompany bonding are much less inevitably associated with it than are the chemical effects associated with other, stronger types of valence.

Experimental

The 2-hydroxy-5-methylbenzophenone oximes have been described previously.[3] Their benzoates and 2,4,6-trimethylbenzoates were prepared in the usual fashion by mixing cold pyridine solutions containing equimolar quantities of oxime and acid chloride. After six hours in the case of the benzoates and twenty hours in the case of the trimethylbenzoates, the reaction mixtures were poured onto ice and dilute hydrochloric acid. When the products

(4) Meisenheimer and Meis, *Ber.*, **57**, 289 (1924).
(5) Blatt and Barnes, THIS JOURNAL, **58**, 1900 (1936).
(6) For a summary of the values of the energy of dissociation of hydrogen bonds see Huggins, *J. Org. Chem.*, **1**, 414 (1936).

were not obtained as solids at this stage of the procedure an ether extraction, followed by appropriate washing, drying and evaporation of the ether, furnished crystalline material. The dibenzoates were prepared by the Schotten–Baumann process using one mole of oxime in ten moles of 2 *N* sodium hydroxide and adding four moles of benzoyl chloride. The solid dibenzoates precipitated promptly. The solvents used for purification, the melting points and the analytical data for the acyl derivatives are given in Table I below.

TABLE I

Substance	Structural formula	Empirical formula	M. p., °C.	Crystallized from	Analyses, % Calcd. C	Calcd. H	Found C	Found H
syn-Phenyl-2-hydroxy-5-methylphenyl ketoxime benzoate	II	$C_{21}H_{17}O_3N$	148–149	Ethanol	76.1	5.1	76.0	5.5
anti-Phenyl-2-hydroxy-5-methylphenyl ketoxime benzoate	VI	$C_{21}H_{17}O_3N$	174–175	Ethanol	76.1	5.1	76.5	5.4
syn-Phenyl-2-benzoyloxy-5-methylphenyl ketoxime benzoate	VII	$C_{28}H_{21}O_4N$	147–148	Ethanol	77.2	4.8	77.1	4.6
anti-Phenyl-2-benzoyloxy-5-methylphenyl ketoxime benzoate	IX	$C_{28}H_{21}O_4N$	132–133	Acetone–petroleum ether	77.2	4.8	77.2	5.0
syn-Phenyl-2-hydroxy-5-methylphenyl ketoxime 2,4,6-trimethylbenzoate	XVI	$C_{24}H_{21}O_3N$	149–150[7]	Ethanol	77.6	6.2	77.54	6.3
anti-Phenyl-2-hydroxy-5-methylphenyl ketoxime 2,4,6-trimethylbenzoate	XVII	$C_{24}H_{23}O_2N$	176–177	Dilute ethanol	77.6	6.2	77.3	6.5

Reactions with Sodium Carbonate.—In the sodium carbonate reactions 0.5 g. of the acyl derivative was suspended or dissolved in 25 cc. of alcohol and 0.5 g. of sodium carbonate dissolved in 5 cc. of water was added. After eighteen hours of mechanical shaking, 70 cc. of water was added and the resulting precipitate filtered and identified. In those reactions in which hydrolysis took place the presence of the ethyl ester of the acid corresponding to the acyl group removed was evidenced by its odor; in those reactions in which rearrangement took place the acid corresponding to the acyl group removed could be isolated.[8] The benzoate (II) furnished 0.3 g. of crude benzoxazole (III); the benzoate (VI) gave no solid material on dilution with water but acidification furnished the non-bonded oxime (V); yield of pure product, 0.15 g. The dibenzoate (VII) furnished 0.17 g. of the monobenzoate (VIII). This monobenzoate is described in the next paragraph. The dibenzoate (IX) was unaffected by sodium carbonate, as was the non-bonded trimethylbenzoate (XVII). The bonded trimethylbenzoate (XVI) furnished benzoxazole and trimethylbenzoic acid.

