seven days. The ether extract yielded 200 g. (80%) of mixed amides, b.p. 125–150° (21 mm.).

Cyclopropylamine.—The following procedure is typical. A solution of 83.5 g. of potassium hydroxide pellets in 90 ml. of water was added to 74.3 g. (0.75 mole) of the amide mixture (from the benzenesulfonate procedure) dissolved in 300 g. of ethylene glycol. The resulting mixture was heated under a 50-cm. Vigreux column and distillate taken off at such a rate as to keep the head temperature below 55°. A Dry-Ice cooled receiver was used to condense the methylamine formed in the reaction. When the hydrolysis was complete, the head temperature rose to 100° and remained constant. The distillate was fractionated using a 30-cm. Vigreux column with a Dry-Ice cooled reflux condenser until all of the methylamine was removed. The yield of methylamine was 2.5 g. (11%). The higher-boiling materials were fractionated using a 30-cm. stainless-steel helix-packed column and yielded 36.5 g. (85%) of cyclopropylamine, b.p. 50°. The benzamide derivative had m.p. 97.5–98.0° (lit.[9] 99°) after crystallization from alcohol–water.

In an attempt to carry out the hydrolysis of the amide mixture by refluxing with 12 *N* hydrochloric acid, extensive decomposition occurred.

(9) M. J. Schlatter, THIS JOURNAL, **63**, 1733 (1941).

CAMBRIDGE 39, MASS. RECEIVED DECEMBER 29, 1950

[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE]

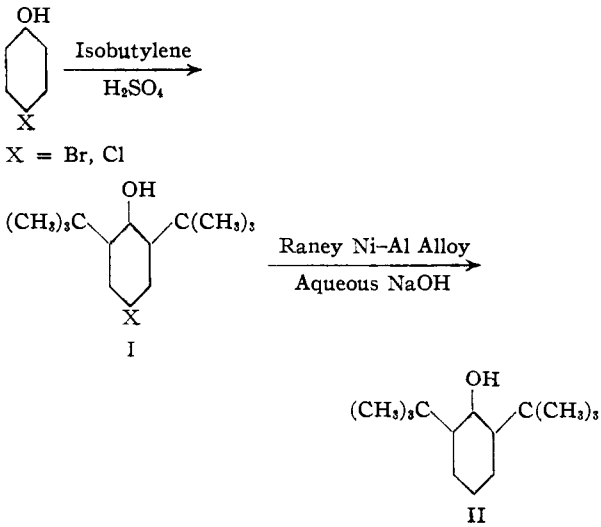
Ortho Alkylated Phenols. 2,6-Di-*t*-butylphenol

BY HAROLD HART AND FRANK A. CASSIS, JR.

In order to study the effect of large substituents ortho to the hydroxyl group on the uncatalyzed alkylation of phenol with *t*-butyl chloride, 2,6-di-*t*-butylphenol was synthesized. Its ultraviolet absorption spectrum and certain of its reactions are described. Nitration at 25–35° in glacial acetic acid (1:1) yielded 4,6-dinitro-2-*t*-butylphenol. Under milder conditions, the oxidation product 3,3′,5,5′-tetra-*t*-butyldiphenoquinone was isolated. Sulfuric acid caused rearrangement to 2,4-di-*t*-butylphenol. The relative rates of alkylation of phenol, 2-*t*-butylphenol, 2,6-di-*t*-butylphenol, *o*-cresol and 2,6-xylenol with *t*-butyl chloride were determined. Possible mechanisms are discussed. The relative rates of alkylation of *m*- and *p*- cresol in the ortho positions are also included.

In order to further elucidate the mechanism of the uncatalyzed alkylation of phenols with tertiary alkyl halides[1] it became desirable to prepare phenols with bulky alkyl groups in the ortho positions but unsubstituted in the para position. Recently[2] the synthesis of 2-*t*-butylphenol in good yield was described. We have now extended this method to the synthesis of 2,6-di-*t*-butylphenol (II) and have studied some of its properties.

