λ max of the hydrolyzed product. The pKa were then calculated in the usual manner.[15]

Acknowledgment. The authors wish to express their gratitude to the Analytical Research Branch of the Research Directorate, U. S. Army Chemical Warfare Laboratories for the analyses herein reported and to Vera Isaacs, Mary D. Pankau, Howard Stroterhoff, Nathan Ingber, Joseph Handelman, and Arthur Jones, Jr., for their technical assistance.

ARMY CHEMICAL CENTER, MARYLAND

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[CONTRIBUTION FROM U. S. ARMY CHEMICAL WARFARE LABORATORIES, PROTECTIVE DEVELOPMENT DIVISION]

A Study of the Physical and Chemical Properties of the Esters of Indophenols. II. Structural Studies of the Isomeric Esters

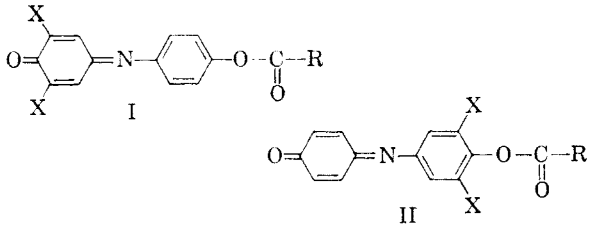
ROBERT M. GAMSON, DAVID N. KRAMER, AND F. M. MILLER

Received May 18, 1959

A study is reported on isomeric dihalo substituted indophenyl esters leading to the identification of a red form as the 2,6 dihalo derivative and an orange form as the 3′,5′ dihalo ester. Structural assignments were made on the basis of comparative preparative methods and hydrolytic and spectral characteristics.

The authors have reported[1] the synthesis and chemical properties of esters of various indophenols for use as synthetic chromogenic substrates for hydrolytic enzymes. As previously indicated, the existence of the isomeric esters I and II was anticipated.

This has been verified by the isolation of two distinct compounds, obtained in red (I) and orange (II) forms. Structural assignments of the two stereoisomeric esters were made on the basis of comparative preparative, hydrolytic, and spectral (u.v., visible, and I.R.) data which are the subject of this report.

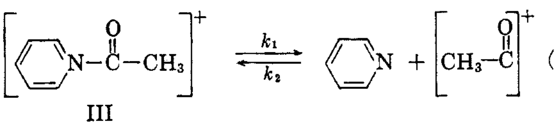


X = Cl, Br   R = CH_3—, $CH_3CH_2CH_2$—

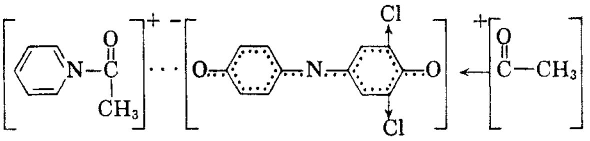
Comparative preparative studies. Of the two isomeric forms, the orange product was obtained by the procedure involving the use of the halogenated sodium indophenol, acyl anhydride, and pyridine catalyst.[1] On the other hand, the red isomer was produced following the procedure employing the acyl anhydride without a catalyst.[2]

Experiments are now in progress to elucidate the mechanism of the acylation reaction with or without pyridine as a catalyst. Preliminary results indicate that the following rationalization may account for the two courses of the reaction.

Pyridine reacts with acetic anhydride to yield an acetylpyridinium complex III.[3] The acetyl pyridinium complex may dissociate as shown in equation 1, where $k_2 > k_1$. Since the esterification employs the sodium salt of the indophenol as the starting material, the acetyl pyridinium ion will associate with the oxygen bearing the highest electron density to form an ion pair as follows:



III   (1)



ion pair

The formation of the ion pair results in an orientation of the dihalo indophenolate ion which, for steric and energetic reasons, prevents attack on the more nucleophilic oxygen and promotes the acylation of the less nucleophilic oxygen. As the acylation step is completed, the ion pair is destroyed. The above is essentially an S_N2 reaction, yielding II.

