1930

A new series of halogenated sulphone-phthaleins.

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A NEW SERIES OF HALOGENATED SULPHONE-PHTHALEINS

By

William Clouser Boyd

(A.B. Harvard, 1925; A.M. Harvard, 1926)

A Dissertation

submitted in partial fulfilment of the
requirements for the
degree of Doctor of Philosophy

GRADUATE SCHOOL
BOSTON UNIVERSITY

1930

To Dr. Allan Winter Rowe, under whose inspiration this work was planned, and with whose vigorous coöperation it was completed, and to Dr. Burnham Sarle Walker, whose kindness was as unfailing as the ingenuity of his suggestions.

VITA

William Clouser Boyd was born March 4, 1903 on a farm near Dearborn, Missouri, the son of William Oliver Boyd and Wilmuth Clouser Boyd. He attended the local grade schools, and entered Dearborn high school in 1918. In 1919 he entered Central high school, St. Joseph, Missouri from which he was graduated in 1922. In the fall of this year he entered Harvard University, concentrated in chemistry and received the degree of A.B. in 1925, and the degree of A.M. in 1926. He then became Teaching Fellow in Physiological Chemistry at the Boston University School of Medicine and carried on the research the results of which comprise this thesis, at the Evans Memorial Hospital, under the direction of Dr. Allan Winter Rowe, in the meantime taking further courses at Harvard and at Boston University. He is the co-author with Dr. S.B. Hooker of a paper "The Chances of Establishing Non-Paternity by the Determination of Blood Groups", (Science, n.s. 68 (no.1773) p. 620; J. Immunol. 16, 451 ('29))

At the suggestion of one of the readers, the names of compounds made for the first time by the author of this thesis are distinguished by being underlined in red.

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ABBREVIATIONS

With the single exception that JACS is used instead of Am.Soc., the abbreviations used in the references to the periodical literature in this thesis are those used in Richter's "Lexikon der Kohlenstoff-Verbindungen", and Stelzner's "Literatur-Register der Organischen Chemie". These abbreviations follow. The names of journals not in this list are either abbreviated in an analogous manner, or written out in full.

The references are made as follows: first, the name of the author, if necessary; second, the abbreviation for the journal; third, the volume; fourth, the page; fifth (in parenthesis) the year (as ('71) = 1871), or the year of the abstract in the Zentralblatt (as(C72) = Zentralblatt, 1872), or Chemical Abstracts, (CA).

A	(Liebig's) Annalen der Chemie
A. ch.	Annales de chimie et physique
Am.	American Chemical Journal
JACS	Journal of the American Chemical Society
A.Pth.	Archiv für experimentelle Pathologie und Pharmakologie
Ar.	Archiv der Pharmazie
B.	Berichte der Deutschen Chemischen Gesellschaft
Bio. Z.	Biochemische Zeitschrift
Bl.	Bulletin de la Société Chimique de France

C	Chemisches Zentralblatt
C. r.	Comptes rendus de l'Academie des sciences
Ch. J	Chemische Industrie
Ch. Z.	Chemiker-Zeitung (Cöthen)
Chem. N.	Chemical News
D.	(Dingler's) Polytechnisches Journal
D.R.P.	Patentschrift des Deutschen Reiches
El. Ch.Z.	Electrochemische Zeitschrift
Fr.	(Fresenius') Zeitschrift für analytische Chemie
Frdl.	(Friedländer's) Fortschrifte der Theerfarbenfabrikation
G.	Gazetta Chimica Italiana
H	(Hoppe-Seyler's) Zeitschrift für physiologische Chemie
J.	Jahresbericht der Chemie
J. pr.	Journal für practische Chemie
J. r.	Journal der russischen physikalisch-chemischen Gesellschaft
J. Th.	Jahresbericht der Thierchemie
L.V.St.	Landwirtschaftliche Versuchsstationen
M.	Monatshefte für Chemie
P.	(Poggendorff's) Annalen der Physik und Chemie
P. C. H.	Pharmaceutische Centralhalle
P.Ch.S.	Proceedings of the Chemical Society
Ph. Ch.	Zeitschrift für physikalische Chemie

R.	Recueil des travaux chimiques des Pays-Bas
R.A.L.	Atti della reale Academia dei Lincei
Soc.	Journal of the Chemical Society
W.	(Wiedermann's) Annalen der Physik
Z.	Zeitschrift für Chemie
Z.a.Ch.	Zeitschrift für anorganischen Chemie
Z. Ang.	Zeitschrift für angewante Chemie
Z.B.	Zeitschrift für Biologie
Z.El.Ch.	Zeitschrift für Electrochemie
Z. Kr.	Zeitschrift für Krystallographie

I

HISTORICAL

"omnis enim color omnino mutatur in omnis;
quod facere haud ullo debent primordia pacto;
immutabile enim quiddam superare necessest,
ne res ad nilum redigantur funditus omnes.
nam quodcumque suis mutatum finibus exit,
continuo hoc mors est illius quod fuit ante.
proinde colore cave contingas semina rerum,
ne tibi res redeant ad nilum funditus omnes.

Lucretius--De Rerum Natura,
Liber II, 749-756

Adolf von Baeyer has described the line of thought leading up to his discovery of the phthaleins in his own words [1]: "Bei Versuchen, eine Kondensation unter Wasserabspaltung zwichen meheren Molekülen von im Pflanzenkörper vorkommenden Phenolen zu bewirken, wollte ich Phtalsäureanhydrid als Entwässerungsmittel anwenden und schmolz dieses mit Pyrogallussäure zussamen."

The discovery of gallein, the first of the series, is described as follows [2]: "Erhitzt man Pyrogallussäure mit Phtalsäure, oder besser mit Phtalsäureanhydrid, so färbt sich die anfangs farblose Flüssigkeit rot und wird nach kurzer Zeit beinahe undurchsichtig. Die Schmelze löst sich in heiszem Wasser zu einer dunkelroten Flüssigkeit, die beim Erkalten kleine, körnige Kristalle eines neuen Farbstoffes, den ich Galleïn nennen will, absetzt.

Zur Darstellung im gröszeren Maszstabe hat es zweckmäszig gezeigt, 1 Tl. Phtalsäureanhydrid mit 2 Tln. Pyrogallussäure einige Stunden auf 190 bis 200° bis zum Dickwerden der Masse zu erhitzen. Man löst die Schmelze dann in heiszem Alkohol, filtriert und verdünnt mit Wasser, wobei sich ein sehr reichlicher, aus beinahe reinum Galleïn bestehender Niederschlag abscheidet, der nur nach einmal aus heiszem, verdünntem Alkohol umkristallisiert zu werden braucht".

---

[1] Adolf von Baeyer, Gesammelte Werke, page xxxii.

[2] B. 4, 457 (1871)

Later on in the same paper he says, à propos of the reaction involved: "Jedenfalls ist hierdurch die Möglichkeit ausgeschlossen, dasz die Phtalsäure mit zur Bildung des Galleïns verwendet wird, und es kann die Reacktion nur auf Wasserentziehung und vielleicht auf Oxydation beruhen."

Thus we see that the discovery of this very important class of compounds was the chance result of an experiment directed at a very different goal. And though Baeyer, like the genius he was, recognized the newness and importance of what had happened, the true reaction involved was confidently declared, at the outset, to be impossible.

In the next paper [1], the transformation of gallein into corulein, and the discovery of fluorescein are described.

In the third paper [2], Baeyer announces that, contrary to his first paper, the phthalic anhydride really enters into the constitution of the product, and describes the use of concentrated sulphuric acid as a condensing agent. He describes experiments with α-naphthol, resorcin, hydroquinone, pyrocatechol, pyrogallol, phloroglucinol, and the preparation of phenolphthalein, which he analyzes as $C_{20}H_{14}O_4$. He describes its reduction, by means of zinc dust and potassium hydroxide, to phenolphthalin. He shows that it contains two hydroxyl groups.