Compound II has been reported by Pardee and Weinrich,[3] and Stillson and Sawyer,[4] the latter authors being the only ones to indicate its method of synthesis. However, no indication of yields was given. The general scheme which we used follows our earlier work.[2] The reduction with Raney Ni–Al alloy and aqueous alkali[2,5] afforded a superior yield to the procedure of Stillson and Sawyer,[4] who used potassium and liquid ammonia.[6]



The ultraviolet absorption spectrum of II in cyclohexane is given in Fig. 1, together with that of 2,6-di-*t*-butyl-4-methylphenol for comparison. The peaks at 271 and 278 mμ indicate the absence of any alkyl group in the para position.[2,7]

Nitration of II with concentrated nitric acid in glacial acetic acid (1:1) at room temperature yielded 4,6-dinitro-2-*t*-butylphenol. This product is identical with that obtained by Ipatieff, Pines and Friedman[8] from the nitration of 2,4-di-*t*-butylphenol. In their case, the 4-*t*-butyl group was cleaved from the ring, whereas in the present instance, one of the 2-*t*-butyl groups was cleaved.

An attempt at nitration without cleavage, using 1:6 nitric–acetic acids at 0° yielded small quantities

(1) H. Hart and J. H. Simons, THIS JOURNAL, **71**, 345 (1949).

(2) H. Hart, *ibid.*, **71**, 1966 (1949).

(3) W. A. Pardee and W. Weinrich, *Ind. Eng. Chem.*, **36**, 595 (1944).

(4) G. H. Stillson and D. W. Sawyer, U. S. Patent 2,459,597 (1949); *C. A.*, **43**, 3459 (1949).

(5) D. Papa, E. Schwenk and B. Whitman, *J. Org. Chem.*, **7**, 587 (1942); E. Schwenk, D. Papa, B. Whitman and H. Ginsburg, *ibid.*, **9**, 1 (1944).

(6) Although the product which we obtained affords the correct analysis and expected properties for 2,6-di-*t*-butylphenol, our product was a colorless liquid. We were unsuccessful in crystallizing this material. Stillson and Sawyer[4] and Pardee and Weinrich[3] claim the product to be a yellow solid, m.p. 38–39°. In this connection, it is of considerable interest to note that when I (X = Cl) was reduced with hydrogen in the presence of palladium chloride on charcoal at *room temperature* and 50 atm., a white solid, m.p. 38–38.5° was obtained. This material was shown to be probably 2,6-di-*t*-butylcyclohexanone (private communication from Dr. R. H. Rosenwald, Universal Oil Products Company). The reduction to the cyclohexanone is to be expected (see A. C. Whitaker, THIS JOURNAL, **69**, 2414 (1947)) but the mild conditions which accomplished the reaction are somewhat surprising and bear further investigation. NOTE ADDED IN PROOF: We have recently succeeded in preparing crystalline 2,6-di-*t*-butylphenol. White prisms were obtained from ethanol, m.p. 37-38°. Admixture with some 2,6-di-*t*-butylcyclohexanone obtained from Dr. Rosenwald resulted in immediate liquefaction at room temperature. Preliminary experiments with crystalline II have shown that its rates of bromination in carbon tetrachloride and coupling with diazotized aniline are negligible when compared with 2,6-xylenol.

(7) H. Hart and E. A. Haglund, *J. Org. Chem.*, **15**, 396 (1950).

(8) V. N. Ipatieff, H. Pines and B. S. Friedman, THIS JOURNAL, **60**, 2495 (1938).

