investigations into these phenomena revealed that of these three isomeric dyes the *m*-isomer was the strongest acid.[3] Thus it appeared that there might be a relationship between protein binding ability and acid strength. Accordingly, this study was undertaken to determine whether the correlation between protein binding ability and acid strength as observed in the case of the anions of the methyl reds could be carried over to the aminobenzoate ions.

Experimental

The systems chosen for study involved bovine serum albumin (BSA) at 0.2% concentration, methyl orange (MO) and the aminobenzoate ions. Considerable difficulty was encountered in attempts to obtain data by equilibrium dialysis of the aminobenzoate ions themselves with the protein, and accordingly the spectral displacement technique and competitive equilibrium dialysis technique as developed by Klotz and his co-workers were used.[4,5] The spectral studies were performed at room temperature and *p*H 7.3. The competitive equilibrium dialysis studies were performed at 0° and *p*H 6.8. Phosphate buffers were used to maintain the *p*H values. In all cases the extent of interaction was measured by studying the effect that the aminobenzoate ions had on the interaction between methyl orange and bovine serum albumin. The molar ratios of aminobenzoate to methyl orange ranged from 62/1 to 925/1. All spectral studies and analyses were performed on the Beckman model DU spectrophotometer. The protein samples were obtained from the Armour Company.

Results and Discussion

A study of protein interactions involving the anions of anthranilic acid and *p*-aminobenzoic acid has been made by Klotz.[4] It was shown at that time that the anthranilate ion was more effective than the *p*-aminobenzoate ion in displacing methyl orange from protein surfaces. The results obtained in this study which pertain to the *o*- and *p*-isomers agree entirely with this earlier work. The *m*-isomer which was not studied at that time yielded very interesting results showing that the *m*-isomer had no greater effect on the displacement of methyl orange than did the *p*-isomer.

TABLE I

CHANGES IN THE MOLAR EXTINCTION COEFFICIENT OF METHYL ORANGE WHEN IN THE PRESENCE OF VARIOUS ADDITIVES (*p*H 7.3, ROOM TEMP.)

Concentration of methyl orange (MO) 1.58×10^{-5} mole/l.; concentration of bovine serum albumin (BSA) and the aminobenzoic acids 0.2%; ratio of aminobenzoic acids to MO 925/1.

System	$\Delta\epsilon$ at 4700 Å.
MO	0
MO, BSA	−4200
MO, BSA, anthranilic acid	−1000
MO, BSA, *m*-aminobenzoic acid	−1800
MO, BSA, *p*-aminobenzoic acid	−1800

TABLE II

BINDING OF METHYL ORANGE AND BOVINE SERUM ALBUMIN IN THE PRESENCE OF THE AMINOBENZOIC ACIDS (*p*H 6.8, 0°)

Concentration of methyl orange (MO) 0.942×10^{-5} mole/l.; concentration of bovine serum albumin (BSA) 0.2%; concentration of the aminobenzoic acids 5.8×10^{-4} mole/l.; ratio of aminobenzoic acids to MO 62/1.

System	Mole of MO bound *per mole protein*
MO, BSA, anthranilic acid	0.16
MO, BSA, *m*-aminobenzoic acid	.33
MO, BSA, *p*-aminobenzoic acid	.33

It has been pointed out by Klotz that the presence of groups capable of hydrogen bonding such as are found in the anthranilic acid molecule enhance protein binding.[4] The ions derived from *m*- and *p*-aminobenzoic acids, however, cannot engage in such bonding and hence acid strength and molecular configuration should be the paramount considerations in their interactions with proteins. The fact that these two isomers show no difference in their ability to displace methyl orange from protein surfaces strongly suggests that the interactions of these two isomeric ions are similar. If this be true then one would suspect that the results observed with the methyl reds might be due to factors other than acid strength. Biochemical implications of these findings are now being investigated.

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(3) R. K. Burkhard, Ph.D. Thesis, Northwestern University, 1950.

(4) I. M. Klotz, THIS JOURNAL, **68**, 2299 (1946).

(5) I. M. Klotz, F. M. Walker and R. B. Pivan, *ibid.*, **68**, 1486 (1946).

Simultaneous Vicinal Dichlorination, a Correction[1]

BY E. CAMPAIGNE AND JOSEPH R. LEAL[2]

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We have been unable to confirm the phenomenon of "simultaneous vicinal dichlorination" previously reported from this Laboratory.[3] Using conditions which were as nearly as possible a duplication of those used in the earlier experiments, complex mixtures of halogenated products were obtained which could not be completely separated. It is possible that small amounts of vicinal chloro derivatives may have escaped detection in these mixtures. The situation is apparently further complicated by the existence of molecular compounds of two or more of the halogenated hydrocarbons which have relatively sharp melting points near those reported for pure compounds.

