Experimental

Reagents.—The preparations of the boron trifluoride catalyst and monoalkylacetylenes have been previously described.[6,7] The alcohols used were C. P. grade, carefully fractionated before use.

Action of *n*-Hexanol on *n*-Butylacetylene.—The catalyst was prepared by heating momentarily 5 g. of red mercuric oxide, 2 ml. of $(C_2H_5)_2O{\cdot}BF_3$ and 2 ml. of methanol. To this was added 120 g. (1.25 moles) of *n*-hexanol and 1 g. of trichloroacetic acid. The mixture was placed in a 1-liter 3-necked flask equipped with a mercury-sealed motor-driven stirrer, a reflux condenser and a dropping funnel. After heating the contents of the flask to 50–60° on a water-bath, 41 g. (0.5 mole) of *n*-butylacetylene was added dropwise at such a rate that the temperature of the reaction mixture did not exceed 70°. After addition of the acetylene, the source of heat (water-bath) was removed and the mixture stirred until it reached room temperature. Then 1 g. of sodium dissolved in a little methyl alcohol was added to neutralize acidity. Fractionation yielded 92 g. (62%) of 2,2-dihexoxyhexane.

Proof of the Structure of 2,2-Dialkoxyalkanes.—Hydrolysis of the 2,2-dialkoxyalkanes with dilute sulfuric acid yielded the corresponding alcohol and methyl *n*-butyl or methyl *n*-amyl ketone. The latter were identified by the melting points of their semicarbazones.

Summary

1. A series of 2,2-dialkoxyalkanes has been prepared from monoalkylacetylenes with the normal monohydric alcohols other than methanol in the presence of mercuric oxide, boron trifluoride and trichloroacetic acid.

2. The ketals obtained from ethanol, *n*-propanol, *n*-butanol, *n*-pentanol and *n*-hexanol with *n*-butyl and *n*-amyl acetylene are described.

3. With branch-chained monohydric alcohols, ketals have not been isolated by this method.

NOTRE DAME, INDIANA RECEIVED NOVEMBER 25, 1935

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[6] Hennion, Hinton and Nieuwland, THIS JOURNAL, **55**, 2858 (1933).

[7] Hennion, Killian, *et al.*, *ibid.*, **56**, 1130 (1934).

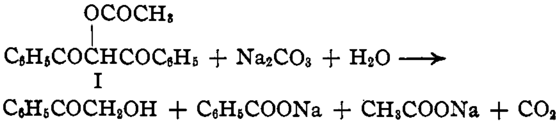
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HOWARD UNIVERSITY]

Hydroxy Polyketones. II. Dibenzoylcarbinol

BY A. H. BLATT AND W. LINCOLN HAWKINS

As part of a study of hydroxy polyketones[1] we have prepared and examined the chemical behavior of dibenzoylcarbinol, one of the simplest of this group of compounds which might reasonably be expected to exist as an ene-diol. While our work was in progress Bigelow, Rule and Black[2] reported the preparation of this carbinol by the catalytic hydrogenation of diphenyl triketone but, evidently having a different interest in the material than we had, these authors did not attempt the reactions reported here.

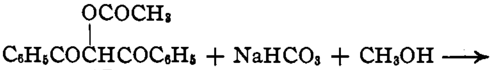
The starting material for our work was dibenzoylcarbinol acetate (I). Neufville and Pechmann,[3] who first prepared this substance, observed that its alcoholic solution gave a characteristic enol coloration with ferric chloride. We find that this alcoholic solution shows by Kurt Meyer titrations constant, though small, amounts of enol (5%). This enolic form, however, does not react with typical hydroxyl reagents. Our principal interest in the acetate was as a source of dibenzoylcarbinol and we have, therefoie, examined its hydrolysis under a variety of conditions. In alkaline solution the acetate undergoes both hydrolysis and cleavage; the products obtained depend upon the solvent employed and upon the hydroxyl-ion concentration. In aqueous sodium carbonate the acetate furnishes benzoic and acetic acids and benzoylcarbinol.



$$\underset{\text{I}}{C_6H_5CO\overset{OCOCH_3}{C}HCOC_6H_5} + Na_2CO_3 + H_2O \longrightarrow$$
$$C_6H_5COCH_2OH + C_6H_5COONa + CH_3COONa + CO_2$$