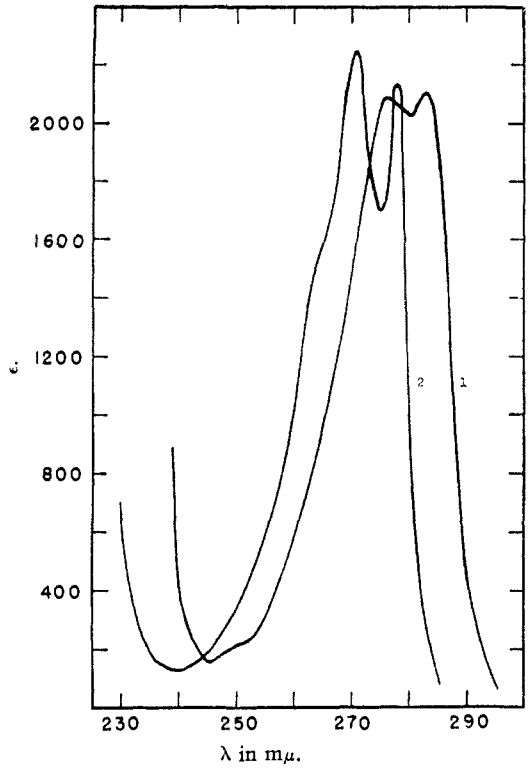


Fig. 1.—Ultraviolet absorption spectra: (1) 2,6-di-*t*-butyl-4-methylphenol, and (2) 2,6-di-*t*-butylphenol, both in cyclohexane solvent.

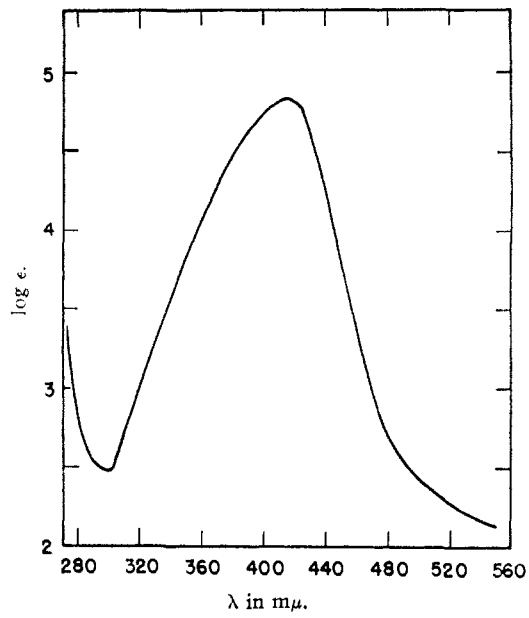


Fig. 2.—Absorption spectrum of 3,3′,5,5′-tetra-*t*-butyldiphenoquinone.

of dark red needles, m.p. 245–247°, which contained no nitrogen. The analysis agrees with the formula for 3,3′,5,5′-tetra-*t*-butyl-*p*-diphenoquinone. Similar oxidation products have been isolated in a number of nitrations of substituted phenols[9] as well as from other phenol oxidations.[10] The major peak in the spectrum of this product (see Fig. 2) at 420 mμ is similar to that obtained by Valyashko and Shcherbak[11] for diphenoquinone, but shifted slightly toward the longer wave length.

Relative Rates of Alkylation of Ortho Alkylated Phenols.—Phenol reacts with *t*-butyl chloride in the absence of any alkylation catalyst to form *p*-*t*-butylphenol and hydrogen chloride.[12] It was of interest to study the rate of this reaction with ortho alkylated phenols for the following reason. If the mechanism is of the S_N1 type, then in an equivalent or identical solvent, the rate of alkylation should not be greatly affected by the presence of large alkyl groups ortho to the hydroxyl. On the other hand, should the reaction involve a concerted mechanism, particularly involving coördination of the phenol with the chlorine of *t*-butyl chloride,[1] one would expect a decrease in the rate of alkylation *in the para position* by alkyl substituents ortho to the hydroxyl.

The experiments which were performed are summarized in Table I. The reactions were carried out by passing nitrogen slowly through a mixture of the reactants and absorbing the evolved hydrogen chloride in sodium carbonate, followed by Volhard determination of the chloride evolved after a given time interval. In spite of the crude method, the results were quite reproducible, as shown in the last column of Table I.

