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

A Study of the Physical and Chemical Properties of the Esters of Indophenols I. Preparation

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

Received May 18, 1959

A series of indophenol esters has been prepared. Methods are given for the acylation of unsymmetrically substituted indophenols. Acyl anhydride alone yields 2,6 dihalo indophenyl esters whereas acyl anhydride and pyridine yields predominately the 3′,5′ dihalo esters. Ultraviolet, visible, and infrared spectra of the esters are reported along with the *p*Ka values of the free indophenols.

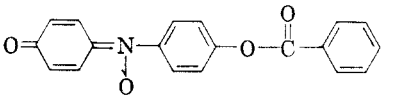
Indophenols have been extensively employed for many years as redox indicators[1] as in the determination of ascorbic acid,[2] and the detection of bacteriological contamination in foodstuffs.[3] Early attempts to use the indophenols as dyestuffs in color photography[4] were unsuccessful because of the instability and high water solubility of the colored indophenolate ion. The conversion of phenols to indophenols is the basis for an extremely sensitive method for the quantitative determination of phenolic compounds.[5,6]

The authors[7] have recently introduced the use of esters of this series of compounds as chromogenic substrates for the estimation of acetylcholinesterase activity. Moreover, Nachlas *et al.*[8] have attempted to employ these substances in the histochemical localization of esteratic enzymes.

Indophenols have been prepared by a variety of procedures. However, as reported by Gibbs, Hall, and Clark,[1] the method of Hirsch[9] or some modification thereof yields the best results. This method is essentially the coupling of the appropriate *N*-chloro quinoneimine with a phenol under alkaline conditions. While previous workers[1,10] encountered difficulties in obtaining pure indophenol salts, we found that esters could be readily made following a modification of the method of Heller[11] and purified from suitable solvents yielding yellow to red crystalline solids with characteristic physical properties.

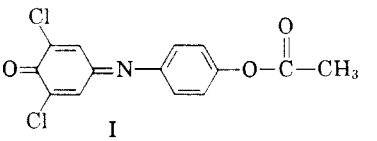
A series of esters of the indophenols was prepared in an endeavor to study the effect of structure on the enzymatic activity of various esterases with particular attention to acetylcholinesterase and serum cholinesterase. The results of these studies will be published elsewhere. Tables I–III list the indophenol esters that have been prepared. The physical constants of these compounds are presented in Table IV.

Other than the *N*(4′-acetoxy phenyl)-*p*-quinoneimine (IPA) reported by Heller,[11] no other esters of the indophenols have been recorded in the literature. However, Meyer and Elbers[12] did prepare the benzoate of the indophenol-*N*-oxide.

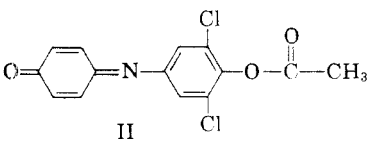


We are likewise preparing a variety of esters in the indophenol-*N*-oxide series and the results of these investigations will be published at a later date.

As there is a possibility for acylation to occur on either oxygen of the unsymmetrically substituted mesomeric ionic salt, two possible isomeric esters can be prepared. In the case of dichloroindophenol acetate, these are I and II. These compounds were actually prepared and identified, one being red and



I



II

(1) W. M. Clark and B. Cohen, Public Health Reports, **38**, 933 (1923) (Reprint no. 834); B. Cohen, H. D. Gibbs, and W. M. Clark, Public Health Reports, **39**, 381 (1924) (Reprint no. 904); B. Cohen, H. D. Gibbs, and W. M. Clark, Public Health Reports **39**, 804 (1924) (Reprint no. 915); H. D. Gibbs, B. Cohen and R. K. Cannan, Public Health Reports **40**, 649 (1925) (Reprint no. 1001); H. D. Gibbs, W. L. Hall and W. M. Clark, Supplement No. 69 to Public Health Reports (1928); W. L. Hall, P. W. Preisler, and B. Cohen, Supplement No. 71 to Public Health Reports (1928); B. Cohen and M. Phillips, Supplement No. 74 to Public Health Reports (1929).