On the other hand, in the absence of pyridine, the course of the reaction proceeds as expected with the attack of the nucleophilic oxygen of the indophenolate ion directly on the acetic anhydride, as shown:

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(1) D. N. Kramer, R. M. Gamson, and F. M. Miller, *J. Org. Chem.*, **24**, 1742 (1959). (Paper I.)

(2) The complete separation of the isomers was confirmed by gas chromatography of the individual compounds and of a mixture of the two. Only one peak was obtained with either form; a mixture produced two well defined peaks.

(3) V. Gold and E. G. Jefferson, *J. Chem. Soc.*, 1409 (1953).

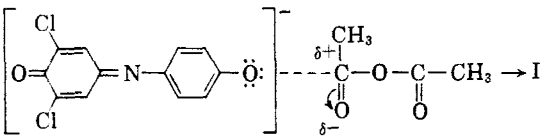


Table I summarizes the physical properties of a number of representative unsymmetrically substituted indophenyl esters. In general, the orange forms have lower melting points than the isomeric red compounds. The mixed melting point of the dibromo butyrate is depressed (58–63° against 79–81° for red and 77° for orange forms) indicating that the two forms are different entities.

TABLE I

Physical Characteristics of Unsymmetrically Substituted Indophenyl Esters

Substituent 2	6	3′	5′	R	Color	Melting[a] Point, °C.
Cl	Cl			CH_3	Red	101
		Cl	Cl	CH_3	Orange	88
Br	Br			CH_3	Red	145
		Br	Br	CH_3	Orange	119–121
Br	Br			nC_3H_7	Red	79–81
		Br	Br	nC_3H_7	Orange	77

[a] Melting points are uncorrected and were determined using a Fisher-Johns melting point apparatus.

Comparative hydrolytic studies. In further studies to elucidate the structure, the esters were spontaneously hydrolyzed. The spontaneous hydrolysis was performed at various temperatures and constant *p*H. The buffer provides a constant hydroxyl ion concentration and the normally second-order hydrolysis becomes pseudo-first order. As one of the hydrolytic products is the intensely colored indophenolate ion, the reaction rate can be readily determined spectrophotometrically. Under these conditions, the percent of hydrolysis was measured as indicated in Table II. Comparing the orange forms of the esters, it can be seen that the hydrolytic rates are slower than the parent indophenyl acetate. This is taken as further evidence that structure II represents the orange form, as steric effects of the halogens ortho to the esteratic bonds would hinder hydrolysis. The dichloro derivatives were hydrolyzed faster than the corresponding dibromo compounds as expected. On the other hand, the red forms of the ester are all hydrolyzed at a greater rate than the parent substance. Thus it is believed that the halogens in the quinoid ring are increasing the hydrolytic susceptibility of the esteratic bond by decreasing the electron density at the carbonyl carbon. Just as there was no appreciable difference in *p*K values of dichloro and dibromo indophenylates,[1] there was no marked difference in the hydrolytic rates of the dihalo red forms.

TABLE II

Hydrolysis of Substituted Indophenyl Acetates in *p*H 8.5 Buffer at Various Temperatures

Substituent	% Hydrolysis 10 minutes 24°	10 minutes 45°	20 minutes 24°	20 minutes 45°
H	2.87	11.75	6.05	21.00
DiBr (orange)	1.25	4.25	1.75	8.75
(red)	5.00	15.00	9.50	21.50
DiCl (orange)	2.25	8.25	4.75	17.50
(red)	4.50	15.00	8.75	22.75

