TABLE I

NITROSATION OF FLUORENE

Solvent	Base	Yield of oxime, %
$(C_2H_5)_2O$, C_6H_6[a]	KOC_2H_5	77,[b] 81[c]
C_2H_5OH	KOH	32
C_4H_9OH	KOH	71
C_4H_9OH	KOH[d]	5
C_4H_9OH	KOH[e]	80, 82, 83
C_4H_9OH	NaOH	11
C_4H_9OH	$NaOC_4H_9$	18
$CH_3CH(OC_2H_7)_2$[f]	KOH	30

[a] Wislicenus conditions. [b] No stirring. [c] Vigorous stirring. [d] 50% aqueous solution. [e] Solvent partially distilled after solution of base to remove water. [f] An excellent solvent for many base-catalyzed reactions. C. Weizmann, E. Bergmann, and M. Sulzbacher, *J. Org. Chem.*, **15**, 918 (1950).

lated zinc, added all at once, as described in the present note.

EXPERIMENTAL

A solution of 100 g. of 85% potassium hydroxide in 500 ml. of butyl alcohol was boiled for 2 hr. under a 20-cm. column with a fractionating head to remove about 30 ml. of water, butyl alcohol being returned. Then 166 g. of technical fluorene was added. This was followed by dropwise addition during 10 min. of 125 ml. (110 g., calcd. 103 g.) of butyl nitrite,[5] and the mixture was boiled for 10 min. It was then diluted with water (two 500-ml. portions) and partially distilled to remove butyl alcohol. The aqueous residue was cooled and extracted with 100 ml. of ligroin,[6] then acidified with acetic acid. There was obtained 143 g. (73%) of fluorenone oxime, m.p. 175–184°, used without purification for reduction. Recrystallization from acetic acid gave tan needles, m.p. 187–188° corresponding to reported values.

A solution of 150 g. of oxime in 450 ml. of warm acetic acid was diluted with 150 ml. of water and heated to about 100°. Then 110 g. of 20 mesh granulated zinc was added, resulting in a smooth reaction which kept the mixture boiling for about 20 min. The mixture was boiled for an additional 20 min., and then decanted from the little remaining zinc into a hot solution of 450 ml. of hydrochloric acid in 1250 ml. of water. Cooling gave gray needles which were pressed on a filter, washed with three 100-ml. portions of ether, and dried. The resulting 9-fluorylamine hydrochloride formed white needles of excellent purity that darkened at 210°, m.p. 220° dec. (reported, 216–217°); yield 128 g. (76%). The yield was raised to 90% by working over the mother liquors, but this was uneconomical.

9-Fluorylamine, m.p. 60–62° was obtained by treatment with base of the salt. It was interesting to discover that although it could be distilled at 20 mm. in quantities of less than 1 g., attempted distillation of larger quantities resulted in much decomposition with formation of dibiphenylene-ethane, -ethylene, and resin.

(5) Butyl nitrite was prepared by adding a slight excess of iced sulfuric acid in portions to a separatory funnel containing ice and 1 equivalent each of butyl alcohol and concentrated aqueous sodium nitrite, with shaking after each addition. The lower layer was discarded, and the product was washed with a little dilute sodium carbonate and stored over solid potassium carbonate; yield, 94%. Preparation of 2 moles of nearly pure butyl nitrite in this way required only a few minutes, and the product kept well. Samples more than 2 years old gave as good results as fresh ones.

(6) This extraction removed 65 g. of dark oil containing 19 g. of fluorene, 35 g. of crude butylidenefluorene (b.p. 200–240°, m.p. 55°, dibromide m.p. 93–94° dec.) and 8 g. of black resin, probably corresponding to impurities in the technical fluorene used.

SCHOOL OF CHEMISTRY
UNIVERSITY OF MINNESOTA
MINNEAPOLIS 14, MINN.