(2) R. E. Buck and W. S. Ritchie, *Ind. Eng. Chem., Anal. Ed.* **10**, 25 (1938); M. H. Menaker and N. B. Guerrant, *Ind. Eng. Chem., Anal. Ed.* **10**, 26 (1938); O. H. Keyes, *Ind. Eng. Chem., Anal. Ed.* **11**, 293 (1939).

(3) J. Tillmans, P. Hirsch, E. Reinshagen, *Z. Unters. Lebensm.* **56**, 272 (1928); *Chem. Abstr.*, **23**, 3277[5] (1929).

(4) P. W. Vittum and G. H. Brown, *J. Amer. Chem. Soc.*, **68**, 2236 (1946).

(5) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).

(6) M. B. Ettinger and C. C. Ruchhoft, *Anal. Chem.*, **20**, 1191 (1948).

(7) D. N. Kramer and R. M. Gamson, *Anal. Chem.*, **30**, 251 (1958).

(8) M. Nachlas, A. Young, and A. M. Seligman, *J. Histochem. and Cytochem.*, **5**, 565 (1957).

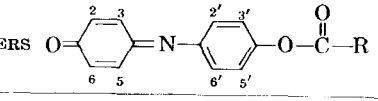
(9) A. Hirsch, *Ber.*, **13**, 1903 (1880).

(10) M. M. Brooks, *J. Am. Chem. Soc.*, **53**, 1826 (1931).

(11) G. Heller, *Ann.*, **392**, 28 (1912).

(12) K. H. Meyer and W. E. Elbers, *Ber.*, **54B**, 343 (1921).