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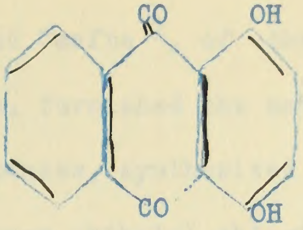
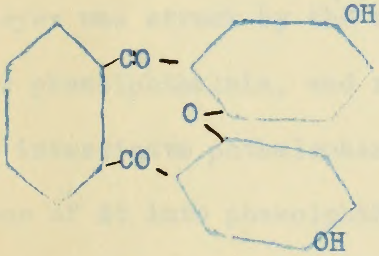
[1] B. 4, 555 ('71)

[2] B. 4, 658, ('87)

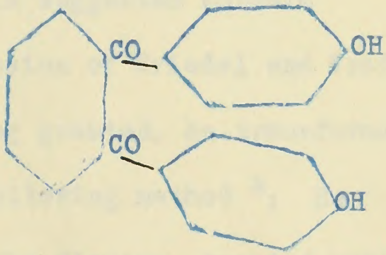
The fact that quinizarin, belongs to the anthraquinone series, the nucleus of which has a symmetrical structure, and arises in the same fus~~y~~ion mixture as hydroquinonephthalein, led Baeyer and Grimm to ascribe an analogous symmetrical structure to the latter, and naturally, to phenolphthalein.



Quinizarin



Hydroquinonephthalein



Phenolphthalein

Baeyer and Caro [1] discovered the tetra-brom derivative of fluorescein which they named eosin, were struck with its properties, and worked out a method for preparing it on a commercial scale which is still used. The Badische Analin - u. Sodafabrik were putting it out as a dyestuff in 1875.

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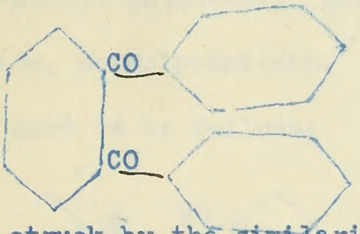
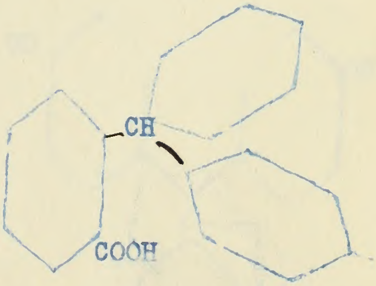
[1] B. 8, 146, ('75)

The publication, in 1877, by Friedel and Crafts [1], of the method of synthesis now known by their names, furnished the next milestone of our story. To one of the substances, synthesized by them by the action of aluminum chloride upon phthalyl chloride and benzene, was ascribed the formula and given the name phthalophenone.



Baeyer was struck by the similarity to his suggested formula for phenolphthalein, and requested permission of Friedel and Crafts to investigate phthalophenone. This being granted, he transformed some of it into phenolphthalein by the following method [2]: He nitrated the phthalophenone, reduced to the diamine, treated with nitrous acid, and warmed in aqueous solution. The resulting product was identical with phenolphthalein.

Some chemists might have stopped here, but Baeyer continued his researches. He boiled the phthalophenone with concentrated alcoholic sodium hydroxide, reduced and found he had obtained a product which he identified with tri-phenyl-methane-carboxylic acid.



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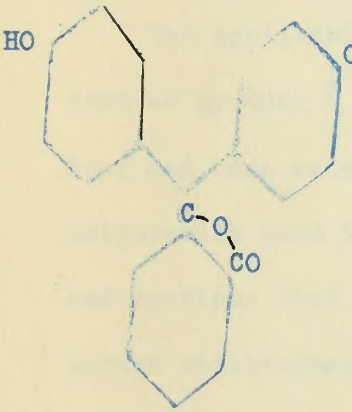
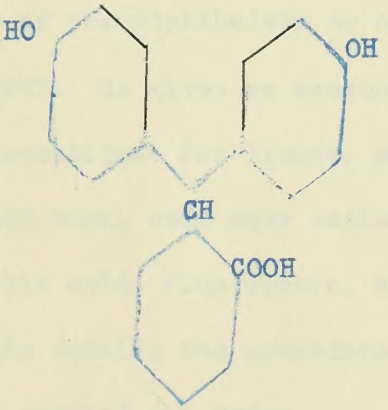
[1] C. 18 Juni ('77)
Revue scientifigue, 2 mars 1878 2
B, 10, 1445
A. ch. 6me. serie, v.l.

[2] B. 12, 642 ('79)
A. 202, 36 ('80)

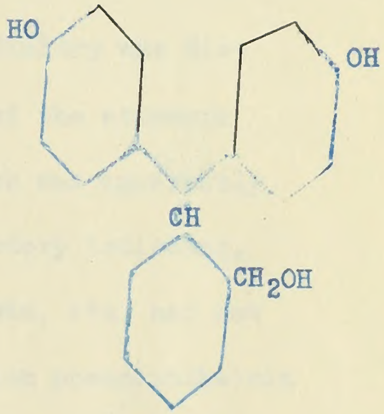
At one stroke, so to speak, he demolished his own theories and those of the obliging gentlemen Friedel and Crafts. The two phenyl residues introduced in the synthesis of phthalophenone are both attached to the same carbon atom, and it is not a phenone at all. (Baeyer renamed it di-phenyl-phthalid.) And the same is therefore true of its derivative, phenolphthalein. The formula of the latter and its derivatives must be as follows:



Phenolphthalein



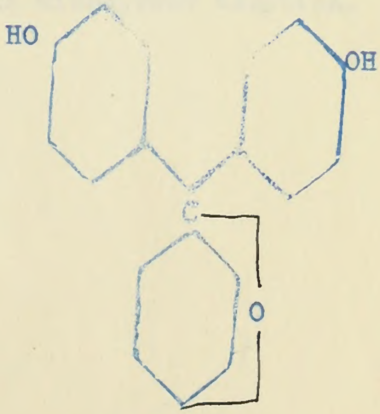
phenolphthalin



phenolphthalol

In this paper Baeyer points out the close similarity between the structure of phenolphthalein and rosolic acid.

The above formulas were also rendered more probable by the discovery made by Hessert [1] in Baeyer's laboratory that phthalaldehyde is not really an aldehyde but the inner anhydride of o-benzyl alcohol-benzoic acid.



rosolic acid.

[1] Hessert, B 10, 1445 ('77)
B 11, 237 ('78)

Then in a magnificent series of three papers [1], Baeyer recounts the progress of his knowledge of these substances, sums up previous work, and gives the results of a stupendous amount of fresh work. Here for the first time we find described: crystallographic data for phenolphthalein, syntheses of phenolphthalein-sulphonic acid, tetra-brom-phenol-phthalein, di-imido-phenolphthalein, p-cresol-phthalein, numerous derivatives of these, and many other substances. The work of a giant, indeed.

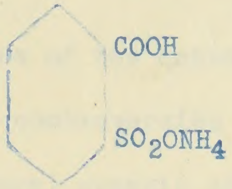
The applicability of phenolphthalein to acidimitry was discovered by Luck [2] in 1877. He gives an account of the attempts that had been made to substitute for litmus, which was apparently universally used in this work, some more satisfactory indicator, and mentions that rosolic acid, fluorescein, cyanin, etc. had not proved satisfactory. He details the procedure with phenolphthalein substantially as it is carried out today.

Immediately following Luck's paper, the only papers published on the use of phenolphthalein as an indicator were distinctly unfavorable, most of the authors criticising one or another feature of the new indicator, but within a few years the increasing number of papers giving results obtained by means of it showed its widespread adoption.

[1] Baeyer, A 202, 36, 153, ('80); A 212, 340

[2] Luck, Fr. 16, 332 ('77)

The sulphone-phthaleins were discovered by Remsen[1]. He reported the synthesis of resorcin-sulphone-phthalein by heating potassium o-sulphobenzoate with resorcin and sulphuric acid. He reported that he could also make the compound from saccharin in the same way. In his next paper [2], however, Remsen reported that the substance he had supposed to be o-sulphobenzoic acid had really been the acid ammonium salt of the acid: He succeeded in really making o-sulpho-benzoic acid, and from it resorcin-sulphone-phthalein. Sohon [3], describes the preparation of o-sulpho-benzoic anhydride and a large number of derived sulphone-phthaleins.



A propos of the production of the sulphone-phthaleins from saccharin, it is of interest to note that it has been rediscovered independently several times since Remsen, and that the same workers have usually rediscovered also the fact that from saccharin can be

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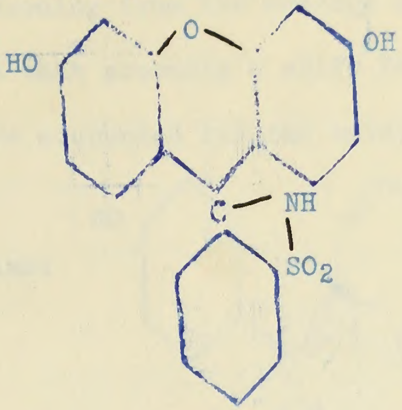
[1] Remsen, Am. Ch. J. <u>6</u>, 180-1, (1884)

[2] Remsen, Am. Ch. J.<u>11</u>, 73 (89)

[3] Sohon, Am. Ch. J.<u>20</u>, 257 (98)

made analogous compounds, variously named sacchareins and sulfamphthaleins, as for example resorcin-saccharein [1,2,3,4].

resorcin-saccharein



In the meantime one of the hottest controversies in the history of a subject where the controversies have always been hotly contested -- in other words, structural organic chemistry -- was raging over the structure of phenolphthalein, the structure of which had apparently just been established by the great Baeyer.