(1)

When the acetate is treated with aqueous sodium hydroxide, it dissolves and can be recovered if the solution is quickly acidified. After standing, however, the solution furnishes benzoic and acetic acids but no benzoylcarbinol. The absence of the carbinol is to be expected for it is destroyed by alkali hydroxides.[4] In aqueous alcoholic solution the cleavage is more interesting. Using sodium bicarbonate in methyl alcohol, benzoylcarbinol and acetic acid are formed as in aqueous solution but the third product is methyl benzoate.



$$C_6H_5CO\overset{OCOCH_3}{C}HCOC_6H_5 + NaHCO_3 + CH_3OH \longrightarrow$$

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[1] For the first paper on this subject see THIS JOURNAL, **57**, 1103 (1935).

[2] Bigelow, Rule and Black, *J. Chem. Soc.*, 83 (1935).

[3] Neufville and Pechmann, *Ber.*, **23**, 3377 (1890).

[4] (a) Hunnius, *ibid.*, **10**, 2010 (1877); (b) Zincke, *Ann.*, **216** 310 (1883).

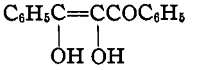
$$C_6H_5COCH_2OH + CH_3COONa + C_6H_5COOCH_3 + CO_2 \quad (2)$$

When ethyl alcohol is used as the solvent ethyl benzoate is formed. Since esters are not formed, from an alcohol and the sodium salt of an acid, this cleavage must involve the addition of alcohol followed by cleavage of the addition product and, from the color changes during the course of the reaction, it is probably the ene-diol (III) which adds alcohol. If the acetate is treated with sodium hydroxide in alcoholic solution, the reaction follows the course outlined by equation 2 except that the benzoylcarbinol is destroyed.

In acid solution the acetate can be hydrolyzed without complete loss by cleavage and furnishes dibenzoylcarbinol (II) in moderate yield. The structure of the carbinol is established by its reaction with acetyl chloride to regenerate the acetate (I) and by its oxidation with nitric acid to diphenyl triketone. The carbinol on treatment in aqueous or alcoholic solution with carbonate or sodium hydroxide undergoes cleavages perfectly parallel to those of the acetate—indicating that the first step in the reaction between the acetate and alkali is hydrolysis of the acetoxy group.

$C_6H_5COCHOHCOC_6H_5$

II



$$C_6H_5\underset{\text{OH}}{\underset{|}{C}}=\underset{\text{OH}}{\underset{|}{C}}COC_6H_5$$

III

$C_6H_5COCH_2OCOC_6H_5$

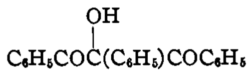
IV

Dibenzoylcarbinol is oxidized with ease. Ammoniacal silver nitrate, Fehling's solution and potassium permanganate are reduced in the cold. An ethereal solution of the carbinol shaken with copper acetate furnishes a copper derivative which almost immediately undergoes oxidation with the formation of cuprous oxide. In warm dilute acetic acid the carbinol is oxidized by copper acetate and furnishes benzil; diphenyl triketone, presumably the first oxidation product, is converted to benzil by copper acetate in acetic acid. Iodine, however, in neutral or acid solution does not oxidize dibenzoylcarbinol. Since oxidation with iodine is so characteristic of the ene-diol system[5] even when highly substituted as in the formoins,[6] we are forced to the conclusion that dibenzoylcarbinol does not exist as the ene-diol (III) except in alkaline solution. In view of the facts that dibenzoylmethane exists completely in the enolic form and that the acetate of dibenzoylcarbinol shows a small enol content by bromine titration, the results of the iodine titrations of dibenzoylcarbinol were unexpected and indicate an unusual resistance to the existence of the ene-diol system in open chain compounds.