TABLE I. ANALYTICAL DATA, INDOPHENYL ESTERS

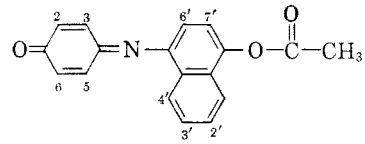


Compound	Substituent 2	3	5	6	2′	3′	5′	R	Molecular Formula	Analyses Calculated C	H	N	Halogen	Found C	H	N	Halogen
1								CH_3	$C_{14}H_{11}NO_3$	69.7	4.6	5.8	...	69.6	5.2	5.7	...
2		CH_3						CH_3	$C_{15}H_{13}NO_3$	70.6	5.1	5.5	...	69.4	5.1	...	...
3		OCH_3						CH_3	$C_{15}H_{13}NO_4$	66.4	4.8	5.2	...	66.1	4.8	...	...
4						CH_3	CH_3	CH_3	$C_{16}H_{15}NO_3$	71.4	5.6	5.2	...	71.3	5.5	5.1	...
5						OCH_3	OCH_3	CH_3	$C_{16}H_{15}NO_5$	63.8	5.0	4.7	...	62.9	4.9	4.6	...
6								C_2H_5	$C_{15}H_{13}NO_3$	70.6	5.1	5.5	...	70.0	5.1	...	...
7A[a]						Br	Br	CH_3	$C_{14}H_9Br_2NO_3$	42.1	2.3	3.5	40.1	42.2	2.6	...	...
B[b]	Br			Br				CH_3						42.3	2.3	4.1	...
8A[a]		CH_3				Br	Br	CH_3	$C_{15}H_{11}Br_2NO_3$	43.6	2.7	3.4	38.7	43.2	2.8	...	...
B[b]	Br			Br	CH_3			CH_3						43.7	3.0	...	...
9	CH_3					Br	Br	CH_3	$C_{15}H_{11}Br_2NO_3$	43.6	2.7	3.4	38.7	43.9	2.6	3.5	38.5
10	Br			Br	C_2H_5			CH_3	$C_{16}H_{13}Br_2NO_3$	45.0	3.0	3.3	37.5	45.4	3.2	...	...
11	C_2H_5					Br	Br	CH_3	$C_{16}H_{13}Br_2NO_3$	45.0	3.0	3.3	37.5	45.0	3.1	...	...
12	OCH_3					Br	Br	CH_3	$C_{15}H_{11}Br_2NO_4$	42.0	2.6	3.3	37.3	42.6	3.0	3.3	37.2
13	CH_3			CH_3		Br	Br	CH_3	$C_{16}H_{13}Br_2NO_3$	45.0	3.0	3.3	37.5	46.6	3.2	3.7	37.9
14	Br			Br		CHCHCOOH		CH_3	$C_{17}H_{11}Br_2NO_5$	43.5	2.4	3.0	34.1	43.5	3.0	3.1	...
15	Br			Br		C_6H_5		CH_3	$C_{20}H_{13}Br_2NO_3$	50.5	2.7	3.0	33.7	51.9	3.1	2.9	...
16	Br			Br		Br		CH_3	$C_{14}H_8Br_3NO_3$	35.2	1.7	2.9	50.2	36.5	2.1	3.0	...
17	Br			Br		Br	Br	CH_3	$C_{14}H_7Br_4NO_3$	30.2	1.3	2.5	57.5	30.8	1.4	...	...
18		$NHCOCH_3$				Br	Br	CH_3	$C_{16}H_{12}Br_2N_2O_4$	42.1	2.6	6.1	35.1	42.0	2.5	6.3	...
19	$NHCOCH_3$					Br	Br	CH_3	$C_{16}H_{12}Br_2N_2O_4$	42.1	2.6	6.1	35.1	42.8	2.7	6.3	...
20		$NHCOC_6H_4NO_2$				Br	Br	CH_3	$C_{21}H_{13}Br_2N_3O_6$	44.8	2.3	7.5	28.4	44.8	2.7	7.6	28.4
21		$NHCOC_2H_4COOH$				Br	Br	CH_3	$C_{18}H_{14}Br_2N_2O_6$	42.0	2.7	5.5	31.1	42.1	2.7	...	...
22A[a]						Br	Br	C_3H_7	$C_{16}H_{13}Br_2NO_3$	45.0	3.0	3.3	37.5	45.2	3.1	...	...
B[b]	Br			Br				C_3H_7						45.2	3.3	...	...
23A[a]						Cl	Cl	CH_3	$C_{14}H_9Cl_2NO_3$	54.2	2.9	4.5	22.9	54.2	2.9	...	...
B[b]	Cl			Cl				CH_3						54.5	3.0	...	...
24	Cl			Cl	CH_3			CH_3	$C_{15}H_{11}Cl_2NO_3$	55.6	3.4	4.3	21.9	54.9	3.8	5.2	...
25	CH_3					Cl	Cl	CH_3	$C_{15}H_{11}Cl_2NO_3$	55.6	3.4	4.3	21.9	55.3	3.5	4.4	...
26	Cl			Cl	OCH_3			CH_3	$C_{15}H_{11}Cl_2NO_4$	52.9	3.2	4.1	20.9	53.1	3.6	4.3	19.6
27	OCH_3					Cl	Cl	CH_3	$C_{15}H_{11}Cl_2NO_4$	52.9	3.2	4.1	20.9	53.1	3.5	...	...
28	CH_3			CH_3		Cl	Cl	CH_3	$C_{16}H_{13}Cl_2NO_3$	56.8	3.9	4.1	21.0	56.8	4.1	4.4	...
29	CH_3			CH_3		Cl	Cl	OC_2H_5	$C_{17}H_{15}Cl_2NO_4$	55.4	4.1	3.8	19.3	55.4	4.0	...	...
30	CH_3		CH_3			Cl	Cl	CH_3	$C_{16}H_{13}Cl_2NO_3$	56.8	3.9	4.1	21.0	56.5	3.9	...	...
31	Cl			Cl		Cl		CH_3	$C_{14}H_8Cl_3NO_3$	48.8	2.3	4.1	30.9	49.1	2.9	3.8	...
32	Cl			CH_3		Cl	Cl	CH_3	$C_{15}H_{10}Cl_2NO_3$	50.2	2.8	3.9	29.7	50.9	3.1	4.0	...
33	$CH_2CH{=}CH_2$					Cl	Cl	CH_3	$C_{17}H_{13}Cl_2NO_3$	58.3	3.7	4.0	20.3	58.5	4.4	3.9	...
34	Cl			Cl		CHCHCOOH		CH_3	$C_{17}H_{11}Cl_2NO_5$	53.7	2.9	3.7	18.7	53.8	3.7	3.4	...
35	C_6H_5					Cl	Cl	CH_3	$C_{20}H_{13}Cl_2NO_3$	62.2	3.4	3.6	18.4	61.9	3.9	...	...
36		$NHCOCH_3$				Cl	Cl	CH_3	$C_{16}H_{12}Cl_2N_2O_4$	52.3	3.3	7.6	19.4	52.4	3.4	7.6	...
37	$NHCOCH_3$					Cl	Cl	CH_3	$C_{16}H_{12}Cl_2N_2O_4$	52.3	3.3	7.6	19.4	52.0	4.3	...	...
38		$NHCOC_2H_5$				Cl	Cl	CH_3	$C_{17}H_{14}Cl_2N_2O_4$	53.5	3.7	7.4	18.6	53.8	3.8	7.5	...
39	Cl			Cl				C_3H_7	$C_{16}H_{13}Cl_2NO_3$	56.8	3.9	4.1	21.0	56.8	3.9	...	...
40						Cl	Cl	C_6H_5	$C_{19}H_{11}Cl_2NO_3$	61.3	3.0	3.8	19.1	61.4	3.2	4.1	...