This particular controversy began with a color -- with the color which phenolphthalein develops in the presence of alkali. Baeyer had assumed this color to be due to the formation of a series of salts, some of them colored, with no breakage of the lactone ring until

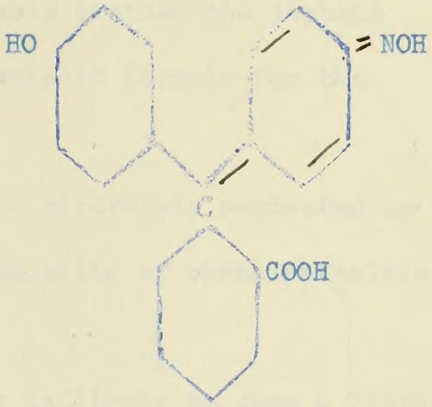
[1] Sisley, (C1897), II 847

[2] (C1899), I 718 (patent)

[3] Monnet and Cartier, Bl. (3) 17, 690 (C97)

[4] Dutt, Soc. 121, 2389 (22)

alkali strong enough to produce decolorization was added. But Bernthsen [1], and Friedländer [2], reasoning from the analogy of fuchsin and rosolic acid, suggested that probably a shift from the lactoid to the quinoid structure accounted for the color. Friedländer supported this idea by forming phenolphthalein-oxime: by the action of hydroxylamine on phenolphthalein in alkaline solution.



For several years these two views, the so-called "ionic" and "quinoid" theories of the color of the phthaleins were both warmly and brilliantly defended by a considerable number of chemists of the first rank.

Friedländer's view was supported by Bistrzycki and Nencki[3], who made dibenzoyl-phenolphthalein from phenolphthalein in alkaline solution, but was objected to by Herzig and Meyer [4], who found that

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[1] Bernthsen, Ch. Z. 104, 1956

[2] Friedländer, B. 26, 172 and 2258 (93)

[3] Bistrzycki and Nencki, B. 29, 131 (96)

[4] Herzig and Meyer, B. 28, 3258 (95)

the white lactoid methyl ether could be made in good yield from the sodium salt of phenolphthalein; and by Meyer and Spengler [1], who placed little confidence in the analogy between phenolphthalein and resorcin-phthalein (fluorescein), and insisted that the alkylation experiments, which incontestably yielded the lactoid ethers, were quite incompatible with a quinoid formula for the salts of phenolphthalein.

The views of Meyer and Spengler were vigorously contested by Green [2], who prepared the colored quinoid salts of phenolphthalein and hydroquinone-phthalein.

This whole phase of the controversy is likely to seem a little unnecessary to a modern chemist, made familiar from the beginning with the concept of dynamic equilibrium. Of course the colorless lactoid ethers could be made from the solutions of the salts of phenolphthalein, because there is an equilibrium there between the lactoid and quinoid forms, and if you remove the lactoid form by esterifying it, the equilibrium will be displaced and more will form, resulting in a reaction that practically runs to an end.

A point more difficult to dispose of was brought up by K. Meyer and Hantzsch [3], who pointed out that phenolphthalein and its lactoid di-methyl ether both give red salts with hydrochloric acid at

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[1] Meyer and Spengler, B. 38, 1318 (C 05)

[2] Green and King, B. 39, 2365, (C 06) B. 40, 3724, (C 07)

[3] Meyer and Hantzsch, B. 40, 3479, (C 07)

the white lactoid methyl ether could be made in good yield from the sodium salt of phenolphthalein; and by Meyer and Spengler [1], who placed little confidence in the analogy between phenolphthalein and resorcin-phthalein (fluorescein), and insisted that the alkylation experiments, which [illegible] yielded the lactoid ethers, were quite incompatible with a quinoid formula for the salts of phenolphthalein.

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[1] Meyer and Spengler, B. 38, 1318 (1905)

[2] Green and King, B. 39, 2365, (1906) B. 40, 3724, (1907)

[3] Meyer and Hantzsch, B. 40, 2472. (1907)

temperatures below -30°C, and with aluminum chloride and with stannic chloride. They concluded that since forms undoubtedly lactoid could give a red coloration under certain conditions, there was no reason to posit the existence of anything else to account for the red colors observed with alkali.

At this stage, which may have seemed critical to some supporters of the quinoid theory, decisive evidence of a totally different sort was brought to bear on the question by R. Meyer and K. Marx [1], who compared the absorption spectra of the alkali salts of phenolphthalein, hydroquinone-phthalein, and fluorescein, and showed them to be quite similar. They assumed all three salts to have a quinoid formula. This has been supported by a considerable amount of later work which has shown that beyond question a deep-seated shift of configuration, without doubt a shift from lactoid to quinoid, takes place when the

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[1] R. Meyer and Karl Marx, B. 40, 3603, (C 07)

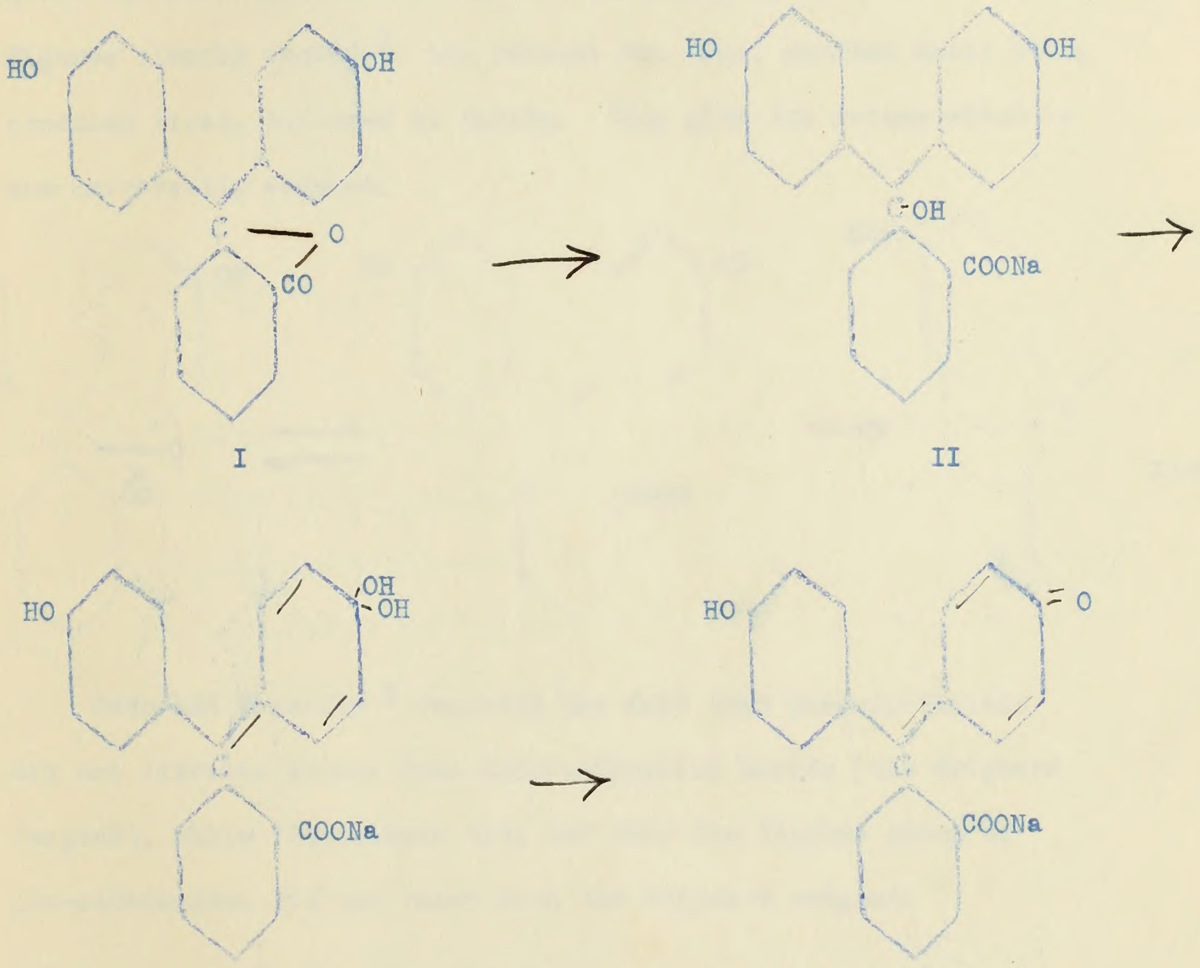
red color with alkalies is developed [1].