Reactions with Sodium Hydroxide.—In general, 10 cc. of 5% sodium hydroxide was added to a suspension of 0.5 g. of the acyl derivative in 20 cc. of alcohol. After a suitable time of reaction, 70 cc. of water was added and the solution was chilled and acidified. The remarks made about the formation of esters in the sodium carbonate reactions apply equally to the sodium hydroxide reactions. The bonded benzoate (II) furnished after ten minutes 0.3 g. of the bonded oxime (I); the non-bonded benzoate (VI) under the same conditions yielded 0.3 g. of the non-bonded oxime (V). The dibenzoate (VII), when the reaction was run for twenty-four hours, furnished 0.25 g. of bonded oxime. The formation of ethyl benzoate in these reactions suggested that the effect of the sodium hydroxide might be largely catalytic and accordingly the use of smaller amounts of alkali was tried successfully in order to bring about partial hydrolysis. Thus when 6.6 g. of the dibenzoate (VII) suspended in 125 cc. of alcohol was shaken for sixteen hours with 5 cc. of 0.98 *N* sodium hydroxide (approximately one mole of alkali to three moles of dibenzoate), 0.45 g. of unattacked material remained undissolved. The filtrate from this material when poured into water furnished 2.5 g. of the oxime (VIII). For analysis the oxime was crystallized from alcohol.

Anal. Calcd. for $C_{21}H_{17}O_3N$: C, 76.1; H, 5.1. Found: C, 76.2; H, 5.2.

***syn*-Phenyl-2-benzoyloxy-5-methylphenyl ketoxime (VIII)** melts at 162–163°. It is hydrolyzed to the bonded oxime on treatment with sodium hydroxide. Pyrolysis gives no definite products. With phosphorus pentachloride in ether it undergoes a Beckmann rearrangement to yield, essentially quantitatively, O,N-dibenzoylaminocresol (XI) melting at 190–191°. Since both O,N-dibenzoylaminocresol and N-benzoylaminocresol are reported as melting at the same temperature[9] we identified the product as the dibenzoyl derivative by hydrolysis to the alkali soluble monobenzoyl derivative and by an analysis.

Anal. Calcd. for $C_{21}H_{17}O_3N$: C, 76.1; H, 5.1. Found: C, 75.7; H, 5.4.

The dibenzoate (IX) after sixteen hours with excess sodium hydroxide furnished the non-bonded oxime (V). Treatment with insufficient sodium hydroxide (one mole of sodium hydroxide to three moles of the dibenzoate) for twenty-four hours yields the oximino benzoate (VI). The bonded trimethylbenzoate (XVI) after ten minutes' treatment with sodium hydroxide furnished benzoxazole (III) and trimethylbenzoic acid. The non-bonded trimethylbenzoate (XVII) after seventy-two hours with sodium hydroxide was recovered almost completely but the formation of traces of trimethylbenzoic acid indicated some reaction. Consequently the reaction time was

(7) The first sample of this material melted at 109–109°. Later preparations melted at 149–150°, and the lower melting material was never obtained again. The reactions of the original and later preparations were identical.

(8) Compare Reference 5.

(9) (a) Auwers and Czerny, *Ber.*, **31**, 2695 (1898); (b) Raiford and Ransome, THIS JOURNAL, **46**, 2312 (1924).

extended until it was found that after nineteen days this trimethylbenzoate was converted to benzoxazol and trimethylbenzoic acid.

Pyrolyses.—To the bare statement of the pyrolysis products given in the discussion little need be added except that the thermal decompositions were carried out with 0.5-g. samples in the vacuum obtained with an oil pump. A brief description of the large scale pyrolyses of the oxime acetates is, however, in order. When 3.0 g. of the bonded acetate was pyrolyzed and the liquid distillate was dissolved in hot alcohol the first, large crop of crystals consisted of the benzisoxazole (IV) melting at 92–93°. The mother liquors deposited succeeding crops which were mixtures, separable by hand, and consisting of benzisoxazole (cubes) and benzoxazole (prisms) melting at 102–103°. The amount of benzoxazole was sufficient only for identification by a mixed melting point. When 2.5 g. of the non-bonded acetate was pyrolyzed there was a considerable amount of brown tarry material formed. The distillate, taken up in hot alcohol and cooled, furnished a small first crop of benzisoxazole. All the succeeding crops were mixtures of benzoxazole and benzisoxazole which melted by 80° and whose melting point was not raised by admixture with isoxazol but was raised by addition of oxazole. Since a separation by crystallization was unsuccessful, the impure solid was boiled for eight hours in a solution of equal volumes of alcohol and concentrated hydrochloric acid. After cooling, diluting with water and extracting with ether, the aqueous layer was made strongly alkaline and benzoyl chloride was added. This resulted in the precipitation of O,N-dibenzoylaminocresol which was identified by a mixed melting point. No attempt was made at an estimate of quantity since, although we found by separate experiments that the benzisoxazole (IV) is not affected by heating with alcoholic hydrochloric acid, the benzoxazole is hydrolyzed, but not quantitatively, by this treatment to aminocresol.