[a] Prepared by Procedure B. [b] Prepared by Procedure A.

TABLE II

ANALYTICAL DATA, INDONAPHTHYL ACETATES

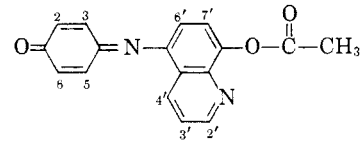


Compound	Substituent[a] 2	6	Molecular Formula	Calculated % C	H	N	Observed % C	H	N
41			$C_{18}H_{13}NO_3$	74.2	4.6	4.8	74.1	4.6	
42	Cl	Cl	$C_{18}H_{11}Cl_2NO_3$	60.0	3.1	3.9	60.7	3.2	4.2
43	Br	Br	$C_{18}H_{11}Br_2NO_3$	48.1	2.5	3.1	48.1	2.5	3.5

[a] Position of acyl group (1 or 8') was not determined.

TABLE III

ANALYTICAL DATA INDOQUINOLINYL ACETATES



Compound	Substituent[a] 2	6	2'	Molecular Formula	Calculated % C	H	N	Found % C	H	N
44				$C_{17}H_{12}N_2O_3$	69.9	4.1	9.6	70.1	4.4	9.6
45	Cl	Cl		$C_{17}H_{10}Cl_2N_2O_3$	56.5	2.8	7.8	56.8	3.1	
46	Cl	Cl	CH_3	$C_{18}H_{12}Cl_2N_2O_3$	57.6	3.2	7.5	58.0	3.3	
47	Br	Br		$C_{17}H_{10}Br_2N_2O_3$	45.3	2.2	6.2	44.2	2.5	6.8
48	Br	Br	CH_3	$C_{18}H_{11}Br_2N_2O_3$	46.6	2.6	6.0	46.5	2.7	6.0

[a] Position of acyl group (1 or 8') was not determined.