The fact that phenolphthalein is dibasic was definitely established by Kober and Marshall [2], who, in a series of papers, described the preparation of the monobasic salts, and showed that

[1] Howe, Phys. Rev. 8, 687 (16)
Howe and Gibson, Phys. Rev. 10, 767, (17)
Orndorff, Gibbs, Scott, and Jackson, Phys. Rev. 17, 437 (21)
Formánek and Knop, Z. anal. Ch. 56, 273 (17)
Moir, Trans. Roy. Soc. S.Afr. 7, 8, 14, (18)
White and Achree, J.A.C.S. 40, 1092, (18)
Brode, J.A.C.S. 46, 585, (24)
Holmes, J.A.C.S. 46, 629, 633, (24)
Orndorff, Gibbs, and Shapiro, J.A.C.S. 47, 2767 (25)
48, 1327, 1994 (26)
49, 1541, 1545, 1588 (27)
50, 819, 1755, 2799 (28)
Thiel and Diehl, Sitzungs. Ges. Beförderung gesamt. Naturwissensch. Marburg 62, 472 (CA, vol. 22, 4403)

[2] Kober and Marshall, J.A.C.S. 33, 62, 1780
34, 424

they were colorless. They pointed out that Gomberg's plausible scheme [1]:



I

II

III

IV

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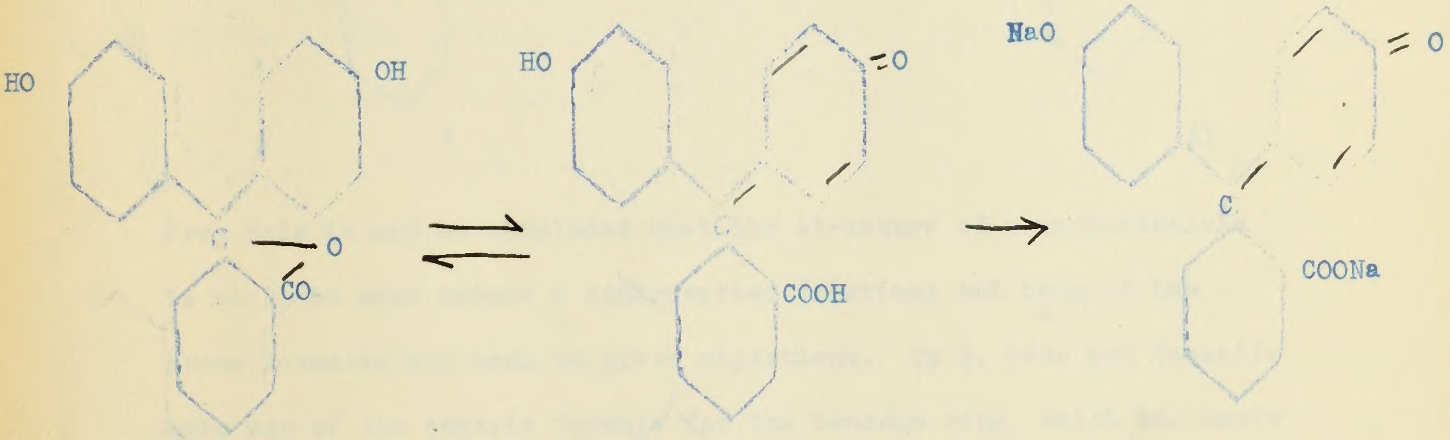
[1] Gomberg, B. 40, 1880 (CA13)

does not agree with the facts. Even disregarding the omission of one Na, the scheme means that in excess of alkali, full color is never reached, most of the compound remaining as II. But, as their figures clearly showed [1], the reverse was true, maximum color being produced first, followed by fading. They give the scheme which is now universally assumed:



Oddo and Vassallo [2] reported the fact that phenolphthalein did not liberate ethane from ethyl-magnesium halide (the Grignard reagent), while fluorescein did, and that the lactone group of phenolphthalein did not react with the Grignard reagent.

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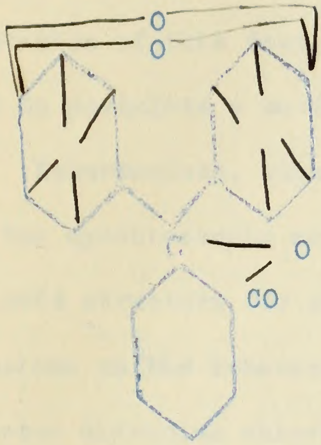
[1] J.A.C.S. 33, 68, (CA 13)

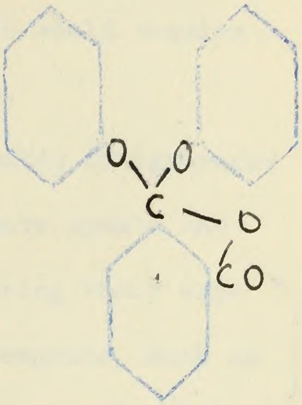
[2] Oddo and Vassallo, G. 42 (II) 204 (CA 13)

They were thus lead to ascribe to phenolphthalein two alternative formulas:



I

or



II

From this it may be concluded that the structure of phenolphthalein is still to some extent a controverted question; but both of the above formulas are open to grave objections. In I, Oddo and Vassallo make use of the centric formula for the benzene ring, which has never proven a conception capable of holding its own with others, such as that of Kekulé, and apparently are not aware of the much earlier work of Haller and Guyot[1], who, by obtaining the diphenyl carbamates of phenolphthalein, established the presence of two functional hydroxyl groups. There is no reason to expect a reaction with the lactone group, as is evident from work such as that of E. P. Kohler on such conjugated systems. In II, the fact that the diethyl ether of phenolphthalein made by the reaction of Friedel and Crafts from phenetole and phthalyl chloride is identical with that made by the

---

[1] Haller and Guyot, C. r. 116, 479 (C 93)

direct ethylation of phenolphthalein [1] is ignored. The interpretation of this fact on the basis of formula II would require one to postulate a hardly credible rearrangement.

Nevertheless, other Italian workers, apparently in ignorance of the spectroscopic evidence to the contrary, have upheld the quinoid structure for phenolphthalein itself, basing their conclusions on the behavior of certain copulating reagents, such as benzene diazonium chloride [2]. Their contentions have produced little impression upon the chemists of other lands, but whether this is due to the fact that the weaknesses in their arguments are universally recognized, or to the fact that few chemists read Italian, it would be difficult to say.

[1] Haller and Guyot, C. r. 120, 296 (C 95)

[2] Consonno and Apostolo, G. 51 (I), 60 (21)

The use of phenolphthalein in acidimetry has already been mentioned. This was an application to the measurement of the *quantity* factor of acidity, and of fundamental importance to analytical chemistry. But an application fully as important for biological chemistry was made possible by the theory of electrolytic dissociation introduced by Arrhenius [1] in 1887. There arose directly from this idea the concept of hydrogen ion concentration, which made it possible to measure the *intensity* factor of acidity. A survey of the indicators suitable for hydrogen ion determination was opened in Nernst's laboratory in 1904 by Salessky [2]. This was extended in the same year by Friedenthal, by Fels and by Salm, and the results were summarized in Salm's famous table [3].

Then in 1909 came the classic work of Sørensen [4], who made an extensive investigation of the correspondence between colorimetric and electrometric determinations, and published a selected list of indicators which were relatively free from the so-called protein and salt errors. This has proven to be of the greatest value to the biochemist and physiologist. The Sørensen list was supplemented by

[1] Arrhenius, Ph. Ch. 1, 631 ('87)

[2] Salessky, Z. El. Ch. 10, 204 (C 04)

[3] Ph. Ch. 57, 451 (06)

[4] Sørensen, Compt. rend. Lab. Carlsberg 8, 1, 396
Bio. Z. 21, 131, 201 (09)

one from Clark and Lubs who studied an extensive series of indicators suggested by the syntheses of Acree and White[1] undertaken for the purpose of obtaining material for the study of the quinoid theory of indicators. The list of Clark and Lubs consisted principally of sulphone-phthaleins.