A study of the use of iodine trichloride as an aromatic halogenating agent and its reaction with benzene under various conditions has been made. When a carbon tetrachloride solution of acetanilide, for example, was refluxed with iodine trichloride, a 17% yield of *p*-chloroacetanilide[4] was found in the aqueous washings of the reaction mixture. The crystalline material remaining after the removal of the solvent, however, was not completely resolved into its components, even after repeated fractional crystallizations from various solvents.

Benzene, on the other hand, gave reaction mixtures from which moderate yields of chlorobenzene

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(1) Contribution No. 568 from the Chemical Laboratories of Indiana University.

(2) Taken from a portion of the thesis submitted by Joseph R. Leal in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

(3) E. Campaigne and W. Thompson, THIS JOURNAL, **72**, 629 (1950).

(4) E. Crepaz, *Atti Reale ist. Veneto sci.*, **94**, 555 (1934–1935) [*C. A.*, **31**, 6209 (1937)] obtained N-dichloroiodoacetanilide by treatment of acetanilide in cold chloroform with potassium chloroiodate (III), $(KICl_4)$.

and iodobenzene, and small yields of *p*-dichlorobenzene and hexachlorobenzene could be separated by ordinary laboratory techniques. The reaction is a complex one requiring rigid control of conditions for duplication of results.[5] Variations in the ratio of iodine trichloride to benzene,[3] the degree of illumination and the state of the reagent influenced the yields and distribution of the products. These results are summarized in Table I.

TABLE I

HALOGENATION OF BENZENE BY IODINE TRICHLORIDE

Expt.	Molar ratio[c]	Illumination	Reagent	C_6H_5-Cl	Product, yield,[g] % C_6H_5I	*p*-$C_6H_4Cl_2$	C_6Cl_6
1[a]	1/11	300-watt lamp	ICl_3[d]	54.2	33.8	4.10	2.10
2[a]	1/11	Diffuse light	ICl_3[d]	17.8	42.2		12.6
3[a]	1/11	Diffuse light	ICl_3[e]	30.2	38.4	8.10	
4[b]	1/1	300-watt lamp	ICl_3[f]	14.3	6.4	13.6	
5[a]	1/4	300-watt lamp	$KICl_4$	66.6	Trace		
6[a]	1/4	300-watt lamp	$HICl_4$	29.3	16.2		

[a] No solvent. [b] CCl_4 solvent. [c] ICl_3/C_6H_6. [d] Commercially prepared sample which had partially deteriorated and was recovered by treatment with chlorine. [e] Commercially prepared sample from sealed bottle. No chlorine treatment given. [f] Laboratory preparation from ICl and Cl_2. [g] Based on 1 g. atom of Cl per mole of ICl_3.

The reaction with anisole yielded a complex mixture of products which could not be readily separated into its components.

Experimental[6]

Iodine Trichloride.—Into approximately 250 ml. of liquid chlorine, contained in a one-liter flask immersed in an acetone–Dry Ice mixture and fitted with a dropping funnel and Hershberg stirrer, was added dropwise 163 g. (1 mole) of iodine monochloride with vigorous stirring. When all of the iodine monochloride had been added, the excess chlorine was allowed to evaporate from the flask. The essentially quantitative yield of dry orange iodine trichloride was used without further purification.

Commercially prepared iodine trichloride[7] was also used. After a bottle had been unsealed, however, the remaining iodine trichloride decomposed rapidly. Treatment of the decomposed material with 10–15 ml. of liquid chlorine restored it to its original orange color. Iodine trichloride apparently contains chlorine in excess of that required by the empirical formula.[8]

Potassium Chloroiodate(III).—Iodine trichloride was added to a cold aqueous solution of potassium chloride.[9] The fine orange crystals that separated were filtered off under suction, pressed between filter paper and used without further drying.

Chloroiodous Acid.—The acid was prepared from iodine trichloride and concentrated hydrochloric acid,[10] but was not crystallized for use in the reaction.

Reaction with Acetanilide.—To a solution of 13.7 g. (0.1 mole) of acetanilide in 500 ml. of carbon tetrachloride was added slowly 46 g. (0.2 mole) of orange iodine trichloride. The mixture was then refluxed for 30 minutes, cooled and washed successively with aqueous sodium bisulfite solution and water. The combined washings contained 4.5 g. of suspended material which was filtered off. Upon recrystallization from water, 3 g. (17% yield) of colorless crystals, m.p. 177–179° were obtained which did not depress the melting point of an authentic sample of *p*-chloroacetanilide.

Upon removal of the solvent present in the decolorized and dried reaction mixture, elemental iodine was liberated, as evidenced by the purple color of the distillate, and 8 g. of product separated from the residual solution. Further distillation allowed the separation of 2.5 g. more of product. Repeated fractional crystallizations from water, 50% acetic acid, carbon tetrachloride, petroleum ether and dilute ethanol finally gave two fractions of m.p. 128–136° and 152–157°, respectively. These fractions were considered to represent impure substances and were not further investigated.