In addition to cleavage and oxidation dibenzoylcarbinol is characterized by the rearrangement which it undergoes on vacuum distillation. The product of this distillation, a colorless solid, is isomeric with the carbinol. It contains no hydroxy group and is not attacked by the reagents which so readily oxidize the carbinol. On alkaline hydrolysis, however, like dibenzoylcarbinol it furnishes benzoic acid. The properties just described for the rearrangement product are those of the benzoate of benzoylcarbinol (IV) and a comparison of the rearrangement product with a synthetic sample of that benzoate showed them to be identical. This rearrangement of an α-hydroxy-β-diketone to the ester of an α-ketonic alcohol is novel in that it involves the shift of a carbon radical from carbon to oxygen. It confirms the interpretation given by Kohler and Erickson[7] for their observation that the addition of one mole of phenylmagnesium bromide to diphenyl triketone furnishes the benzoate of benzoin (V) instead of the expected phenyldibenzoylcarbinol (VI). In the case of phenyldibenzoylcarbinol rearrangement takes place so readily that the carbinol could not be isolated; dibenzoylcarbinol is stable except at elevated temperatures. In connection with this rearrangement attention should be called to the explanation given by Wolff[8] for the hydrolysis of diazo anhydrides. In this hydrolysis Wolff postulated the transient existence of analogs of dibenzoylcarbinol and assumed that they would undergo a shift of the hydroxyl group and an alkyl group, then lose carbon dioxide to furnish monoketones. This process with dibenzoylcarbinol would involve the migration of a phenyl group and would lead to desoxybenzoin. Under the conditions of our experiments we have never observed such a rearrangement.

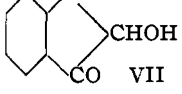
$C_6H_5COCH(C_6H_5)OCOC_6H_5$

V



$$C_6H_5CO\overset{\text{OH}}{\overset{|}{C}}(C_6H_5)COC_6H_5$$

VI



VII

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(5) Herbert, Hirst, *et al.*, *J. Chem. Soc.*, 1270 (1933); Micheel and Jung, *Ber.*, **66**, 1291 (1933); Euler and Martius, *Ann.*, **505**, 73 (1933).

(6) Karrer, *Helv. Chim. Acta*, **18**, 273, 1140 (1935).

(7) Kohler and Erickson, THIS JOURNAL, **53**, 2305 (1931).

(8) Wolff, *Ann.*, **325**, 144 (1902).

Dibenzoylcarbinol stands in the same relationship to diphenyl triketone as does the, as yet unknown, hydroxy diketone (VII) to ninhydrin. Since the substance VII is frequently assumed[9] to be an intermediate in the ninhydrin reaction, we thought it worth while to try the behavior of dibenzoylcarbinol toward certain typical amino acids. Dr. V. J. Tulane has been kind enough to make tests with alanine, glycine, tryptophan, histidine, methionine, glutamic acid and tyrosine and finds that dibenzoylcarbinol does not give a color test with these amino acids. In view of these results we are attempting the preparation of the cyclic compound (VII) in order to examine its behavior toward amino acids under the conditions of the ninhydrin reaction.

Experimental

Dibenzoylcarbinol acetate in 95% ethyl alcohol at room temperature shows by indirect bromine titrations an average enol content of 5.1% (individual titrations: 5.1, 5.0, 5.3). The acetate is not affected by acetyl chloride, by anisoyl chloride in pyridine or by acetic anhydride alone or with sulfuric acid. It furnishes no copper derivative with copper acetate.

Action of Alkali on the Acetate (I).—When a solution of 14.0 g. of the acetate in 125 cc. of warm methanol was mixed with 4.5 g. of sodium bicarbonate in 30 cc. of hot water a yellow solution resulted. After five minutes' heating the color faded and the reaction mixture was poured onto ice and water and extracted with ether. The only organic material in the aqueous layer was sodium acetate. The washed and dried ethereal extract left on evaporation 12.5 g. of oily residue which was vacuum distilled. The first fraction, 5.7 g., smelled strongly of oil of wintergreen. It had the composition of methyl benzoate (*Anal.* Calcd. for $C_8H_8O_2$: C, 70.6; H, 5.9. Found: C, 70.5; H, 5.8) and its identification as that ester was completed by conversion to triphenylcarbinol with phenylmagnesium bromide and by hydrolysis to benzoic acid. The second fraction, 4.0 g., solidified on cooling. After crystallization from alcohol it melted at 88°, reduced ammoniacal silver nitrate[10] and had the composition of benzoylcarbinol. *Anal.* Calcd. for $C_8H_8O_2$: C, 70.6; H, 5.9. Found: C, 70.3; H, 5.9. Its identification as benzoylcarbinol was completed by conversion to the oxime.[11]

When the acetate was treated with sodium carbonate in aqueous ethyl alcohol some benzoic acid was found in the water layer after decomposition and extraction with ether. Ethyl benzoate and benzoylcarbinol were present in the ether extract and, since they could not be separated by distillation, the carbinol was removed with bisulfite and identified by a mixed melting point, then the residual ester was identified by its alkaline hydrolysis to furnish benzoic acid.