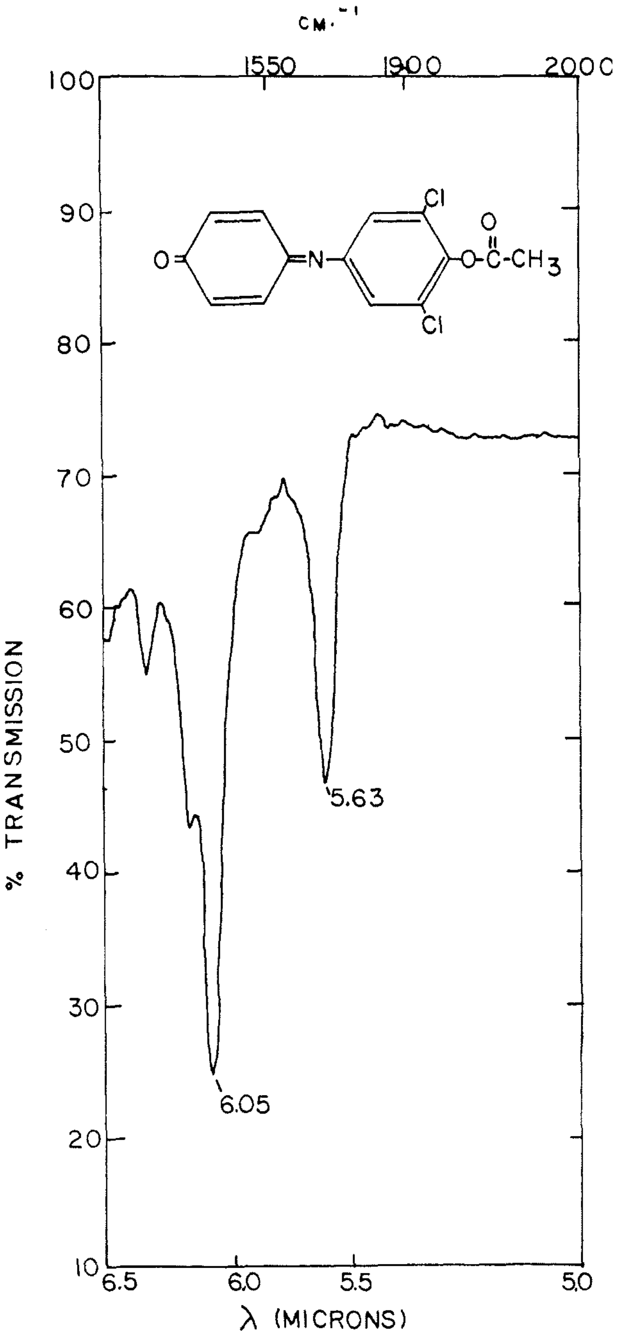


Fig. 1. Infrared spectrum of orange form of 2,6-dichloroindophenyl acetate in the 6 micron region

Comparative spectral studies. The λ_{max} of the red forms (both the dibromo and dichloro, 7B and 23B, respectively[1]) in the visible portion of the spectrum is at 470 mμ and that of the orange forms (7A