Reactions with Acetyl Chloride.—When 1.0 g. of the bonded oxime (I) was dissolved in 3 cc. of acetyl chloride at room temperature, left for one week and then the reagent evaporated in vacuum over alkali, the solid residue consisted of the benzoxazole (III). This result is not surprising since acetyl chloride has long been known to bring about Beckmann rearrangements.[10] When 0.5 g. of the bonded oxime was dissolved in acetyl chloride and heated at the boiling point of the reagent for thirty minutes, then cooled and the acetyl chloride removed, the crystalline residue was found to be the acetate of the bonded oxime. This acetate dissolved in cold acetyl chloride and left for a week, or dissolved in acetyl chloride at its boiling point and heated for six hours, furnishes the non-bonded oxime diacetate (XII); the first of these two methods gives only a fair yield, the second gives a quantitative yield. For analyses the diacetate was crystallized from alcohol.

Anal. Calcd. for $C_{18}H_{17}O_4N$: C, 69.45; H, 5.5. Found: C, 69.5; H, 6.1.

***anti*-Phenyl-2-acetoxy-5-methylphenyl ketoxime acetate (XII)** melts at 100°. It is hydrolyzed by sodium carbonate, sodium hydroxide and hydrochloric acid to furnish the non-bonded oxime (V). Its behavior toward acetic anhydride and sulfuric acid is described in the next section.

(10) Beckmann, *Ber.*, **20**, 1507, 2580 (1887).

The non-bonded oxime with acetyl chloride either at room temperature for one week or at the boiling point of the reagent for thirty minutes furnishes in excellent yield the diacetate just described. Similarly, the non-bonded acetate on comparable treatment furnishes the same diacetate.

Reactions with Acetic Anhydride and Sulfuric Acid.—The reagent used in these reactions consisted of 2 cc. of acetic anhydride to which five drops of concd. sulfuric acid had been added and which had been allowed to cool to room temperature. When to this reagent was added 0.5 g. of the bonded oxime, the non-bonded oxime, the bonded acetate, the non-bonded acetate, or the non-bonded diacetate, the solid dissolved to yield a pale yellow solution. After an hour the addition of water furnished in excellent yields O-acetyl-N-benzoylaminocresol (XIII) melting, after crystallization from alcohol, at 135–136°.[11] This product was identified by comparison with a sample prepared by acetylating N-benzoylaminocresol and by hydrolysis to N-benzoylaminocresole.

Reactions with Benzenesulfonyl Chloride.—When 0.9 g. of benzenesulfonyl chloride was added to a cold solution of 1.2 g. of the bonded oxime in 6 cc. of pyridine, considerable heat was evolved. The flask was cooled in tap water, left for a half hour and the contents then poured on ice and dilute hydrochloric acid. The precipitate after drying weighed 1.0 g. and a mixed melting point showed it to consist of benzoxazole.

When 0.9 g. of benzenesulfonyl chloride was added to a solution of 1.2 g. of the bonded oxime in 25 cc. of 20% potassium hydroxide much heat was again evolved. The reaction mixture was shaken for two hours during which time a yellow precipitate formed and the odor of the reagent disappeared. The precipitate was filtered and the filtrate on acidification furnished a very small amount of unchanged oxime. The main precipitate, which weighed 1.1 g., was crystallized from methanol; it melted at 92–93° and was identified by a mixed melting point as the benzisoxazole (IV).