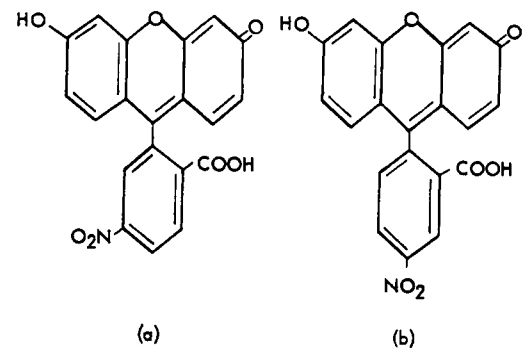
Spectral Evidence for the Structures of the Nitrofluorescein Isomers

FELIX BOREK

Received July 20, 1960

In 1905 Bogert and Wright[1] reported that condensation of 4-nitrophthalic acid with resorcinol yields a nitro derivative of fluorescein. One might expect from this reaction two isomeric products differing in the position of the nitro group (Fig. 1), which would result from the condensation of one or the other carboxyl groups of 4-nitrophthalic acid. The authors, however, have not indicated the existence of such isomers.

In 1942 Coons and co-workers[2] repeated the above reaction with the eventual aim of obtaining a derivative of fluorescein which could be used as a fluorescent label for antibody proteins. They apparently believed the crude product to be 4′-nitrofluorescein (Fig. 1b). In 1950, however, Coons



(a) (b)

Fig. 1. (a) 5′-nitrofluorescein; (b) 4′-nitrofluorescein

and Kaplan[3] found the product to be a mixture of two isomers and succeeded in separating them by fractional crystallization of the 3,6-diacetates. They called the isomer which was less soluble in benzene-ethanol mixture "nitrofluorescein diacetate I," and the more soluble one "nitrofluorescein diacetate II." Henceforth all other derivatives prepared from these compounds received designations of I or II, respectively. No attempt has been made to determine the position of the nitro group in either isomer, but the separation of the two

(1) R. T. Bogert and R. G. Wright, *J. Am. Chem. Soc.*, **27**, 1310 (1905).

(2) A. H. Coons, H. J. Creech, R. N. Jones, and E. Berliner, *J. Immunol.*, **45**, 159 (1942).

(3) A. H. Coons and M. H. Kaplan, *J. Exp. Med.*, **91**, 1 (1950).

TABLE I

COMPARISON OF INFRARED ABSORPTION BANDS[a]

Nitrofluorescein I	MNX	Nitrofluorescein II	PNX
1032 cm.$^{-1}$ (m)	1032 cm.$^{-1}$ (m)	—	—
Nitrofluorescein I Diacetate	**MNX Diacetate**	**Nitrofluorescein II Diacetate**	**PNX Diacetate**
—	—	703 cm.$^{-1}$ (m)	703 cm.$^{-1}$ (m)
Dichloronitrofluorane I	***p*-$NO_2PhCOOH$**	***p*-NO_2PhCHO**	
875 cm.$^{-1}$ (s)	872 cm.$^{-1}$ (s)	—	
828 cm.$^{-1}$ (m)	825 cm.$^{-1}$ (w)	—	
742 cm.$^{-1}$ (s)	—	740 cm.$^{-1}$ (s)	
Dichloronitrofluorane II	***m*-$NO_2PhCOOH$**	***m*-NO_2PhCHO**	
826 cm.$^{-1}$ (v.s.)	826 cm.$^{-1}$ (s)	826 cm.$^{-1}$ (m)	
812 cm.$^{-1}$ (m)	811 cm.$^{-1}$ (m)	812 cm.$^{-1}$ (s)	
735 cm.$^{-1}$ (s)	—	732 cm.$^{-1}$ (s)	

[a] The infrared spectra were determined with a Perkin-Elmer Infracord Spectrophotometer. The samples were Nujol mulls.

isomers was found to be the best way for the purification of the product. De Repentigny and James[4] devised an efficient separation of aminofluoresceins I and II prepared by the catalytic hydrogenation of the nitro derivatives. They used chromatography on a kieselguhr column in phosphate buffer, with a mixture of *n*-butyl alcohol and cyclohexane as eluent. In this system isomer II was found to be the more mobile component.