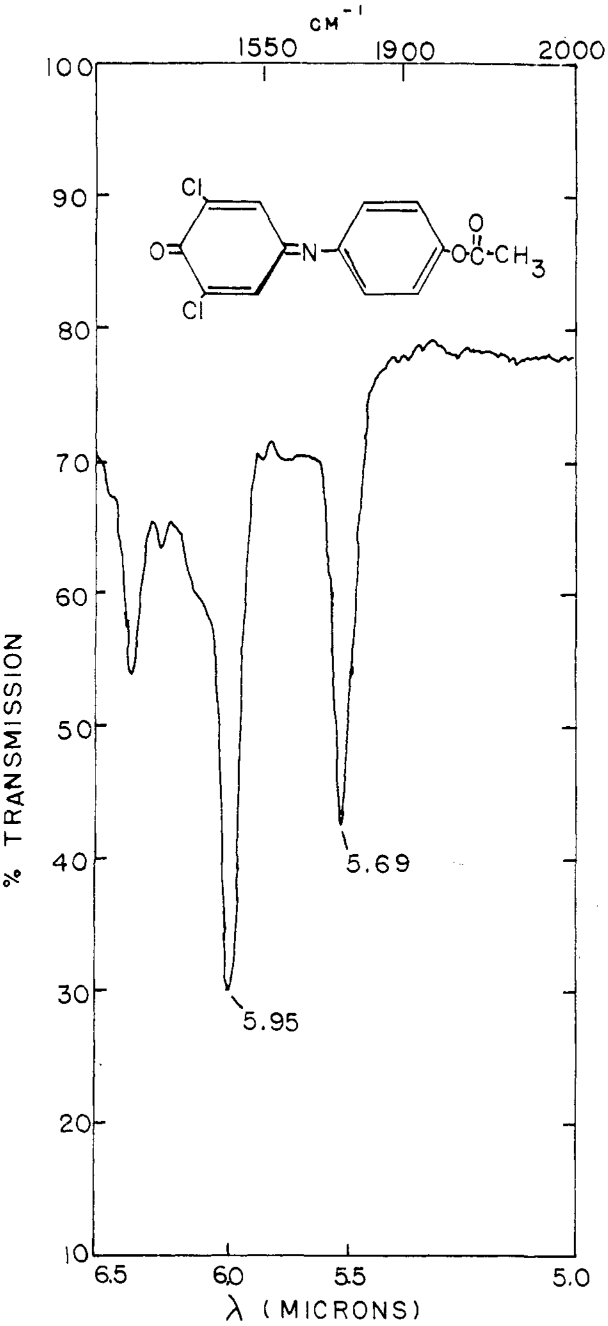
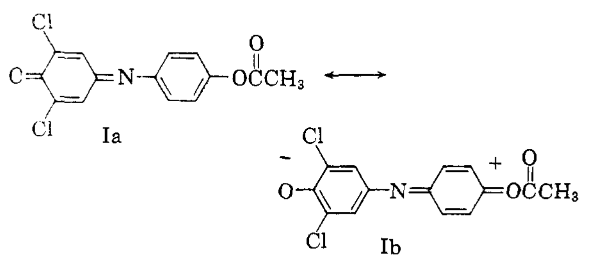
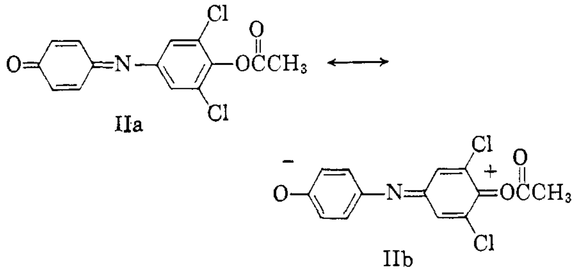


Fig. 2. Infrared spectrum of red form of 2,6-dichloroindophenyl acetate in the 6 micron region

and 23A respectively[1]) is at 445 mμ compared to 460 mμ for the unsubstituted indophenyl acetate. In an analogous study in the indaniline series, Vittum and Brown[4] found a similar shift resulted from halogen substituents in the quinoid ring. This is attributed to the stabilization of an excited state such as Ib by the inductive withdrawal of the halogens. Such stabilization would not be anticipated for IIb. Indeed, IIb should be destabilized by the inductive effect of the halogens and therefore absorb at shorter wavelengths. A comparable absorption at longer wavelengths by the red form of dichloro indophenyl acetate is also observed in the ultraviolet spectra of the two isomers. The λ_{max} of the red form are at 229, 274s and 305 (compound No. 23B[1]) while those of the orange form are at 226, 264 and 274s (compound No. 23A[1]). Support for this concept is found in the spectra of quinones having halogen substitution[5] and in the spectra of N-chloroquinoneimines,[6] where in both cases the effect of dihalogen substitution is a bathochromic shift of 30 mμ.[7]