The lists both of Sørensen and of Clark and Lubs will be found in the well known book by Clark[2], from which has been taken most of the material for this brief survey of the history of the use of indicators for hydrogen ion determinations.

Sørensen, to avoid the somewhat awkward exponential expression initially used, also introduced the invaluable symbol p_H^+, now usually written, for typographical convenience, pH. It may be of interest to quote the words of the original: "......, la grandeur de la concentration des ions hydrogène s'exprime par le facteur de normalité de la solution par rapport aux ions hydrogène, facteur indiqué sous la forme d'une puissance negative de 10.

..... le facteur de normalité de la solution sous le rapport des ions hydrogène ou, en d'autres termes, le nombre d'atomes-grammes d'ions hydrogène par litre est plus petit que 1 et peut être posé égal à 10^{-p},

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[1] Acree and White Am. Ch. J. 39, 529 (08)

[2] Clark, W.M., "The Determination of Hydrogen Ions"
Williams and Wilkins, 1928. p. 92, p. 94.

où pour le nombre p je propose le nom d'exposant des ions hydrogène et la désignation p_H. Par exposant des ions hydrogène (p_H^+) d'une solution, nous entendons donc le logarithm Brigg de la valeur réciproque du facteur de normalité de la solution relativement aux ions hydrogène.

Comme il n'est d'ordinaire pas question de solutions d'ions hydrogène plus fortes qu'une solution normale, j'ai choisi la définition ci-dessus de l'exposant des ions hydrogène, qui par suite sera généralement un nombre positif; il ne sera négatif que dans les cas bien rares où l'on a affaire à des solutions plus fortes que la normale."

At the same time the theories of buffer action were being developed. The power of certain solutions to resist changes in reaction was commented upon by Fernbach and Hubert[1], who, writing in French, likened the resistance of phosphate solutions to a "tampon". The word was adopted by Sørensen, and in the German rendition of his paper it became "Puffer", and thence the cognate English "buffer". Important among those who contributed to the understanding of the theory of this action may be mentioned L. J. Henderson[2].

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[1] Fernbach and Hubert, C. r. 131, 293 (00)

[2] Henderson, L.J., Am. J. Physiol. 15, 257, (06)
J.A.C.S. 30, 954 (08)
Am. J. Physiol. 21, 173 (08)
Erg. Physiol. 8, 254 (09)

Buffer solutions systematically covering the pH scale have been prepared by Sørensen[1], and Clark and Lubs[2]. These also are described in detail in Clark's book.

This work on indicators made the phthaleins indispensible to the biologist as well as the chemist, and provided an impetus to further studies, and the preparation of new compounds.

According to a well-known story, of which it appears to be impossible to trace the sources, the purgative effects of phenolphthalein were discovered in the following manner. The tax collectors of the Hungarian government, in order to have a means of checking up on the "bootleggers" of the day, developed the practice of adding phenolphthalein to wine upon which the tax had been duly paid. Then, if any given sample of wine were suspected of being "moonshine", the government inspectors had only to take a small amount, dilute it, and add alkali. If the red color appeared, the tax had been paid, and if it did not, the wine was illicit. The wine makers, apparently being less skilled chemically than the bootleggers of to-day, did not know what the substance was that the government officials added, and consequently could not sophisticate their wines in this respect.

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[1] Sørensen, Prozesse. Ergeb. Physiol. 12, 393 (12)

[2] Clark and Lubs, J. Biol. Chem. 25, 479 (16)

The consumers of the wines, however, while they did not exactly discover the formula of the esoteric substance, did discover something about it, namely, that a large steady consumption of wine containing it produced continued diarrahoea.

This may be only a story, but it would seem that Liebermann did at any rate propose the use of phenolphthalein for such purposes, as other authors refer to his suggestion in their papers.

The first scientific study of the purgative effects of phenolphthalein is in a paper by Zaltón and Vámossy[1] from the Pharmacological Institute of the Royal Hungarian University at Buda-Pesth. The authors do not state how the phenomenon was brought to their attention, but detail experiments upon themselves and some of their friends which demonstrate quite clearly the efficacy of the substance.

Later articles by Vámossy[2], Tunnicliffe[3], Fleig[4], Dornblueth[5], and Pattiez[6] laid the foundations of our pharmacological knowledge of this substance. According to Pattiez, it is unaltered in the stomach,

[1] Zaltón and Vámossy, Ch. Z. 24, 679 (C 00)

[2] Vámossy, Ther. Gegenw. 43, 203 (02)

[3] Tunnicliffe, Brit. Med. J. 2181, 1221 (02)

[4] Fleig, Arch. inter. Pharm. 18, 327 (08)

[5] Dornblueth, Münch. med. Woch. no. 52 (03)

[6] Pattiez, J. pharm. Belg. 3, 685 (21)

but is acted upon by the alkaline secretion of Brünner's glands in the small intestine, rendered soluble, and absorbed to the circulation. It is excreted by the feces and in the urine in traces. (Fleig). It is in part removed by the liver, where it is hydrolyzed, and the products oxidized to water and carbon dioxide, according to Pattiez.

The work of Vámossy led Abel and Rowntree[1] to investigate the pharmacology of a number of halogenated derivatives of phenolphthalein, in the hope of finding a suitable hypodermic purgative. They investigated a number of the products prepared by Orndorff[2], Remsen[3], and Acree. Their chief interest was in the phenol-tetra--chlor-phthalein, which they found to be a mild purgative, even when administered hypodermically, and to be excreted entirely by way of the bile, with some reabsorption in the large intestine. At Rowntree's suggestion, Whipple, Mason, and Peightal studied the excretion of this substance in the bile when the liver was subjected to artificial lesions; Rowntree, Marshall, and Chesney applied the results clinically and developed the phenol-tetra-chlor-phthalein test of liver function. (For the details of this test see Kahn[4].) It may be doubted if this test has any considerable diagnostic value.

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[1] Abel and Rowntree, J. Pharm. and Exp. Ther. 1, 231 (09)

[2] Orndorff and Black, Am. Ch. J. 41, 349, (09)

[3] Remsen, loc. cit.

[4] Kahn, Functional Diagnosis, W.F.Prior Co. 1920. p. 114

A modification of this test destined to be of considerable importance was made by Graham[1], searching for some substance which would make possible X-ray photography of the gall-bladder, or, as it is usually called, cholecystography. Graham and Cole[2] hit upon this substance, which Abel and Rowntree (v.supra) had shown to be excreted through the bile. They tried tetra-iodo-phenolphthalein, but probably because of an impure sample, believed it to be too toxic, and recommended tetra-brom-phenol-phthalein as the best compromise.

In the meantime, Whitaker[3] had been working in the same field, and had employed tetra-iodo-phenol-phthalein. His paper[4] and that of Graham[5], both describing the successful use of somewhat purer samples of tetra-iodo-phenol-phthalein, appeared practically simultaneously. The test as at present employed, consists of the intravenous, oral, or simultaneous intravenous and oral administration of the dye, followed by the taking of pictures after an interval of 6 to 12 hours. The X-ray findings have proved to be of the greatest value in diagnosis.

[1] Graham and Cole, JAMA 82, 613, (24)

[2] Graham and Cole, JAMA 82, 1777 (24)

[3] Whitaker and Milliken, Surg., Gyn., and Obst. 40, 17, (25)
646, (25)

[4] Whitaker and Milliken, Surg., Gyn., Obst. 40, 17, Jan. 1925

[5] Graham, Cole, Copher, JAMA 84, 14, Jan. 1925

In the paper by Abel and Rowntree already referred to, they detail the results of an investigation of a sample of phenol-sulphone-phthalein supplied them by Remsen, and reported that it was excreted practically entirely in the urine, and did not seem to injure the kidneys of the experimental animals used. Then in a later paper by Rowntree and Geraghty[1], the now well known phenol-sulphone-phthalein (or, as it is often abbreviated, P. S. P.) test of renal function is proposed. (See Kahn, p. 246)

As carried out to-day, the test usually consists of the injection intramuscularly of a definite amount (1 c.c. of solution containing 6 mg.) of the phthalein, the collection of urine at the end of the first and second hours, and the reading of the amount of dye appearing in the urine, by means of making the urine alkaline, making up to a definite volume, and reading in the colorimeter against a standard. In normal renal function, by the usual procedure, at least 50% of the dye should be eliminated during the first two hours. It was these two function tests which suggested the present study. Knowing that sulphone-phthaleins were eliminated through the kidneys, and that halogens could be introduced into phthaleins so as to increase their density, and hence their opacity to X-rays, it was hoped that substances might be prepared which would permit X-ray pictures to be taken of the kidney while functioning. In any case, it was thought that a new series of a class of compounds which had already been proven so important and interesting could not fail to be of some value.

