

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF H. KOHNSTAMM AND COMPANY, INC.]

The Structure of Dichlorofluorescein

BY ROBERT F. MILLIGAN AND FRED J. HOPE

In the recent list of coal-tar colors permitted by the United States Government¹ for use in the manufacture of drugs and cosmetics there appeared a new dye, D and C Orange No. 8, which is stated to be 4,5-dichloro-3,6-fluorandioli. No reference could be found to substantiate the validity of this configuration since the only dichlorofluorescein heretofore prepared containing chlorine in the "resorcinol part" is that of Kolthoff, Lauer and Sunde² which is described as being 2,7-dichloro-3,6-fluorandioli. However, the differences in physical appearances and the disparity of melting points of the diacetates (232° as against 237°)³ from these two products seemed significant enough to warrant investigation.

When fluorescein, or its analogs, are hydrolyzed with strong alkalis⁴ scission takes place at the triphenylmethane carbon and at the ether linkage to yield a resorcinol and an *O*-(2,4-dihydroxybenzoyl)-benzoic acid. The positions of the substituent groups in the hydrolysis products are in direct relation to their positions in the original molecule. When eosine⁵ is so treated the products are 2,4-dibromoresorcinol and *o*-(2,4-dihydroxy)-3,5-dibromobenzoyl)-benzoic acid. Thus the identification of the hydrolysis products from this type of compound is indicative of its configuration. From this analogy D and C Orange No. 8 should yield 2-chlororesorcinol and *o*-(2,4-dihydroxy-3-chlorobenzoyl)-benzoic acid to conform to the proposed structure for this compound.

Degradation of D and C Orange No. 8 and subsequent separation of the products yielded a chlororesorcinol which corresponded to the product previously reported⁶ and an organic acid. The latter product has not been described in the literature and had to be synthesized by the Friedel-Crafts reaction.

Experimental

The D and C Orange No. 8 used in this work was of the grade certifiable to the Food and Drug Administration and was used without further purification.

Hydrolysis and Isolation.—Ten grams of the dye was dissolved in a sodium hydroxide solution, prepared by dissolving 100 g. of sodium hydroxide in 100 cc. of water, and heated with gentle stirring, at 120°, until a test drop diluted with water no longer showed fluorescence. The mixture was cooled, poured over 300 g. of ice and acidified with 250 cc. of concd. hydrochloric acid. To remove the hydrolysis products from the water phase, five successive portions of ether were then used. The extractions were

combined and washed with 2% sodium bicarbonate solution and the ethereal solution was evaporated.

The chlororesorcinol thus obtained melted at 95°. Purification by sublimation gave a product m. p. 97–98° (uncor.).

Anal. Calcd. for C₆H₅O₂Cl: Cl, 24.54. Found: Cl, 24.50.

Acidification of the sodium bicarbonate, followed by extraction with ether and subsequent evaporation of the ethereal solution yielded the second scission product. Recrystallization from 10% alcoholic solution gave pale yellow crystals, m.p. 218–219° (uncor.).

Anal. Calcd. for C₁₄H₉O₃Cl: Cl, 12.12. Found: Cl, 12.15.

The diacetate was made by refluxing this acid with acetic anhydride and recrystallizing the product from alcohol, m. p. 154–155° (uncor.).

Anal. Calcd. for C₁₈H₁₃O₇Cl: Cl, 9.41. Found: Cl, 9.40.

Preparation of *o*-(2,4-Dihydroxy-3-chlorobenzoyl)-benzoic Acid.—A modification of the Friedel-Crafts reaction as outlined by Desai and Figueroa⁷ gave the desired product by the following method. To a mixture of 14 g. of anhydrous aluminum chloride in 75 cc. of nitrobenzene contained in a 500-cc. glass-stoppered flask was added 7.35 g. of 2-chlororesorcinol at room temperature. Agitation for thirty minutes resulted in a clear solution to which was added slowly 7 g. of finely powdered phthalic anhydride. After standing at room temperature for thirty-six hours the mixture was heated on the steam-bath for two hours and allowed to cool. Thirty cc. of concd. hydrochloric acid was mixed with 100 g. of ice and poured into the reaction mixture. The nitrobenzene was removed by steam distillation and the residual material concentrated to about 100 cc. Upon cooling, the solid was collected, dissolved in alcohol and treated with decolorizing carbon. After filtration the solution was diluted with hot water until a turbidity resulted. On cooling yellow crystals were obtained, which after two additional recrystallizations resulted in a yield of 6 g. The crystals melted at 218–219° (uncor.).

Anal. Calcd. for C₁₁H₉O₃Cl: Cl, 12.12. Found: Cl, 12.20.

There was no depression of the melting point on mixing this product with the acid obtained by the degradation of the dye.

The diacetate was made by refluxing this compound with acetic anhydride. Crystallization of the diacetate from alcohol gave the pure compound, m. p. 154–155° (uncor.).

Anal. Calcd. for C₁₈H₁₃O₇Cl: Cl, 9.41. Found: Cl, 9.37.

There was no depression of the melting point on mixing the diacetate obtained from the two methods.

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Summary

D and C Orange No. 8 has been shown to be a dichlorofluorescein with the chlorine atoms occupying the 4 and 5 positions in the molecule. The proposed structure for this compound has

(7) Desai and Figueroa, *Proc. Indian Acad. Sci.*, **14A**, 605–608 (1941).

(1) Federal Security Agency, Food and Drug Administration, Service and Regulatory Announcements, Food, Drug and Cosmetic No. 3.

(2) Kolthoff, Lauer and Sunde, *THIS JOURNAL*, **61**, 3273 (1929).