The structural similarity of the two isomers is reflected in their physical properties, which include absorption spectra—visible as well as infrared. However, it was found in our laboratory that when the nitrofluoresceins were converted into their 3,6-dichloroderivatives, their infrared spectra showed distinct differences in the fingerprint region. Some of these differences proved to be analogous to those in the spectra of structurally related compounds, namely *p*- and *m*-nitrobenzoic acid and *p*- and *m*-nitrobenzaldehyde. On the other hand, spectra of the original nitrofluorescein isomers were compared with those of independently prepared *p*- and *m*-nitrophenyl derivatives of 3,6-dihydroxyxanthene (PNX and MNX, respectively), and of the corresponding diacetates. This comparison is shown in Table I and in Fig. 2.

From these data it is evident that in the region of 810–830 cm.$^{-1}$ the spectrum of dichloronitrofluorane I shows a close similarity to the spectra of *m*-nitrobenzoic acid and *m*-nitrobenzaldehyde, while it is markedly different from the spectra of *p*-nitrobenzoic acid and *p*-nitrobenzaldehyde in that region. On the other hand, the spectrum of dichloronitrofluorane II has a strong band near 870 cm.$^{-1}$, in common with that of *p*-nitrobenzoic acid. Further, the 1000 cm.$^{-1}$ region in the spectrum of nitrofluorescein I is similar to that of *m*-nitrophenyl-3,6-dihydroxyxanthene while near 700 cm.$^{-1}$ there is a similarity between the spectrum of nitrofluorescein II diacetate and that of *p*-nitrophenyl-3,6-dihydroxyxanthene diacetate.

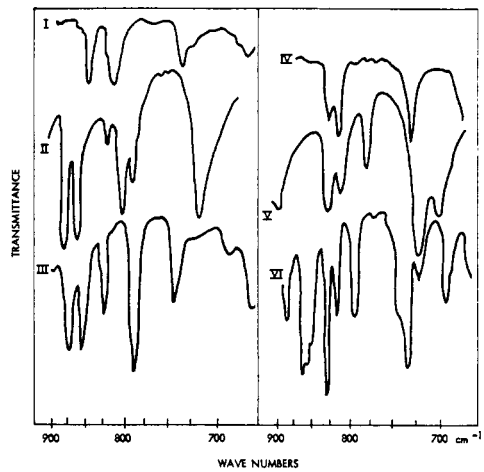


Fig. 2. Infrared spectra. I. *p*-$NO_2C_6H_4CHO$; II. *p*-$NO_2C_6H_4COOH$; III. dichloronitrofluorane I; IV. *m*-$NO_2C_6H_4CHO$; V. *m*-$NO_2C_6H_4COOH$; VI. dichloronitrofluorane II

In the absence of other evidence, the above-mentioned similarities of infrared data suggest that the structure shown in Fig. 1a (5′-nitrofluorescein) can be assigned to nitrofluorescein I, and the structure in Fig. 1b, (4′-nitrofluorescein), to nitrofluorescein II.

EXPERIMENTAL[5]

3,6-Dichloronitrofluorane I. A suspension of 10 g. (0.0265 mole) of nitrofluorescein I in 15 ml. of phosphorus oxychloride was heated to 100° for 0.5 hr. Then 12 g. (0.0517 mole) of phosphorus pentachloride was added, and the mixture maintained at 100° for 0.5 hr. After the evolution of hydrogen chloride had ceased, the mixture was poured slowly and with vigorous stirring into 1.5 l. of water and then heated

(4) J. De Repentigny and A. T. James, *Nature*, 174, 927 (1954).

(5) The melting points were determined on a Fisher-Johns apparatus and are uncorrected. Microanalyses were done in part by Dr. H. A. Bright at the National Bureau of Standards, Washington, D. C., and in part by Mr. J. F. Alicino, Metuchen, N. J.

to boiling for 10 min. The solid precipitate was filtered with suction, redissolved in 1.5 l. of boiling water, filtered again hot, and dried. The yield of the crude product was 10 g. (92%). Several recrystallizations from 1:1 absolute ethanol-benzene gave a white crystalline solid,[6] m.p. 221–222.5°.