In the experiments (1–7) the phenol being alkylated was used in large excess as the solvent. The reaction medium could hardly be considered as equivalent from one experiment to another, although in several cases (say 6 and 7) the solvent properties of the phenol should not change markedly.

The large decrease in the rate of alkylation of phenol caused by ortho alkyl groups may be the result of several factors. In addition to the decrease of the electron density at the position para to the hydroxyl because of the inductive effect of the alkyl groups, these substances (particularly the dialkylphenols) would be less associated solvents[13] and less efficient at solvating carbonium ions or coördinating the halogen of *t*-butyl chloride.

Meta and para cresol (experiments 6 and 7) both alkylate ortho to the hydroxyl[14] and therefore represent comparable reactions. These phenols should be very similar in solvent properties.[15] The large enhancement in the rate of meta cresol over the para isomer may be ascribed here to the increased electron density at the 6-position caused by the inductive and/or hyperconjugative effect of the methyl group.

If the reactions could be studied in dilute solution in an inert solvent, one should not encounter the above multiplicity of possible explanations. These reactions decrease rapidly in rate, however, when solvents such as xylene, etc., are used.[1] An attempt

(9) H. E. Albert, Abstracts of 118th Meeting of the American Chemical Society, 42N (1950).

(10) S. L. Cosgrove and W. A. Waters, Abstracts of 117th Meeting of the American Chemical Society, 75L (1950).

(11) N. A. Valyashko and M. M. Shcherbak, *J. Gen. Chem.* (*U. S. S. R.*), **8**, 1641 (1938).

(12) J. H. Simons and H. Hart, THIS JOURNAL, **66**, 1309 (1944).

(13) N. D. Coggeshall, *ibid.*, **69**, 1620 (1947).

(14) A. Tchitchibabine, *Bull. soc. chim.*, [5] **2**, 497 (1935).

(15) Compare, for example, the dielectric constants of these compounds; "International Critical Tables," McGraw-Hill Book Company, Inc., New York, N. Y., 1929, Vol. 6, p. 92.

TABLE I

RELATIVE RATES OF ALKYLATION OF SUBSTITUTED PHENOLS WITH t-BUTYL CHLORIDE

Expt.	t-Butyl chloride, mole	Substituent on phenol	Moles of phenol	Solvent	Mole of solvent	Total reaction time (t), hr.	Temp., °C.	Alkylation,[a] %
1	0.01	None	0.1	.....	..	2	50	41 ± 2[b]
2	.005	2-t-Butyl	.05	.....	..	2	50	2.3
3	.01	2-Methyl	.1	.....	..	2	50	2.7 ± 0.2
4	.01	2,6-Di-methyl	.1	.....	..	2	50	1.4 ± 0.2
5	.005	2,6-Di-t-butyl	.05	.....	..	2	50	0.0
6	.01	4-Methyl	.1	.....	..	2	50	2.2 ± 0.1
7	.01	3-Methyl	.1	.....	..	2	50	22 ± 2[c]
8	.1	None	.1	Nitrobenzene	0.25	24	75	9 ± 1[c]
						48		14 ± 1[c]
9	.1	2,6-Di-t-butyl	.1	Nitrobenzene	.25	24	75	0.8
						48		1.3
10	.1	2,6-Di-t-butyl with 0.1 mole phenol	.1	Nitrobenzene	.25	48	75	13.7

[a] Calculated as (moles of HCl evolved in time t/moles of t-butyl chloride) × 100. [b] Average of three runs. [c] Average of two runs.

was made to use nitrobenzene as the solvent, but here again the rate was very slow, even at 75°. Although the solutions are rather concentrated in the phenol, nevertheless the results of experiments 8 to 10 are more suitable for comparison than those cited above. We again observe the marked decrease in rate for the 2,6-di-t-butylphenol.

Experiments are now in progress in an attempt to obtain precise kinetic data for the alkylation of substituted phenols, in dilute solution in an inert solvent. These will involve higher boiling halides than t-butyl chloride, so that the reaction rate may be increased by elevation of the temperature. It is for this reason that the data involving t-butyl chloride are reported here.