When the non-bonded oxime in pyridine or in 25% potassium hydroxide solution was treated with benzenesulfonyl chloride exactly as described for the bonded oxime, the product obtained was *p*-cresotinic anilide (XV) identified by its melting point and by a mixed melting point with a synthetic sample.[12]

Summary

The behavior of the isomeric 2-hydroxy-5-methylbenzophenone oximes toward acylating agents and the behavior of the resulting acyl derivatives are described. From the behavior patterns thus established it is seen that bonded oximes and their acyl derivatives differ significantly in their chemistry from their non-bonded stereoisomers and that these differences are a result of bonding. The effect of bonding is primarily to facilitate or retard certain reactions, not to

(11) Auwers and Eisenlohr, *Ann.*, **369**, 225 (1909). See also reference 9b.

(12) Auwers and Jordan, *Ber.*, **58**, 34 (1925).

make them inevitable or impossible, and steric factors may either accentuate or mask this effect. Specifically, it has been shown that the direct conversion of an *o*-hydroxybenzophenone oxime, whose configuration is such that the oximino hydroxyl group is *anti* to the phenolic hydroxyl group, to a benzisoxazole can be effected.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE AND THE PARKE, DAVIS & COMPANY RESEARCH LABORATORIES]

Sterols. XXVIII. Pregnanetriols from Pregnancy Urine

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In the course of our work on the isolation of various sterol derivatives from pregnant mares' urine we have isolated two triols possessing the empirical formula $C_{21}H_{36}O_3$. In some respects they appear to bear the same relationship to each other as do *allo*-pregnanediol and pregnanediol. Provisionally we are referring to them as pregnanetriol-A and pregnanetriol-B. Compound A is less soluble than the isomer in ethyl alcohol and so separates first in the isolation procedure. Both compounds melt in the neighborhood of 300° with slight decomposition. Compound A forms a triacetate melting at 136° whereas Compound B forms a triacetate melting at 168°. It was of interest to find that in the form of their acetates the solubility relationship is reversed and that the acetate of Compound B is less soluble in methyl and ethyl alcohols than is Compound A.

A search of the literature shows that Haslewood, Marrian and Smith,[1] in an investigation of the neutral carbinols of mares' pregnancy urine, obtained a saturated alcohol which they suggested might be a pregnanetriol. From the physical properties above presented it appears that B may be identical with the compound already described by Haslewood, Marrian and Smith.

The neutral fraction of mares' pregnancy urine, consisting mainly of esters of carbinols and hydrocarbons, was hydrolyzed with an excess of alkali and extracted with a large volume of benzene. After removal of the solvent the tarry residue was allowed to stand for a prolonged period in order to solidify and crystallize. The tar was removed by washing with an equal volume of ether and the crystalline residue fractionated by recrystallization from ethyl alcohol. The more insoluble fraction (pregnanetriol-A) was obtained in about twice the quantity of the more soluble fraction. A more satisfactory and almost quantitative separation can be made by acetylating the crude mixture and separating the two isomeric triacetates by means of solvents. The triacetate of pregnanetriol-A is quite soluble in methanol and can be crystallized only upon dilution with water whereas the triacetate of pregnanetriol-B is quite insoluble in methanol. These acetates give depressions in melting points when mixed with each other, and upon hydrolysis give the original triols with much sharper melting points.

Both triols give an orange red color with a green fluorescence when warmed with concentrated sulfuric acid, which is also characteristic of the pregnanediols. Neither isomer precipitates with alcoholic digitonin solution.

That these compounds have an angular methyl group between rings A and B is indicated by the fact that when heated with platinum black under the same conditions under which neoergosterol is transformed into dehydroneoergosterol[2] no naphthalene derivative is obtained.

Compound A contains an —OH group in the 20-position as is shown by the fact that it gives a positive iodoform reaction showing the presence of a CH_3CHOH— group. One of the other hydroxyl groups is probably at the usual 3-position, and since the third hydroxyl also can be acetylated, one can assume that it is not a tertiary hydroxyl. From the reactivity of the third hydroxyl group, about which we expect to publish later, we have indications that it is in the same position in the nucleus as the unreactive nuclear-OH group in the cortical hormone derivative of Reichstein.[3] Thus we may speculate on the possibility of this new compound being derived from the hormone of the adrenal cortex. Papers dealing with the structures

(1) Haslewood, Marrian and Smith, *Biochem. J.*, **28**, 1316 (1934).

(2) Honigmann, *Ann.*, **511**, 292 (1934).

(3) Reichstein, *Helv. Chim. Acta*, **19**, 29, 223, 402, 979, 1107 (1936).