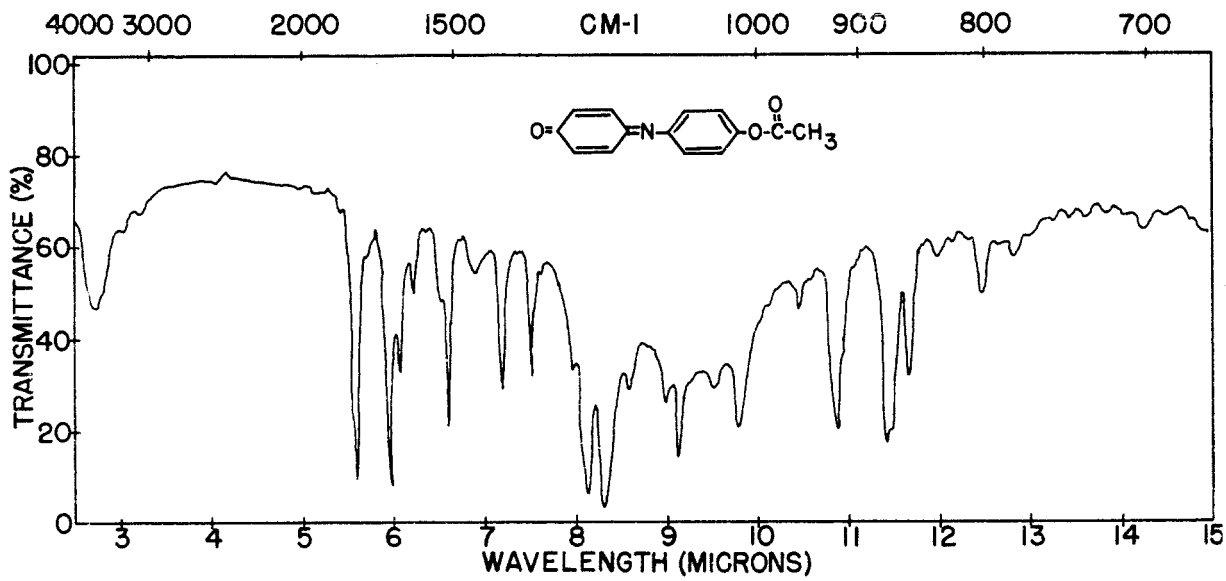


Fig. 1. Infrared spectrum of indophenyl acetate in a potassium bromide pellet

one orange. The red form is predominantly obtained by procedure A and the orange form by procedure B. The isolation of the two isomers and assignment of structures will be discussed in another publication.[13]

The infrared spectrum of indophenyl acetate is given in Figure 1.

EXPERIMENTAL

Preparation of Indophenol Sodium Salts. Some of these compounds are available from the Eastman Kodak Co. and National Aniline and Dye Co. The other salts were prepared by the following procedure: A mixture of 0.1 mole of appropriate phenol and 0.21 mole of sodium carbonate was dis-

(13) R. M. Gamson, D. N. Kramer and F. M. Miller, *J. Org. Chem.*, in press (paper II).