Reaction with Benzene.—In experiment 1, 233 g. (1 mole) of yellow iodine trichloride was added slowly with vigorous stirring to a two-liter flask equipped with stirrer, reflux condenser and addition tube and containing 858 g. (11 moles) of anhydrous thiophene-free benzene. A 300-watt lamp approximately 30 inches away illuminated the reaction vessel during the addition of the reagent and during the subsequent one-hour refluxing period. After refluxing, the dark-colored mixture was cooled, decolorized with aqueous sodium bisulfite solution, washed with water, dried and rectified through a heated distillation column packed with glass helices. During the distillation, elemental iodine was liberated; consequently, after the unreacted benzene had been removed, the reaction mixture was again decolorized, washed and dried. Subsequent distillation of the mixture, now weighing 200 g., gave 4.5 g. of forerun, b.p. 80–128° and 61 g. (54.2%) of chlorobenzene, b.p. 128–129°, identified by conversion to a bromochlorobenzene, which did not depress the melting point of an authentic sample of *p*-bromochlorobenzene,[11] m.p. 65–66°, and a dinitrochlorobenzene, m.p. 49°, which did not depress the melting point of 2,4-dinitrochlorobenzene.[12] Further distillation under reduced pressure gave 11 g. of material boiling over the range 39–65° at 20 mm. and 5 g. of crystals (A) which had condensed in the column and still-head. Recrystallization of (A) yielded 3 g. (4.1%) of *p*-dichlorobenzene, m.p. 53–55°, which did not depress the melting point of an authentic sample of *p*-dichlorobenzene. After removal of the *p*-dichlorobenzene and resumption of the distillation, free iodine was again liberated, coloring the 79 g. of distillate (B) collected at 65–70° at 20 mm. Refractionation of (B) yielded 69 g. (33.8%) of iodobenzene, b.p. 69–70° at 20 mm., brominated to a crystalline compound, m.p. 164–165°, which did not depress the melting point of 2,4,5-tribromoiodobenzene, m.p. 164–165°.[13] It was also converted to 2,4-dinitroiodobenzene, m.p. 86–88°, as shown by mixed melting point with an authentic sample.[14] Passage of chlorine into a portion of (B) in chloroform gave an almost quantitative yield of phenyliodo dichloride.[13]

The residue, which amounted to 18 g. after removal of 2 g. of crystals (C), m.p. 225°, gave 12 g. more of distillate collected over the range 76–96° at 8 mm. Recrystallization of (C) gave 1 g. (2.11%) of hexachlorobenzene, m.p. 226–227°, which did not depress the melting point of an authentic sample of hexachlorobenzene.

In experiments 2 and 3, the reaction vessel was shielded from direct light rays during the addition of the iodine trichloride as well as the refluxing period. Benzene was dropped into a mixture of carbon tetrachloride and iodine trichloride in experiment 4, while in experiments 5 and 6, the reagents involved were added to an excess of benzene. Table I summarizes the results of these experiments.

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[5] Other investigators have reported the products as: (a) mono-, di- and trichlorobenzenes, H. Müller, *J. Chem. Soc.*, **15**, 41 (1862); (b) hexachlorobenzene, V. Thomas and P. Depuis, *Compt. rend.*, **143**, 282 (1906); (c) chlorine substitution products of benzene and small amounts of iodobenzene, V. Arreguine, Jr., and E. D. Garcia, *Anales asoc. quím. argentina*, **9**, 121 (1921); (d) chlorobenzenes, chlorocycloolefins and benzene hexachloride, G. Calingaert, M. E. Griffing, E. R. Kerr, A. J. Kolka and H. D. Orloff, THIS JOURNAL, **73**, 5224 (1951).

[6] Melting points and boiling points are uncorrected.

[7] Obtained from the Fairmount Chemical Company.

[8] Iodine trichloride varied in color from bright yellow to red-brown. Chlorine/iodine determinations indicated a ratio of 3.03 for a partially decomposed red-brown sample and 4.04 for a bright yellow sample formed by treating the decomposed material with excess chlorine. Other lots of the orange reagent varied between $ICl_{3.2}$ and $ICl_{3.4}$.

[9] Fritz Ephraim, "Inorganic Chemistry," 5th English edition by P. C. L. Thorne and E. R. Roberts, Gurney and Jackson, London, 1948, p. 254.

[10] H. W. Cremer and D. R. Duncan, *J. Chem. Soc.*, 1857 (1931).

[11] W. Körner, *Gazz. chim. ital.*, **4**, 342 (1874).

[12] E. H. Huntress, "Organic Chlorine Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 1081.

[13] C. Willgerodt, *J. prakt. Chem.*, [2] **33**, 154 (1886).

[14] W. Körner, *Gazz. chim. ital.*, **4**, 323 (1874).