Acknowledgment.—We wish to express our gratitude to the Research Corporation for a Frederic Gardner Cottrell grant which financed this work.

Experimental

4-Bromo-2,6-di-t-butylphenol (I, X = Br).—A mixture of p-bromophenol (86.5 g. or 0.5 mole), 200 ml. of benzene and 4 ml. of 98% sulfuric acid was treated at 65 ± 5° for ten hours with two moles of isobutylene in an alkylation vessel similar to that used by Stillson, Sawyer and Hunt.[16] After extraction with 20% alkali to remove unreacted p-bromophenol or monoalkylated product, the benzene solution was washed with water (neutral to litmus), dried over anhydrous sodium sulfate and the solvent removed *in vacuo* (any t-butylbenzene formed during the reaction was also removed at this point). The residue was fractionally distilled at 4 mm. through a Vigreux column. The distillate, boiling 126–128°, yielded white crystals on cooling, which, recrystallized from aqueous ethanol, melted 83–83.5°; yield 51 g. (35.6%).

Anal. Calcd. for $C_{14}H_{21}OBr$: Br, 28.03. Found: Br, 27.95, 27.75.

When p-xylene was used as the solvent, the yield was 47%. At 70° in the absence of a solvent and with isobutylene under 200 p.s.i. in a steel bomb, 23% of the desired product was obtained. In each case, about 20–25% of 4-bromo-2-t-butylphenol was recovered from the initial alkali extraction.

4-Chloro-2,6-di-t-butylphenol (I, X = Cl).—A mixture of p-chlorophenol (64 g. or 0.5 mole) and 100 g. of 85% sulfuric acid was treated at 70 ± 5° over a period of three hours with 1.5 moles of t-butyl alcohol in a three-necked 1-l. flask equipped with a stirrer and thermometer. Fifty grams of 98% sulfuric acid was added rapidly at the end of each of the first two hours, in order to maintain the concentration of the acid. After stirring for an additional hour, the sirupy red solution was poured onto cracked ice and the product extracted with benzene. The benzene solution was washed with bicarbonate and extracted with Claisen solution.[16] The benzene layer was dried over anhydrous potassium carbonate and the solvent removed *in vacuo*. The residue was fractionally distilled at 3 mm. through a Vigreux column. The distillate, boiling 111–115°, yielded white crystals on cooling, which, recrystallized from ligroin melted 75–76°; yield 29 g. (24%).

Anal. Calcd. for $C_{14}H_{21}OCl$: Cl, 14.76. Found: Cl, 14.61, 14.70.

About 5% of 4-chloro-2-t-butylphenol was recovered from the Claisen solution extraction. When the above procedure was followed with p-bromophenol, 5% of 4-bromo-2-t-butylphenol was recovered, but the benzene extract yielded no 4-bromo-2,6-di-t-butylphenol, only polymeric residues being obtained.

2,6-Di-t-butylphenol (II). **Raney Ni–Al Alloy and Aqueous Alkali Reduction.**[2]—From 10 g. (0.035 mole) of I (X = Br) (or an equivalent quantity of I, X = Cl) there was obtained 6.7 g. (93%) of (II), b.p. 94–98° at 5–6 mm.

Anal. Calcd. for $C_{14}H_{22}O$: C, 81.5; H, 10.68. Found: C, 81.6, 81.35; H, 10.80, 10.85.

Potassium in Liquid Ammonia Reduction.[4]—In a 1-l. two-necked flask 400 g. of liquid ammonia was condensed on 10 g. (0.035 mole) of (I, X = Br). Potassium metal was introduced (mixture being stirred) until the solution took on a permanent blue color. Ammonium chloride was added to destroy the unreacted potassium and the ammonia was allowed to evaporate overnight. The residue was dissolved in petroleum ether, washed with water until neutral and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue fractionated at 5–6 mm. through a Vigreux column. The product, boiling 94–98°, weighed 3.5 g. (48.6%). We were unsuccessful at crystallizing this material.[6]

The product from both methods of reduction yielded a deep blue color when treated with phosphomolybdic acid and ammonium hydroxide.[17] (II) gave no color change with 1% ferric chloride. It was insoluble in 10% sodium hydroxide.