Anal. Calcd. for $C_{20}H_8O_5NCl_2$: C, 58.0; H, 2.2; N, 3.4; Cl, 17.1. Found: C, 58.2; H, 2.6; N, 3.1; Cl, 17.3.

3,6-Dichloronitrofluorane II. This material was prepared from nitrofluorescein II, using a procedure analogous to that described above. The yield of the crude product was 9 g. (82%), and the recrystallized material[6, 7] melted at 215–216°.

Anal. Calcd. for $C_{20}H_8O_5NCl_2$: C, 58.0; H, 2.2; N, 3.4; Cl, 17.1. Found: C, 58.1; H, 2.4; N, 3.1; Cl, 17.0.

3,6-Dihydroxy-9-(m-nitrophenyl)-xanthene (MNX) and its diacetate. A mixture of 3.8 g. (0.025 mole) of *m*-nitrobenzaldehyde and 5.5 g. (0.050 mole) of resorcinol was fused at 195–200° and maintained at that temperature for 3 hr. during which the mixture hardened into a dark mass. The melt was ground in the mortar and heated on a steam bath with 15 ml. of 6*N* hydrochloric acid for 1 hr. The solid was filtered, dissolved in *N* sodium hydroxide, and reprecipitated with *N* hydrochloric acid. The product, m.p. 185–188° dec., showed blue fluorescence in alkaline solution which was quenched on acidification.

One gram of the product was dissolved in 5 ml. of pyridine and treated with 5 ml. of acetic anhydride. The solution was heated on a steam bath for 0.5 hr., left at room temperature overnight, and then poured into iced water. The resulting precipitate was filtered and dried. After the diacetate had been recrystallized several times from acetone-ethanol, it melted at 185–190° dec. It was found to be hygroscopic.

Anal. Calcd. for $C_{23}H_{17}O_7N.1^1/_2H_2O$: C, 61.89; H, 4.51. Found: C, 62.30; H, 4.05.

3,6-Dihydroxy-9-(p-nitrophenyl)-xanthene (PNX) and its diacetate. These compounds were prepared, using *p*-nitrobenzaldehyde and resorcinol as starting materials, by the procedures described above. Both products did not melt below 300° and were hygroscopic.

Anal. (diacetate) Calcd. for $C_{23}H_{17}O_7N.H_2O$: C, 63.16; H, 4.38; N, 3.20. Found: C, 63.68; H, 3.97; N, 3.21.

Acknowledgment. The author wishes to thank Mr. P. Berrigan for his assistance in the preparation of some of the compounds.

ARMED FORCES INSTITUTE OF PATHOLOGY
WASHINGTON 25, D. C.

(6) A γ-lactone form was indicated by the strong infrared absorption band at 1760 cm.$^{-1}$ (*cf.* ref. 7), lack of color and fluorescence, and low solubility in water and alkalis.

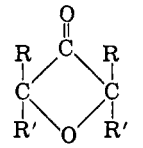
(7) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Wiley, Inc., New York, 1954, p. 159.

The Preparation of Oxetanones

JAMES L. HARPER[1,2] AND CHARLES T. LESTER[3]

Received July 27, 1960

Within recent years several oxetanones have been synthesized.[4] We wish to report that we have completed the synthesis of 2,4-dimethyl-2,4-diethyl-3-oxetanone[5] (Ia) and 2,2,4,4-tetraphenyl-3-oxetanone[4b] (Ib) by the method used to synthesize 2,2,4,4-tetramethyl-3-oxetanone[4c,f] (Ic).



Ia. R = CH_3; R′ = C_2H_5
Ib. R,R′ = C_6H_5
Ic. R,R′ = CH_3

Included in this report are descriptions of the synthesis of 2,2,4,4-tetramethyl-3-oxetanol (II) and 2,2,3,4,4-pentamethyl-3-oxetanol (III). Both of these have been prepared from Ic.