It is of interest to compare the infrared spectra of the two isomeric dichloroindophenyl acetates as shown in Figs. 1 and 2. The red form has a carbonyl absorption at 5.95 μ, whereas the corresponding absorption of the orange form is at 6.05 μ. Weissberger[8] states that halogenation in the α position to a ketone raises the carbonyl frequency. This is additional evidence that structure I is consistent with the red form of the ester. Moreover, a similar effect is noted in the carbonyl ester absorbance, which is reported to be about 5.69 μ.[9] It would be expected that the ester corresponding to structure II would absorb at a lower wave length because of the proximity of the halogens. Figure 1 shows that the orange form has an absorbance peak at 5.62–5.64 μ and Fig. 2 indicates that the red form has a peak at 5.68–5.70 μ. These results are confirmed by de Borst *et al.*,[10] who also report that a

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[4] P. W. Vittum and G. H. Brown, *J. Am. Chem. Soc.*, 68, 2237 (1946).

[5] E. A. Braude, *J. Chem. Soc.*, 490 (1945).

[6] Unpublished results of this laboratory.

[7] The λ_{max} of the quinoneimines are: N-chloroquinone imine, 286 mμ; 2,6-dibromo-N-chloroquinoneimine, 319 mμ; and 2,6-dichloro-N-chloroquinoneimine, 308 mμ.

[8] A. Weissberger, *Technique of Organic Chemistry, Chemical Applications of Spectroscopy*, Interscience, New York, 1956, Volume IX, p. 472.

[9] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*, Wiley and Sons, New York, 1954, p. 153, 156.

[10] C. de Borst, F. N. Hooge, G. J. Arkenbout, *Nature*, 182, 1017 (1958). This work was done independently following a visit by one of the authors (DNK) to the Chemical Laboratory, National Defense Research Council, TNO, Rijswijk, Netherlands.

similar shift due to C—O—C vibration was observed in the 8.3 μ region.[9]

EXPERIMENTAL

The esters were prepared by the procedure of Kramer *et al.*[1] Procedure A was used to obtain the red isomer while procedure B, using pyridine yielded the orange product. The analyses, melting points, and spectral characteristics of the esters have been previously reported.[1]

Spontaneous hydrolysis. A $2 \times 10^{-3}M$ solution of the ester in dioxane was aliquoted and diluted with tris buffer *p*H 8.5 so as to obtain a $2 \times 10^{-5}M$ solution. This solution was placed in a DU spectrophotometer cell at room temperature and its absorbance obtained at the λ_{max} of the hydrolysis product at 10 minute intervals for 1 hr. *vs.* a blank containing no ester. This was repeated at 5° intervals, from 25° to 45°. The buffer was thermostated at the working temperature and adjusted to *p*H 8.5 against standard buffer which was maintained at room temperature. This thermostated buffer was then used in the hydrolysis study. The Beckman DU spectrophotometer's cell compartment was maintained at the investigative temperature by the use of thermospacers and a circulating bath. The extinction coefficient and the absorbance values were used to calculate the concentration of hydrolysis product.

Spectral studies. Ultraviolet, visible and infrared spectra were obtained using a Perkin-Elmer Model 13U Spectrophotometer. The ultraviolet and visible spectra were determined in peroxide-free dioxane[11] at concentrations of $2 \times 10^{-5}M$ and $2 \times 10^{-4}M$ respectively. The infrared spectra were determined in potassium bromide pellets (1 mg. of ester per 200 mg. of bromide) using a sodium chloride prism.

Gas chromatography. The samples were chromatographed on a one foot column with a packing of 30% silicone grease on 40–100 mesh Celite 545. The block temperature was 240° and the column temperature was 200°. A helium flow of 45 ml. per min. was used with 400 ma. on the bridge circuit and a chart speed of 10″/hr. The orange form emerged in 2.5 minutes and the red form in 3.75 minutes. No other peaks appeared. A mixture of the red and yellow samples gave two peaks.