TABLE IV

PHYSICAL CONSTANTS OF INDOPHENYL ESTERS

Compound	Appearance	M.P.[a] °C.	Ester	Indophenylate Ion		
					*p*Ka	
			λ_{max} (log ϵ)[b,c]	λ_{max} (log ϵ)	Observed	Lit.[a]
1	Red needles, red plates	115–118	233(3.94), 262(4.23), 290(4.13), 460(3.52)	625(4.50)	8.1	8.10
2	Red needles	110–112	263(4.13), 290(4.00), 460(3.38)	650(4.32)	8.8	8.55
3	Red-orange needles	109	285(3.83), 455(3.45)	605(4.00)	...	...
4	Red platelets	82–84	233(3.81), 285(4.14), 443(3.49)	595(4.23)	8.7	8.9
5	Orange-red microcrystals	175	222(4.12), 313(4.26), 444(3.55)	600(4.18)	9.0	...
6	Red needles	48–49	225s(3.74), 256(3.89), 455(3.05)	...	...	...
7A	Orange needles	119–121	213(4.44), 266(4.43), 433(3.47)	620(4.30)	5.8	5.70
B	Red-black, lustrous needles	145	226(4.19), 266(4.21), 274s(4.17), 470(3.76)			
8A	Red-orange microcrystals	110–112	227(4.19), 268(4.27), 445(3.34)	600(4.11)	5.6	5.9
B	Deep red	171	233(4.01), 233s(3.88), 316(3.94), 495(3.64)			
9	Orange microcrystals	130	226(4.12), 272(4.29), 440(3.40)	595(4.24)	5.4	5.4
10	Deep-red microcrystals	132–136	225(4.27), 450(3.11)	595(3.33)	6.4	...
11	Red-orange microcrystals	108	226(4.24), 270(4.23), 440(3.45)	595(4.20)	5.2	...
12	Orange microcrystals	98–100	227(4.18), 298(4.16), 420(3.35)	575(3.99)	6.0	5.6
13	Orange needles	129	226(4.23), 282(4.46), 432(3.47)	580(3.18)	5.4	...
14	Brick-red microcrystals	142–146	225(4.32), 274(4.26), 302s(4.19), 475(3.49)	625(4.01)	5.7	...
15	Red microcrystals	64–68	226(4.36), 302(4.00), 460(3.64)	610(4.18)	6.1	...
16	Red microcrystals	64	228(4.18), 313(4.14), 465(3.57)	650(4.05)	5.4	5.1
17	Deep-red microcrystals	190	227(4.28), 311(4.21), 470(3.45)	630(4.09)	6.1	...
18	Yellow microcrystals	108–111	226(3.50), 398(3.55)	...	...	...
19	Yellow-orange microcrystals	163	215(4.41), 282(4.19), 443(3.48)	...	...	...
20	Orange microcrystals	100–102	229(4.37), 268(4.34), 452(3.32)	...	...	...
21	Red-orange microcrystals	104–105	229(4.09), 275(3.95), 305s(3.89), 436(3.36)	...	...	...
22A	Orange microcrystals	77	267(4.39), 435(3.48)	...	...	...
B	Red microcrystals	79–81	313(4.23), 470(3.77)			
23A	Orange needles	88	226(4.23), 264(4.20), 274s(4.15), 445(3.44)	615(4.30)	5.8	5.70
B	Red needles	101–103	229(4.07), 274s(4.00), 305(4.07), 470(3.76)			
24	Deep-red microcrystals	70–74	223(4.26), 280(4.12), 492(3.46)	595(3.56)	5.9	...
25	Orange microcrystals	102–103	227(3.96), 269(4.20), 435(3.42)	590(4.28)	5.7	5.50
26	Red microcrystals	113	228(4.09), 262s(3.86), 275(3.91), 460(3.29)	595(3.88)	6.2	...
27	Red-orange microcrystals	135	223(4.23), 283(3.95), 433(3.32)	596(3.85)	5.6	...
28	Red-orange needles	118–119	226(3.83), 284(4.27), 435(3.39)	575(4.18)	5.9	...
29	Orange needles	107	221(4.25), 282(4.41), 434(3.35)	...	...	...
30	Red-orange microcrystals	75	228(3.76), 283(4.28), 435(3.29)	590(4.10)	6.1	...
31	Orange-red microcrystals	125	227(4.18), 278(4.17), 457(3.45)	650(4.11)	5.7	5.80
32	Red-brown microcrystals	119	227(4.13), 283(4.18), 447(3.33)	620(4.15)	5.5	...
33	Orange platelets	95–96	217(4.29), 274(4.26), 445(3.46)	600(4.26)	...	...
34	Red microcrystals	74–76	225(4.35), 288(4.31), 475(2.81)	632(4.34)	5.6	...
35	Red-orange needles	116	229(4.37), 262(4.29), 434(3.56)	620(4.27)	5.8	...
36	Yellow microcrystals	205–208	229(4.29), 276(4.05), 404(3.56)	...	...	...
37	Yellow-orange microcrystals	154–156	232(4.06), 293(4.06), 443(2.26)	...	...	...
38	Orange needles	138	229(4.31), 273(4.03), 400(3.56)	...	...	...
39	Red-black lustrous plates	75	305(4.18), 475(3.66)	...	...	...