When 1.4 g. of the acetate was shaken for twenty hours with 25 cc. of saturated sodium carbonate solution and the reaction mixture then extracted with ether, the aqueous layer furnished 0.5 g. of benzoic acid and the ether extract furnished benzoylcarbinol.

Using 5% sodium hydroxide as the reagent the acetate furnished benzoic acid when water was the solvent and methyl benzoate when methyl alcohol was the solvent. No benzoylcarbinol could be found.

Preparation and Properties of Dibenzoylcarbinol (II).—Five and nine-tenths grams of the acetate was added to 50 cc. of chilled sulfuric acid and the resulting orange-red solution was immediately poured through the shaft of a centrifugal stirrer into ice and water. The yellow solid which separated was extracted with peroxide-free ether and the ethereal extract washed free from acid and dried. The ether was removed in vacuum in a desiccator and the residue of dibenzoylcarbinol was filtered on a sintered glass filter and washed with small portions of cold alcohol until colorless. The filtrate, after removal of the solvent, was vacuum distilled and furnished 1.0 g. of benzoic acid and 0.6 g. of diphenyl triketone. The carbinol was crystallized from alcohol and melted at 111–112°; yield, 1.3 g. or 25%.

Anal. Calcd. for $C_{15}H_{12}O_3$: C, 75.0; H, 5.0. Found: C, 75.0; H, 5.1.

Dibenzoylcarbinol as prepared in this fashion agrees in its properties with the description given by Bigelow, Rule and Black. To that description and to the description given in the first part of this article can be added the following facts. The carbinol is destroyed by extended contact with sulfuric acid or with potassium cyanide. It does not furnish a product of the hydrindantin type when warmed in alcoholic solution with diphenyl triketone. With diazomethane only oily products are obtained. In alcoholic solution it is not oxidized by iodine even when the solution has been made acid with dilute sulfuric acid. The carbinol is quantitatively converted to its acetate by warming for thirty minutes with acetyl chloride. Thus 0.208 g. of carbinol furnished, after evaporation of excess acetyl chloride, 0.243 g. of the acetate instead of the calculated 0.244 g. The carbinol is oxidized to benzil by copper acetate in acetic acid. Thus 0.4131 g. of carbinol in 60 cc. of 60% acetic acid heated for one hour with an excess of copper acetate gave 0.25 g. of benzil which was identified by analysis and mixed melting point. *Anal.* Calcd. for $C_{14}H_{10}O_2$: C, 80.0; H, 4.76. Found: C, 80.0; H, 4.63. The cuprous oxide formed was filtered and on ignition furnished 0.2650 g. of cupric oxide. This corresponds to 0.967 atom of oxygen consumed per mole of carbinol.

Action of Alkali on the Carbinol.—To 25 cc. of saturated sodium carbonate solution there was added 1.2 g. of the carbinol and the reaction mixture was shaken for sixty hours, then extracted with ether. The aqueous layer furnished 0.5 g. of benzoic acid and the ether extract furnished benzoylcarbinol. When aqueous sodium hydroxide was used the carbinol furnished benzoic acid but no benzoylcarbinol. In methyl alcohol solution using sodium bicarbonate or hydroxide methyl benzoate was obtained.

Rearrangement of the Carbinol.—A sample of 0.2 g. of the carbinol was distilled at 4-mm. pressure. The distillate was a yellow oil which furnished a colorless solid on standing. It was crystallized for analysis from alcohol,

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[9] Harding and Warneford, *J. Biol. Chem.*, **25**, 319 (1916).

[10] Hunaeus and Zincke, *Ber.*, **10**, 1488 (1877).

[11] Meyer and Nägeli, *ibid.*, **16**, 1624 (1883).

in which it was only sparingly soluble, and the purified material melted at 120°.

Anal. Calcd. for $C_{16}H_{12}O_3$: C, 75.0; H, 5.0. Found: C, 75.0; H, 5.06.