Nitration of II. 4,6-Dinitro-2-t-butylphenol.—To 1 g. of II was added, cautiously, 5 ml. of a 1:1 mixture of concentrated nitric and glacial acetic acids. The solution was warmed for one minute, then poured onto cracked ice. After filtration and recrystallization from dilute methanol, yellow plates were obtained, m.p. 124–125° (lit. value[6] for 4,6-dinitro-2-t-butylphenol 123–124°); mixing melting point with picric acid 85–100°.

Anal. Calcd. for $C_{10}H_{13}O_5N_2$: N, 11.66. Found: N, 11.82, 11.73.

(16) G. H. Stillson, D. W. Sawyer and C. K. Hunt, THIS JOURNAL, **67**, 303 (1945).

(17) V. M. Plarkovskaya and S. G. Vatkina, *J. Applied Chem. (U. S. S. R.)*, **10**, 202 (1937); *C. A.*, **31**, 4232 (1937).

Attempted Nitration of II without Cleavage of *t*-Butyl Group.—To 1.03 g. (0.005 mole) of I was added a solution of 0.315 g. (0.005 mole) of concentrated nitric acid in 2 ml. of glacial acetic acid, the temperature being kept below 0°. After several minutes, the mixture was poured onto cracked ice. The reddish-brown oil was washed by decantation with water and sodium bicarbonate, then dissolved in 95% ethanol. On dilution with water, crystals separated which, on recrystallization from aqueous ethanol, gave dark red needles, m.p. 245–247°.

Anal.[18] Calcd. for $C_{28}H_{40}O_2$: C, 82.2; H, 9.85. Found: C, 82.5, 82.7; H, 9.69, 9.60.

This product is probably 3,3′,5,5′-tetra-*t*-butyldiphenoquinone.

Rearrangement of II.—The warm solution of 5 drops of 98% sulfuric acid in 1 ml. of II was further heated for several minutes, then poured into cold water. The organic product was taken up in petroleum ether, and the latter washed with 10% aqueous alkali to extract any rearranged product. Acidification, followed by extraction with ligroin and evaporation of the solvent yielded crystals which, recrystallized from ligroin, melted 55–55.5°; mixed with an authentic sample of 2,4-di-*t*-butylphenol, 55–56°.

(18) Performed by Clark Micro Analytical Laboratories, Urbana, Illinois.

The absorption spectra were determined with a Beckman spectrophotometer (model DU) using 1-cm. quartz cells. The cyclohexane solvent (for the phenols) was freed of benzene by passage through silica gel, followed by fractionation. Absolute alcohol was the solvent for the quinone. The 2,6-di-*t*-butyl-4-methylphenol was obtained through the generosity of the Koppers Company, Pittsburgh, Penna., and was purified by distillation (146° at 20 mm.) and recrystallization from ligroin, m.p. 69–70°.

Relative Rates of Alkylation.—The apparatus consisted of a 125-ml. erlenmeyer flask equipped with a nitrogen bubbler and a condenser, the outlet of which was connected to small absorption tubes containing sodium carbonate. The flask was immersed in a constant temperature bath. The mixture of *t*-butyl chloride and substituted phenol was allowed to react, with a slow stream of dry nitrogen bubbling through the mixture. At the end of the time interval, the sodium carbonate tube was removed, its contents dissolved in distilled water, and an aliquot was acidified with nitric acid and analyzed for chloride by the Volhard method.

When nitrobenzene was used as the solvent, a blank experiment (in which the phenol was omitted) showed no dehydrohalogenation of the *t*-butyl chloride under the conditions of the experiment.