TABLE IV (*Continued*)

Compound	Appearance	M.P.,[a] °C.	Ester λ_{max} (log ϵ)[b,c]	Indophenylate Ion λ_{max} (log ϵ)	Indophenylate Ion *p*Ka Observed	Indophenylate Ion *p*Ka Lit.[d]
40	Deep-orange needles	136–137	233(4.32), 266(4.35), 440(3.45)	...	...	...
41	Red-orange microcrystals	149–152	230(4.20), 260(4.30), 455(3.50)	598(4.13)	9.1	...
42	Orange-red platelets	150–153	227(4.18), 262(4.25), 428(3.41)	580(3.97)	6.8	...
43	Orange red platelets	146–148	227(4.38), 263(4.35), 436(3.40)	585(4.06)	6.9	...
44	Red needles	151	242(4.26), 445(3.51)	620(4.23)	9.5	...
45	Orange microcrystals	183	231(4.21), 268(4.29), 425(3.42)	590(4.03)	5.9	...
46	Red-orange plates	192–194	223(4.27), 268(4.37), 425(3.43)	585(4.06)	5.9	...
47	Orange plates	212–214	228(4.42), 265(4.39), 428(3.42)	590(4.10)	6.0	...
48	Yellow-orange needles	197–200	225(4.36), 268(4.40), 420(3.49)	595(3.98)	6.0	...

[a] All melting points are uncorrected. Determined using Fisher-Johns melting point apparatus. [b] Dioxane solutions. [c] *Anal. Chem.*, **31**, 42A (1959). [d] W. L. Hall, P. W. Preisler, and B. Cohen, Supplement No. 71, Public Health Reports (1928).

solved in 100 ml. of water. This solution was placed in a round bottom flask, immersed in an ice bath and was stirred magnetically until solution was complete, small amounts of dioxane being added if necessary. The appropriate *N*-chloroquinoneimine (0.1 mole) was dissolved in 100 ml. of dioxane and added dropwise to the cooled phenolic solution over a period of about 30 minutes. Mixing was continued for another 15 minutes. The solid sodium salt was filtered and air dried. If no solid formed, the solution was evaporated to dryness. No attempts were made to further purify the sodium salts.

Preparation of Esters. Procedure A. The following procedure is typical for the compounds described in Table I. The dry sodium salt (0.1 mole) was placed in an Erlenmeyer flask and 0.3 mole of acid anhydride added. The flask was then shaken on a mechanical wrist-action shaker for 2 hr. and allowed to stand at room temperature for 1 hr. It was poured onto crushed ice (600 g.) and after 1.5 hr. was filtered and the solid precipitate was washed with water. Glasses were sometimes obtained and washed with water, taken up in ether, and dried over sodium sulfate. The dried ether was concentrated on a steam bath to about 10 ml., diluted with four volumes of petroleum ether until a cloudiness appeared, and filtered. The filtrate was cooled in a freezer and the crystalline ester filtered. It was recrystallized from ether–petroleum ether.

Procedure B. The dry sodium salt (0.1 mole) was placed in a flask and 0.3 mole of acid anhydride and 0.1 mole of pyridine were added. The mixture was stirred for about 30 minutes, poured onto crushed ice and stirred for 1 hr. at room temperature until the excess acetic anhydride had hydrolyzed. The product was extracted with ether and the extracted portion washed free of acetic acid and pyridine and dried over anhydrous magnesium sulfate. The ether was removed under vacuum and a glassy product obtained. It was triturated with methanol and a yellow-orange solid thus formed was collected by filtration. Recrystallization from hot methanol gave the desired substance.