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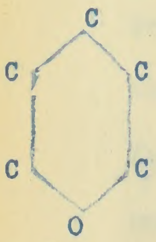
[1] Rowntree and Geraghty, J. Pharm. and Exp. Ther. 1, 579 (10)

To conclude this historical introduction, it only remains to speak of two further uses for substances of this class. Eosin, though the best known, is only one of a large series of the phthaleins which are employed as dyes[1]. The fluorescence of eosin lends it the special applicability of being used to produce the so-called "changeable silk". The fluorescence of organic compounds, in other words the transformation of certain wave lengths of radient energy into certain longer wave lengths (Stokes' rule of fluorescence) had been investigated by several workers, e.g. Meyer[2], who pointed out that it practically always accompanies the presence of certain "fluorphores" in the compound. He named the following groups as fluorophores.



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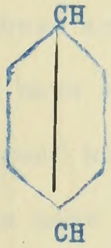

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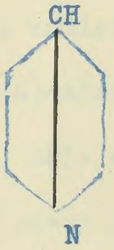

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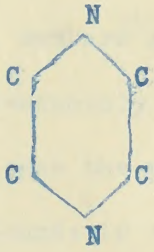

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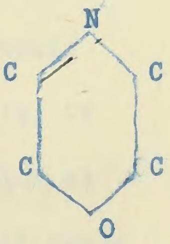

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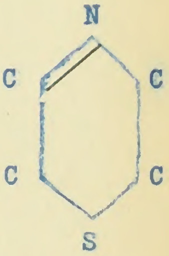

VI

Of these, it is I, the pyrane ring, which accounts for the fluorescence of eosin and the compounds next to be mentioned.

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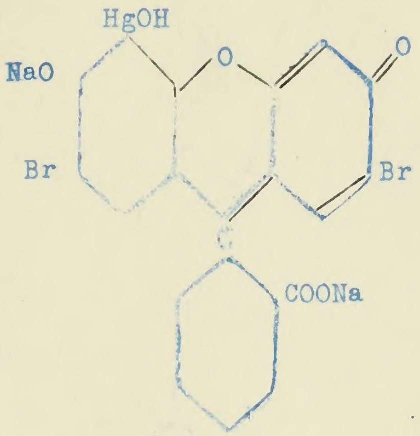
[1] Cain and Thorpe "The Synthetic Dyestuffs"
Charles Griffin & Co., London, 1920, pp 100 - 113

[2] Meyer, Festschrift, d. Tech. Hochschule Braunschweig (1897)
Meyer, Ph. Ch. 24, 468 (C 98)

White[1] has produced the mercury derivatives of a number of phthaleins, among which the best known is mercurochrome 220 (di-brom-hydroxy-mercuri-fluorescein), probably:



Some of these are powerful antiseptics, but are so non-toxic that they have even been injected intravenously. Surely, if further proof were needed, this establishes the phthaleins as one of the most versatile class of compounds in the whole realm of organic chemistry.

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[1] White, JAMA <u>73</u>, 1483 (19)
JACS <u>42</u>, 2355 (20)

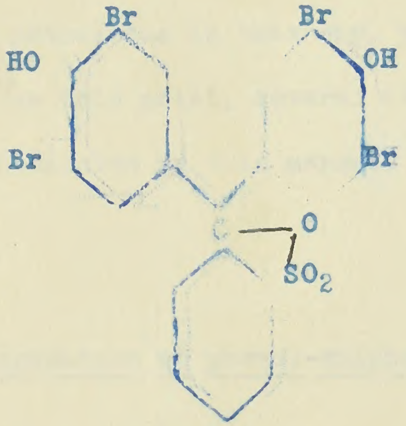
PART II

EXPERIMENTAL

A. IODIZED PHENOL SULPHONE PHTHALEINS

1. TETRA-IODO-ORTHO-SULPHO-BENZOIC ANHYDRIDE

Halogenated sulphone-phthaleins having the halogen substituted in the phenolic residues have been known for some time. One of them, tetra-brom-phenol-sulphone-phthalein



is widely used as an indicator under the name of brom phenol blue.[1] These compounds may be prepared with comparative ease from the corresponding sulphone-phthalein. Bromine, and often iodine, can be substituted in the phenolic residues by boiling the phthalein with a solution of the halogen in glacial acetic acid. Iodine is usually substituted from a cold solution of iodine in aqueous potassium iodide solution, which is kept alkaline, the phthalein itself serving as the indicator.

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[1] JACS 38, 2776, 2779; 40, 1092; 41, 1205

These methods are not vigorous enough to effect substitution in the phthalic anhydride residue, or the ortho-sulpho-benzoic anhydride residue, and the employment of more vigorous methods results in the destruction of the phthalein structure; so that four atoms of halogen per molecule of phthalein is apparently the maximum which can be attained starting from the phthalein. There is no record in the literature of any successful attempt to prepare more highly halogenated phthaleins in this way, but in order to obtain direct information on this point, several attempts were made to prepare halogenated phthaleins in this manner.

Halogenation of phenol-sulphone-phthalein

Bromination. 7g. (.02 mol) of pure phenol-sulphone-phthalein were dissolved in 100 c.c. glacial acetic acid, and brought to boiling. Then a solution of 13g. (.18 mol) of bromine gradually added. A reflux condenser was used to prevent escape of bromine and acetic acid. After refluxing the mixture for five hours, it was poured into water, washed, and recrystallized from alcohol. The product consisted of flesh colored crystals melting at 269-270°C. It gave a blue color with alkali, and had a pH range 3.0 - 4.6. It was obviously tetra-brom-phenol-sulphone-phthalein.

Iodination. 7g. (.02 mol) of pure phenol-sulphone-phthalein were dissolved in 1L. of 1% sodium hydroxide solution, cooled, and a solution of 22 g. iodine in 30 g. of potassium iodide in 200 c.c. of water gradually added. The mixture was cooled by ice and stirred mechanically during the addition of the iodine, and kept alkaline by the addition of sodium hydroxide solution (10%). Five hours were allowed for completion of the reaction, the crude phthalein precipitated by the addition of acid, washed with potassium iodide solution, and recrystallized from alcohol. It consisted of reddish-blue crystals of high but indefinite melting point, and gave a dark blue color in alkali. Preliminary analyses showed that it certainly did not contain more than 59% iodine, and was therefore doubtless tetra-iodo-phenol-sulphone-phthalein.

Attempts were then made to employ a more vigorous method.

Method of Datta and Chatterjee.[1] 7g. phenol-sulphone-phthalein were dissolved in nitric acid, and 22g. of iodine added. When the product of the first run, at the end of four hours, was poured into water, washed, and recrystallized, it gave a mixture of unchanged phenol-sulphone-phthalein and di- and tetra-nitro-phenol-sulphone-phthaleins. The second run was heated to 100° for four hours, and no substance giving the reactions of a phthalein could be detected in the mixture.

[1] JACS 39, 435, (17)

Method of Juvalta[1]. 7g. phenol-sulphone-phthalein dissolved in 100 c.c. of fuming sulphuric acid containing 50% free SO_3, 22g. iodine added, and the mixture heated to 110° for four hours. No phthalein could be recovered from the mixture.

It was concluded that, in harmony with the findings of Pratt and Shupp[2] in preparing phthaleins halogenated in the phthalic anhydride residue, it would be necessary to introduce the halogen into the anhydride molecule before condensing it with the phenolic substance to form the phthalein.

Pratt and Shupp employed a modification of the method due to Juvalta. By a slight modification of their method it was found possible to prepare in good yield tetra-iodo-ortho-sulpho-benzoic anhydride, which is the parent substance of all the iodized sulphone-phthaleins prepared for the first time in the course of the present study. The details of a typical run are given below.