The rearrangement product was shown to be the benzoate of benzoylcarbinol (IV) by a mixed melting point with an authentic sample of that carbinol prepared by the method of Rather and Reid.[12]

Summary

Dibenzoylcarbinol has been prepared and its chemical behavior described. The carbinol, which does not exist as an ene-diol, is very readily oxidized. It is cleaved by alkali and rearranges on heating to furnish the benzoate of benzoylcarbinol.

WASHINGTON, D. C. RECEIVED NOVEMBER 25, 1935

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[12] Rather and Reid, THIS JOURNAL, **41**, 75 (1919).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Study of the Influence of Heavy Water upon the Activity and upon the Stability of Pancreatic Amylase

BY M. L. CALDWELL, S. E. DOEBBELING AND S. H. MANIAN

Since the discovery of heavy water there have been conflicting reports as to its influence upon biological processes including enzyme action. The data so far obtained are too meager to be properly evaluated but sufficient to indicate the need for further carefully controlled work with typical enzymes.[1]

A quantitative study is reported briefly here of the influence of heavy water upon the activity and upon the stability of pancreatic amylase. Such studies are of special interest because the amylases, of which this one is typical, catalyze hydrolytic reactions in which water is an important factor. Moreover, there were available for this work, preparations of pancreatic amylase of exceedingly high purity and activity.[2] This makes the systems more sensitive and the interpretation of the results in terms of the influence of heavy water upon the enzyme more convincing than would be the case if less highly purified enzyme preparations had been used.

The heavy water was kindly supplied by Professor H. C. Urey. The methods for its concentration, purification, and analysis have been described previously.[3] It was further purified for the enzyme work by three redistillations: the first, from permanganate made alkaline by the addition of anhydrous sodium carbonate; the second, after the addition of a few milligrams of phosphorus pentoxide; and the last, without further treatment. The distillations were carried out slowly, under atmospheric pressure, in all Pyrex glass apparatus equipped with suitable traps. The first and last few cubic centimeters of the distillate were not used for the enzyme work. The heavy water was found to be free from oxidizing substances (ozone and peroxides) as shown by the potassium-iodide-starch test, which was found to be sensitive to one part of hydrogen peroxide in 500,000. The ordinary distilled water used was treated in the same way in the same apparatus.

Influence of Heavy Water upon the Activity of Pancreatic Amylase.—The activity of the enzyme was studied both by determinations of its so-called amyloclastic and of its saccharogenic action. For the former, a modification of the Wohlgemuth method[4] was used. In the latter, the reducing sugar formed in the reaction mixtures was determined iodimetrically[5] and calculated to maltose. In all cases, the conditions were those which have been found most favorable to the activity and stability of the enzyme.[6] Every effort was made to keep the measurements in the presence of heavy and of ordinary water strictly comparable and to eliminate as far as possible the influence of any other variable factors.

Briefly, equal weights of the enzyme preparation were dissolved under the same conditions at 0° in purified 100% (99.9 +)[3] heavy water and in similarly purified ordinary water. Equal portions of these two enzyme solutions then reacted side by side at 40 ± 0.02° with equal portions of each

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[1] (a) Barnes and Larson, THIS JOURNAL, **55**, 5059 (1933); (b) *Protoplasma*, **22**, 431 (1934); (c) Hughes, Yudkin, Kemp and Rideal, *J. Chem. Soc.*, 1105 (1934); (d) Steacie, *Z. physik. Chem.*, **27B**, 6 (1934); **28B**, 236 (1935); (e) Pacsu, THIS JOURNAL, **56**, 245 (1934); (f) Fox, *J. Cell. and Comp. Physiol.*, **6**, 405 (1935).

[2] Sherman, Caldwell and Adams, *J. Biol. Chem.*, **88**, 295 (1930).

[3] (a) Brown and Dagget, *J. Chem. Phys.*, **3**, 216 (1935); (b) Crist, Murphy and Urey, *ibid.*, **2**, 112 (1934).

[4] (a) Wohlgemuth, *Biochem. Z.*, **9**, 1 (1908); (b) Sherman and Thomas, THIS JOURNAL, **37**, 623 (1915).

[5] Caldwell, Doebbeling and Manian, unpublished data.

[6] Sherman, Caldwell and Adams, THIS JOURNAL, **50**, 2529, 2535, 2538 (1928).