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[11] W. Dasler and C. D. Bauer, *Ind. and Eng. Chem. Anal. Ed.*, **18**, 52 (1946).

[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Some Applications of the Nenitzescu Reaction

EDGAR A. STECK[1] WITH R. PAULINE BRUNDAGE AND LYNN T. FLETCHER

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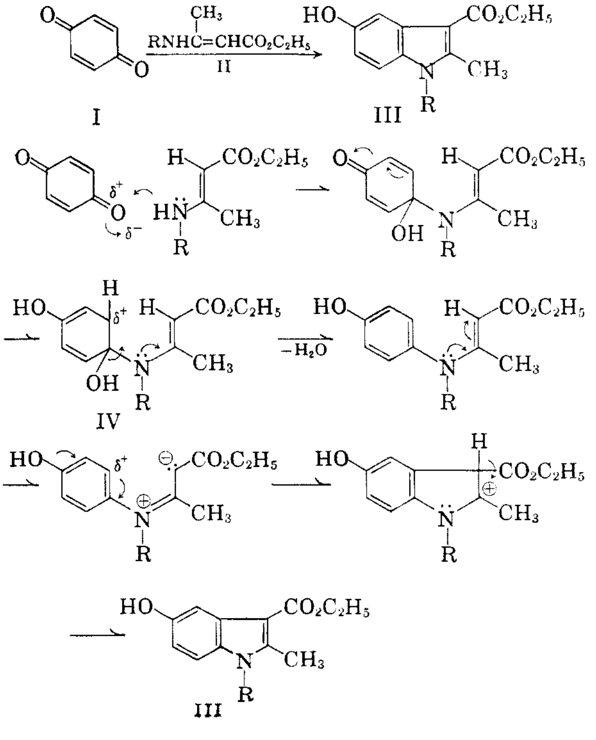
A group of 1-substituted 3-carbethoxy-5-hydroxy-2-methylindoles was prepared from ethyl *N*-substituted β-aminocrotonates and a mechanism proposed for the Nenitzescu reaction.

Several recent publications,[2–7] especially those of Grinev and co-workers,[2–5] have rendered it desirable that we record certain findings in our applications of the Nenitzescu[8,9] reaction for the synthesis of 5-hydroxyindole types. Our interest lay in the use of the indole derivatives as intermediates for other syntheses, and attempts were



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[1] Present address: McNeil Laboratories, Phila. 32, Pa.

[2] A. N. Grinev, N. K. Kul'bovskaya, and A. P. Terent'ev, *Zhur. Obshacei Khim.*, **25**, 1355 (1955); *Chem. Abstr.*, **50**, 4903g (1956).

[3] A. N. Grinev, N. E. Rozdevich, and A. P. Terent'ev, *Zhur. Obschei Khim.*, **27**, 1690 (1957); *Chem. Abstr.*, **52**, 3762b (1958).

[4] A. N. Grinev, I. A. Zaĭtsev, N. K. Venevtseva, and A. P. Terent'ev, *Zhur. Obschei Khim.*, **28**, 1853 (1958); *Chem. Abstr.*, **53**, 1299b (1959).

[5] A. N. Grinev, V. N. Ermakova, and A. P. Terent'ev, *Doklady, Akad. Nauk S.S.R.*, **121**, 862 (1958); *Chem. Abstr.*, **53**, 1167e (1959).

[6] H. J. Teuber and G. Thaler, *Chem. Ber.*, **91**, 2264 (1958).

[7] J. H. Koehneke and M. E. Speeter, U. S. Patent **2,707,187**; *Chem. Abstr.*, **50**, 5035e (1956).

[8] C. D. Nenitzescu, *Bull. Soc. Chim. Romania*, **11**, 37 (1929); *Chem. Abstr.*, **24**, 110[8] (1930).

[9] R. Beer, K. Clarke, H. Davenport, and A. Robertson, *J. Chem. Soc.*, 2029 (1951).