Preparation of Tetra-iodo-ortho-sulpho-benzoic anhydride

108 g. o-sulpho-benzoic anhydride (Eastman, practical grade) and 300 g. of powdered iodine were mixed in a long necked flask (an 800 c.c. Kjeldahl serves very well), and 500 c.c. of fuming sulphuric acid containing 50% free SO_3 added, with stirring. (Acid of this strength is ordinarily partly or completely solid, and may require melting). The flask, into the neck of which a test tube was loosely

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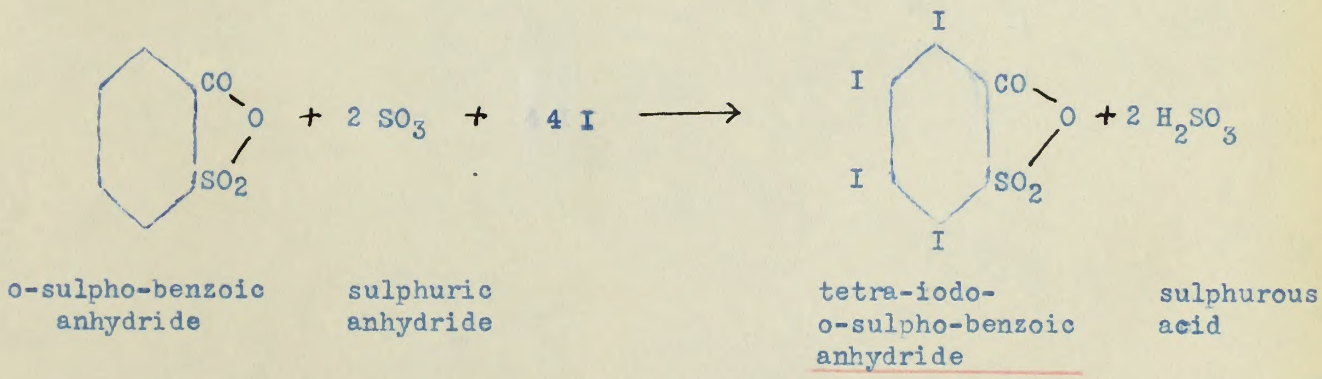
[1] DRP 50, 177; Fortschr. Teerfarb.-fabrik 2, 93 (89)

[2] JACS 40, 257 (18)

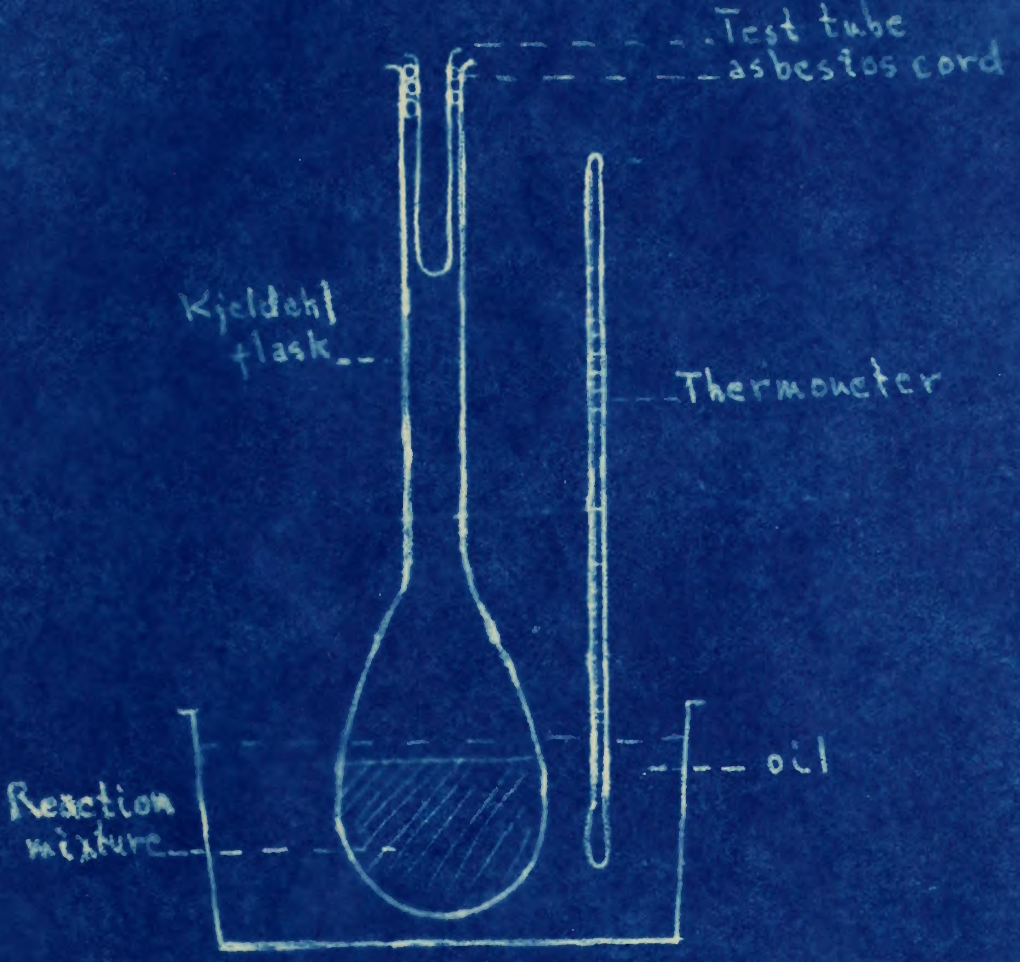
fitted by means of asbestos cord, to prevent the escape of too much iodine or sulphuric acid, was slowly heated in an oil bath, and the temperature kept between 110° and 120° until the evolution of SO_2 had practically ceased (5 to 10 hours). The temperature was then raised to 175° - 180° and held for about thirty minutes. The whole apparatus was then allowed to cool, and when cool, the practically solid contents poured into about 1.5 L. of water, and washed with successive portions of water containing sulphurous acid to remove the excess iodine. It was allowed to stand overnight with the final portion of wash water, the solid filtered off, washed with a little water, and dried in air. Yield 88%, calculated on the amount of o-sulpho-benzoic anhydride. The product so obtained is pure enough for making sulphone-phthaleins.

The reactions involved in this preparation may be summarized as follows:

(a)

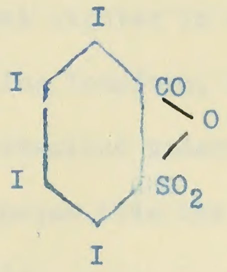


(b) $H_2SO_3 \longrightarrow SO_2\uparrow + H_2O$



APPARATUS for IODINATION

Properties of tetra-iodo-ortho-sulpho-benzoic anhydride and purification of tetra-iodo-ortho-sulpho-benzoic anhydride.



Tetra-iodo-o-sulpho-benzoic anhydride is somewhat soluble in water, nitrobenzene, acetic acid, tetrachlorethane, chloroform, benzene. It is quite soluble in acetone and in alcohol, but cannot be recrystallized from either. When dissolved in acetone it forms a dark brown mass, owing, apparently, to the formation of an addition product, since tetra-iodo-o-sulpho-benzoic anhydride can be recovered from it by the action of warm nitric acid. In alcohol it supersaturates, forming, when concentrated, a yellow varnish-like mass, which is quite unmanageable. When recrystallized from chloroform it has a melting point of 132° (uncorr.), as against the melting point of the crude product of about 260°; indicating the probable formation of some sort of addition product.

It can, however, be recrystallized from a mixture of alcohol and acetic acid. (60 g. will require about 350 c.c. of a mixture consisting of 100 c.c. of 95% ethyl alcohol and 250 c.c. of glacial acetic acid.)

The product so obtained does not melt, but decomposes about 305° to 310°. It is somewhat lighter in color than tetra-iodo-phthalic anhydride, resembling iodoform, except for a faint greenish tinge. The crystals, when examined under the microscope, are small, imperfect platelets, more opaque than the coffin shaped crystals of tetra-iodo-phthalic anhydride.

The recrystallized product was prepared for analysis by drying in the oven at 140° for 6 hours. Iodine analyses were done by the method of Baubigny and Chavanne[1].

Analysis of tetra-iodo-ortho-sulpho-benzoic anhydride

The obvious analytical procedure for this compound is the determination of the percentage of iodine. The determination of carbon is attended with great difficulties because of the great stability of the substance, and serious difficulties are caused by the iodine and sulphur contained in it.

	found	calc.
I_2	73.8 74.1	74.0%
S	4.8	4.6

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[1] "Organic Laboratory Methods" - Lassar-Cohn, Oesper-Adams-Clarke

The customary procedure for the determination of halogens in the organic laboratory is that due to Carius. But this method is well known to be somewhat unsatisfactory for iodine[1], and in this case gave extremely erratic and unreliable results, perhaps because of the drastic treatment required to destroy this very stable compound. After numerous attempts to use this method, experiments were undertaken to determine whether another method, at the same time more accurate for iodine and less time consuming, could not be found.