EXPERIMENTAL[6]

The preparation of 2,5-dimethyl-2,5-diethyltetrahydro-3-furanone (IV). The procedure of Richet[7] gave 145 g. (70%) of ketone, b.p. 190–196°, from 209 g. of 3,6-dimethyl-4-octyne-3,6-diol.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.55; H, 10.59. Found: C, 70.65; H, 10.75.

The preparation of 2,5-dimethyl-2,5-diethyltetrahydro-furan-3,4-dione (V). Thirty-one grams of IV was added dropwise to a suspension of 24 g. of selenium dioxide in 400 ml. of dioxane and 20 ml. of water. The mixture was stirred and heated to reflux temperature during the addition of IV and for 12 hr. thereafter. The selenium was removed by filtration and the dioxane evaporated under reduced pressure. The residue was dissolved in ether and the solution stored over Drierite. After filtration and evaporation of the solvent, the residue was purified by distillation, yielding 27 g. (68%) of V, b.p., 56–63° at 1 mm.

Anal. Calcd. for $C_{10}H_{16}O_3$: C, 65.19; H, 8.75. Found: C, 65.31; H, 8.82. Several attempts to prepare V by way of the dibromo derivative[4c] of IV were unsuccessful.

The preparation of 2,4-dimethyl-2,4-diethyl-3-oxetanol-3-carboxylic acid. (VI). A procedure previously described for rearranging a diketone[4c] was followed. This produced 5.7 g. (30%) of VI from 17.7 g. of V. After several crystallizations from carbon tetrachloride, the acid melted at 127–128°.

Anal. Calcd. for $C_{14}H_{18}O_4$: C, 59.46; H, 8.92; neut. equiv., 202. Found: C, 59.69; H, 8.77; neut. equiv., 200.4.

The preparation of Ia. The lead tetraacetate oxidation of VI was employed as in the preparation of Ic.[4c] A yield of 4.2 g. (60%) of Ia, b.p., 133–134° resulted from the oxidation of 10 g. of VI. Ia solidified on cooling; m.p. of resublimed product, 56–57°.

Anal. Calcd. for $C_9H_{16}O_2$: C, 69.87; H, 10.26. Found: C, 70.46; H, 9.90. The oxetanone readily formed a 2,4-dinitrophenylhydrazone derivative,[8] m.p., 135–136°.

(1) Taken from the Ph.D. Dissertation of James L. Harper, Emory University, 1957.

(2) Tennessee Eastman Fellow, 1956–57.

(3) To whom inquiries should be sent.

(4)(a) J. T. Marshall and J. Walker, *J. Chem. Soc.*, 467 (1952). (b) G. B. Hoey, D. O. Dean, and C. T. Lester, *J. Am. Chem. Soc.*, **77**, 391 (1955). (c) B. L. Murr, G. B. Hoey, and C. T. Lester, *J. Am. Chem. Soc.*, **77**, 4430 (1955). (d) W. S. Allen, S. Bernstein, M. Heller, and R. Littell, *J. Am. Chem. Soc.*, **77**, 4784 (1955). (e) G. A. Bailey, G. I. Poos, R. Walker, and S. M. Chermeda, *J. Am. Chem. Soc.*, **78**, 4814 (1956). (f) C. Sandris and G. Ourisson, *Bull. Soc. Chim., France*, 958 (1956). (g) J. Maxwell, Ph.D. dissertation, Emory University, 1957.

(5) No effort has been made to resolve this or any related compounds into the various stereoisomers.

(6) All melting and boiling points are uncorrected. Microanalyses were done by Drs. G. Weiler and F. B. Strauss, Oxford, England.

(7)(a) H. Richet, R. Dulon, and G. Dupont, *Bull. Soc. Chim., France*, 693 (1947). (b) H. Richet, *Ann. chim.*, [12] **3**, 317 (1948).