(3) Sandin, Gillies and Lynn, *ibid.*, **61**, 2919 (1939).

(4) Baeyer, *Ann.*, **183**, 23–25 (1876).

(5) Baeyer, *ibid.*, **183**, 56, 57 (1876).

(6) Milligan and Hope, *THIS JOURNAL*, **68**, 544 (1941).

been shown to be correct. A new compound *o*-(2,4-dihydroxy-3-chlorobenzoyl)-benzoic acid has been synthesized and characterized.

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The Use of Sodium Methoxide in the Claisen Reaction

BY E. EARL ROYALS

Freri has reported¹ that the condensation of diethyl oxalate with acetone or acetophenone by means of sodium methoxide in absolute methanol gives the methyl ester of the corresponding acylpyruvate in excellent yield and that the reaction requires less care than the condensation using sodium ethoxide. In the present investigation the corresponding reactions have been carried out with four other methyl ketones. The results are summarized in Table I.

flask fitted with a reflux condenser, a dropping funnel and a modified Hershberg stirrer.⁴ The reaction flask was protected from atmospheric moisture by drying tubes filled with absorbent cotton previously dried at 100°. The solution was cooled to room temperature. The reaction vessel was immersed in a basin of tap water, the stirrer was started and a mixture of one-half mole each of diethyl oxalate and the required ketone was run in from the dropping funnel during one hour. Stirring was continued for four to six hours. The reaction mixture was allowed to stand overnight at room temperature. It was then cooled in an ice-bath, and an ice-cold solution of 15 g. of concd.

TABLE I

PREPARATION OF METHYL ACYLPYRUVATES

Acyl substituent	Yield, %	°C.	B. p.,		n_D^{20} ^b	Calcd.	Equivalent wt.	
			mm.				Found	
Aceto-	69.7	93-97 ^a	9-12			72.06	71.75	71.65
Propiono-	42.1	90-95	4		1.4722-1.4751	79.10	79.30	79.75
Butyro-	51.5	110-112	7-8		1.4725-1.4750	86.10	87.07	87.24 ^c
Isovalero-	84.0	103	4		1.4719-1.4729	93.10	95.02	95.07 ^c
Pivalo-	74.5	112-113	11		1.4720	93.10	92.55	92.97

^a This product melted at 61.5-62.5° after a single crystallization from benzene. Reported (ref. 1) m. p. 63°. ^b The variations in refractive index represent variations in individual samples. ^c It is possible, as was suggested by the Referee, that these high values may indicate contamination of the methyl ester with some of the ethyl ester.

It is assumed that, in common with other base-catalyzed condensations,² the present reactions involve the methyl rather than the methylene group of the ketone to form methyl acylpyruvates having the structure $\text{RCOCH}_2\text{COCO}_2\text{CH}_3$. On boiling with dilute aqueous sodium hydroxide, these acylpyruvates were found to be cleaved quantitatively according to the equation



The equivalent weights given in the final column of Table I were calculated on the basis of this reaction.

Experimental

Materials.—Diethyl oxalate, prepared by a standard procedure, was distilled through a modified Widmer column under reduced pressure and used without further purification. C. P. grade acetone was dried for one week over calcium chloride and distilled through a modified Widmer column. A middle cut of accepted boiling point and refractive index was taken for use. The other ketones used were Eastman Kodak Co. practical grade products purified as described for acetone. Methanol was dried just prior to use by the method of Lund and Bjerrum.³

Condensation Procedure.—One-half gram atom of sodium was cut into thin strips and dissolved in 150 cc. of absolute methanol contained in a 500-cc., three-necked

sulfuric acid in 100 cc. of water was rapidly run in from the dropping funnel. The mixture was stirred for five to ten minutes, then poured into 500 cc. of water. The liberated methyl acylpyruvate was extracted with three 50-cc. portions of benzene. The benzene extracts were combined and washed with two 50-cc. portions of water. The benzene was removed by distillation at atmospheric pressure, and the residue was distilled under reduced pressure from an ordinary Claisen flask. The crude product was collected over a somewhat wider range than that recorded in Table I and was redistilled from a modified Claisen flask.⁵

A modification of the above procedure was necessary in the condensation of acetone with diethyl oxalate because of the unusually heavy precipitation of the sodium salt of methyl acetopyruvate during the reaction. In this case, 300 cc. of methanol was used as solvent for a run of the size described above. After reaction was complete, the precipitated sodium salt of the product was filtered from the reaction mixture and sucked dry on the filter. It was then returned to the reaction flask and worked up as described above. The product was distilled under reduced pressure from a modified Claisen flask fitted with an air condenser.

The last four compounds listed in Table I were colorless to light yellow oils; the yields and physical properties recorded are for products redistilled as described above. Methyl acetopyruvate was solid at room temperature. Crystallization of this compound was difficult because of its tendency to oil out of solution. It could, however, be crystallized from benzene with considerable loss due to its solubility in this medium. The yield reported in Table I is for the uncrystallized compound, while the analytical data were determined on a crystallized sample.

(1) Freri, *Gazz. chim. ital.*, **68**, 616 (1938).

(2) See Tracy and Elderfield, *J. Org. Chem.*, **6**, 70 (1941); see also Hauser and Adams, *THIS JOURNAL*, **66**, 345 (1944).

(3) Lund and Bjerrum, *Ber.*, **64**, 210 (1931).

(4) Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 313 (1936).

(5) Adkins and Rainey, "Organic Syntheses," Vol. 20, 1940, p. 9.

(6) Noyes and Skinner, *THIS JOURNAL*, **39**, 2718 (1917).