The results of these experiments are summarized in Table I.

EAST LANSING, MICH. RECEIVED JANUARY 5, 1951

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Binding of Organic Ions by Proteins. Optical Evidence of Coöperative Interactions with Hydrogen Ions

BY IRVING M. KLOTZ AND JEAN M. URQUHART

Addition of sodium dodecyl sulfate to a buffered solution of iodinated human serum albumin or of iodinated bovine serum albumin produces a decrease in optical density of the protein in the region of 313 mμ. This drop in absorption demonstrates the uptake of hydrogen ions by the protein concurrently with the binding of anions. The magnitude of the effect is smaller than might be expected from simple electrostatic considerations.

The effect of bound anions on the acid-titration curves of proteins was first emphasized by Steinhardt[1] who estimated in addition the affinity of the protein for various anions. Changes in *p*H of isoionic albumin accompanying its interactions with dodecyl sulfate anions were also demonstrated by Putnam and Neurath.[2] More recently Scatchard and Black[3] have examined the uptake of hydrogen ions by isoionic albumin in the presence of a variety of anions and have used the observed shifts in *p*H to compute the number of bound anions. Similarly Longsworth and Jacobsen[4] have shown from their electrophoretic studies that anion binding may be accompanied by the uptake of protons.

From a complementary point of view, the electrostatic effect of bound hydrogen ions upon the affinity of proteins for anions has been considered in some detail by Scatchard, Scheinberg and Armstrong.[5] Experimentally, these investigators[5] have shown a marked increase in binding of chloride ion by cationic albumin as compared to anionic albumin molecules. Similar results have been reported[6] in studies with methyl orange at *p*H's acid to the isoelectric point of serum albumin. The increased binding of anions by positively-charged albumin can be accounted for quantitatively by electrostatic theory.[5]

In the present paper, changes in the optical properties of an iodinated serum albumin have been used to demonstrate proton uptake by the protein molecule (in buffered solutions) concurrently with the binding of anions. Quantitative estimates of the extent of combination of the protein with hydrogen ions, however, are smaller in magnitude than might be anticipated from calculations based on simple electrostatic considerations.

Experimental

Three iodinated albumins were used in these investigations, two of these being derivatives of human albumin and one of bovine albumin.

The derivatives of human origin were obtained through the kindness of Dr. W. L. Hughes, Jr., and Dr. R. Straessle[7] of the Department of Physical Chemistry of the Harvard Medical School. One sample ("50% iodinated") contained sufficient iodine to convert one-half of the (eighteen) tyrosine residues in human albumin to diiodotyrosine. The second sample ("100% iodinated") contained enough iodine to saturate all eighteen tyrosine groups, but chemical evidence indicates that only about thirteen were covered.[7]

An estimate of the number of diiodotyrosine groups in these protein derivatives can be made also from their optical absorption. The optical density of a 0.100% solution of the 100% iodinated material at 313 mμ was approximately 0.83 at *p*H 11.1. From the data of Crammer[8] and Herriott[9] on diiodotyrosine, one may estimate a value of

(1) J. Steinhardt, *Ann. N. Y. Acad. Sci.*, **41**, 287 (1941).

(2) F. W. Putnam and H. Neurath, THIS JOURNAL, **66**, 692 (1944).

(3) G. Scatchard and E. S. Black, *J. Phys. Colloid Chem.*, **53**, 88 (1949).

(4) L. G. Longsworth and C. F. Jacobsen, *ibid.*, **53**, 126 (1949).

(5) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., THIS JOURNAL, **72**, 535, 540 (1950).

(6) I. M. Klotz and J. M. Urquhart, *ibid.*, **71**, 1597 (1949).

(7) W. L. Hughes, Jr. and R. Straessle, *ibid.*, **72**, 452 (1950).

(8) J. L. Crammer and A. Neuberger, *Biochem. J.*, **37**, 302 (1943).

(9) R. M. Herriott, *J. Gen. Physiol.*, **31**, 19 (1947).