Spectra. Ultraviolet and visible spectra were determined in C.P. dioxane with a Perkin-Elmer Model 13U Spectrophotometer. Concentrations were $2 \times 10^{-5}M$ and $2 \times 10^{-4}M$ respectively. Spectra of the hydrolyzed product were determined immediately after hydrolysis of the dioxane solutions with 0.1*N* NaOH and dilution to volume so as to obtain $2 \times 10^{-5}M$ solutions. Infrared absorption spectra were obtained with a Perkin-Elmer Infracord using a sodium chloride prism and potassium bromide pellets.

pKa Values.[14] The *p*Ka values were obtained spectrophotometrically by the addition of 0.2 ml. of $2 \times 10^{-3}M$ dioxane solutions of the substrate to 4 ml. of 0.1*N* sodium hydroxide. After a predetermined time to obtain maximum hydrolysis, a solution of 0.1*M* potassium dihydrogen phosphate was added until the solution turned from blue to purple. It was sometimes necessary to add hydrochloric acid to effect the color change. The solutions were diluted to 10 ml. with deionized water and the *p*H determined. The absorption of the solution was simultaneously obtained at the

(14) Some difficulties were noted in obtaining the *p*Ka values. As the rate of hydrolysis varied between compounds, a determination was made of the time required for complete hydrolysis of each compound. After hydrolysis for the required period using a second sample, the solution was immediately neutralized to its intermediate color. This color was usually purple, but in some cases a grey range was obtained and these compounds were adjusted to the grey end of the blue range. During neutralization, the solution was not rendered strongly acid as the indophenols are unstable in their acidic forms.[1] However, best results were obtained by making the solution just pink and then adding dilute alkali to obtain a purple solution.

λ max of the hydrolyzed product. The *p*Ka 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.

(15) E. Salm, *Z. physik. Chem.*, **57**, 471 (1907); L. Flexser, L. P. Hammet, A. Dingwall, *J. Am. Chem. Soc.*, **57**, 2103 (1935).

ARMY CHEMICAL CENTER, MARYLAND

[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

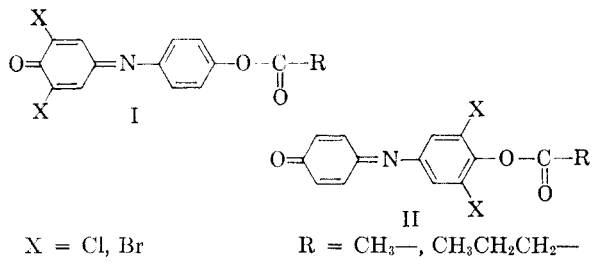
ROBERT M. GAMSON, DAVID N. KRAMER, AND F. M. MILLER

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



I

II

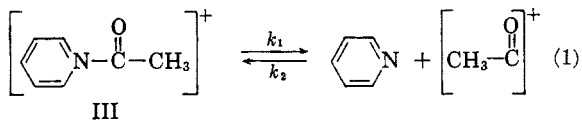
X = Cl, Br R = CH_3—, $CH_3CH_2CH_2$—

(u.v., visible, and I.R.) data which are the subject of this report.

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]

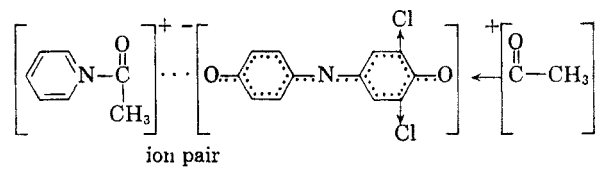
Experiments are now in progress to elucidate the mechanism of the acylation reaction with or without pyridine as a catalyst. Preliminary results indicate that the following rationalization may account for the two courses of the reaction.

Pyridine reacts with acetic anhydride to yield an acetylpyridinium complex III.[3] The acetyl



III (1)

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:



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 SN_2 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:

(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).