The researches involved in this part of the work were partly bibliographical, and partly experimental. The number of proposed methods for the determination of iodine in organic compounds is very great, and practically every issue of Chemical Abstracts contains references to three or four new ones--a fact in itself suggestive of the widespread dissatisfaction with the existing methods. It was necessary to investigate this literature, test those methods which seemed to hold promise of applicability to the present problem, and test out modifications and combinations which might make them more applicable.

The results, both of the author's original experiments and of statements found in the literature, relating to methods which seem at all promising, are summarized below. So far as the author is aware, no such summary has before been published, and it is hoped that the present one might have some value for persons who may have occasion to analyze similar compounds.

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[1] Clarke, "Handbook of Organic Analysis" Longmans, Green and Co., London, 1926. p.306

Analysis of Iodine Compounds

I. Destruction of the substance to be analyzed.

1. In sealed tubes. The principal example of this is the Carius method. It does not give trustworthy values, for reasons difficult to determine. Possibly the prolonged heating to a high temperature with fuming nitric acid, which is necessary, results in the formation of some silver iodate, which is less soluble than silver iodide. Methods involving sealed tubes are, in general, inconvenient, time-consuming, and not without a certain element of danger.

2. In open tubes

(a) With nitric acid (b) With sulphuric acid. With either of these methods, of course, iodine escapes. Attempts to trap the escaping iodine are unsatisfactory, and if successful, would not insure volatization of all the iodine, as a certain amount of iodic acid would be formed.

(c) With nitric acid and silver nitrate (d) With sulphuric acid and silver nitrate. If the mixture is heated sufficiently to insure complete decomposition, iodine escapes.

(e) With nitric acid and potassium permanganate. This method does not result in complete decomposition.

(f) With sulphuric acid, potassium dichromate, and silver nitrate. This is the method of Baubigny and Chavanne[1], which has proven the most satisfactory in the present work. It will be described more in detail later.

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[1] "Organic Laboratory Methods", Lassar-Cohn, Oesper-Adams-Clarke. Williams & Wilkins, Baltimore, 1928. p.443

(g) With aqueous sodium hydroxide. This is not vigorous enough to destroy the compound.

(h) With alcoholic sodium hydroxide. This will remove the iodine quantitatively from aliphatic compounds, but is too slow for ring compounds.

(i) With solid sodium hydroxide. Does not destroy the compound completely and is difficult to control, usually resulting in loss of material, or of iodine.

(j) With solid sodium hydroxide and sodium nitrate. This is also too violent, resulting in loss of material.

(k) With sodium carbonate. Incomplete.

(l) With sodium carbonate and sodium nitrate. This procedure is reasonably satisfactory, but must be carefully controlled, or iodine will be lost.

(m) With sodium peroxide.

(n) With barium peroxide. These methods are both too violent, and the decomposition is often not complete.

II. Treatment of the resulting solution.

1. Oxidation of the iodide to iodate.

2. Reduction of the iodate to iodide.

(a) With sulphurous acid. (b) With nitrous acid. These methods were shown to be satisfactory by experiments with known amounts of inorganic compounds.

3. Releasing the iodine with sulphuric acid, and trapping the free iodine. This was unsatisfactory, because, to remove the iodine completely, the mixture had to be heated so hot that sulphuric acid distilled over, and interfered seriously.

III. Estimation of the resulting iodine compound.

1. Gravimetric.

(a) Precipitation in acid solution of silver iodide or iodate. This is satisfactory in the method of Baubigny and Chavanne, but cannot be done if the compound were destroyed by fusion with an alkaline mixture in glass or quartz, for in that case, silicic acid precipitates.

(b) Precipitation in alkaline solution of silver iodide. If it is attempted to do this without the presence of sulphite, the mixture darkens, and the results are quite incorrect. If sulphite is present, the darkening does not occur, but the results are always too low. This was proven by experiments using a known amount of inorganic iodide.

2. Volumetric.

(a) Titration of the iodine liberated by the addition of iodate. The acidity control must be quite exact, and this is usually difficult in the mixtures involved, so the results are quite variable.

(b) Titration of the excess silver nitrate after precipitation of the iodine as silver iodide.

α. With thiocyanate. The end point is not satisfactory.

β. With potassium iodide solution (or direct titration of the iodide with silver nitrate). This is not impossible but is far from satisfactory, owing to indefinate end points.

In general, volumetric methods do not seem to be satisfactory for the solutions obtained after any of the permissable methods of decomposition.

Analysis of tetra-iodo-ortho-sulpho-benzoic anhydride by the method of Baubigny and Chavanne.

In 40 c.c. of concentrated sulphuric acid (d. 1.84) there is dissolved by warming 1 g. of silver nitrate and 8 g. of powdered potassium dichromate, and the mixture cooled. The sample to be analyzed, (about .5 g), which has been weighed out in a tiny beaker of pyrex, is introduced, and dissolved gradually by heating and shaking. The decomposition may be performed in a long necked flask of hard glass, such as a 300 c.c. Kjeldahl digestion flask. To insure complete decomposition, the mixture is finally heated to boiling. After the mixture has cooled, it is transferred to a beaker, about 150 c.c. water added, and then reduced by the addition of an excess of concentrated sodium sulphite solution. (Sulphurous acid may be used, but so much is required that it increases the volume of the solution unduly.)

The silver iodide is filtered off on a Gooch crucible, washed, the crucible dried at 120° in the oven, and weighed. The calculations are the same as in the Carius method. Any chlorine or bromine in the substance does not interfere, as they are evolved quantitatively as such during the decomposition.

The average accuracy obtained by this method may be judged from the following figures for tetra-iodo-phthalic anhydride, which was used to test the method. Its calculated percentage of iodine is 77.9%.

78.0%
77.6
77.6
77.7

(b) Biological properties of tetra-iodo-ortho-sulpho-benzoic anhydride.

The compound has a faint, slightly unpleasant taste . It is quite non-toxic. 200 mg. injected intravenously into a rabbit weighing 2 Kg. apparently produced no bad effects whatever. This corresponds to a dose of between 6 and 7 grams for a man. The author took 0.5 g. by mouth without any effect. Attempts to demonstrate the substance in the urine following this experiments were not successful.

Effect on bacteria. To cultures of B.Coli communis, there was added varying amounts of the substance in solution so that the resulting concentrations were, respectively, (a) 40 mg./cc., (b) 4 mg./cc., (c) 0.4 mg./cc. From these, subcultures were made at intervals of 1 min., 5 min., 30 min., 8 hours. Only in the case where the bacteria had been exposed to action of the 40 mg./cc. for 8 hours, did the subculture fail to grow. So it was concluded that tetra-iodo-ortho-sulpho-benzoic anhydride had no marked bacteriocidal action on B. Coli communis.

The author is indebted to Miss Elizabeth Tayian for assistance in this work.

2. METHODS OF CONDENSING TETRA-IODO-ORTHO-SULPHO-BENZOIC ANHYDRIDE WITH PHENOLIC SUBSTANCES

In the matter of condensing agents in the preparation of phthaleins, there is a wide choice. Baeyer, it will be remembered, discovered the first phthalein by simply fusing the mixture, without any condensing agent at all, and in fact Sohon[1] recommends that in the preparation of phenol-sulphone-phthalein, no condensing agent be used. Baeyer himself[2] came to the conclusion that in general, zinc chloride was the best, but in view of the high cost of this substance, the less satisfactory sulphuric acid could be recommended. Fuming stannic chloride[3], stannous chloride[4], and other compounds have been used. It was thought that before attempting the preparation of a series of these compounds, in large amounts, it would be advisable to conduct some experiments with the object of determining the best condensing agent for use with this particular series.

Preliminary experiments disclosed the fact that the tetra-iodo-ortho-sulpho-benzoic anhydride was much more difficult to condense with phenolic substances than the unhalogenated ortho-sulpho-benzoic anhydride, and that some condensing agent was necessary, but that the amount of the condensing agent was not especially important.

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[1] Am. Ch. J. 20, 257

[2] Ann. 202, 36

[3] Ann. 202, 153

[4] J. Ch. Ind. Russia 1, 2628 (25)

Comparison of condensing agents. Tetra-iodo-ortho-sulphobenzoic anhydride and freshly distilled phenol were mixed in the proportion of 1 g. of the former to 4 g. of the latter, and the mixture heated to bring about complete solution. 3 c.c. of this mixture was pipetted into a test tube, and 1 c.c. of a condensing agent added. The following were used: (a) no condensing agent, (b) fuming stannic chloride, (c) concentrated sulphuric acid, (d) fuming sulphuric acid containing 20% free SO_3, (e) zinc chloride, (f) aluminum chloride, (g) lead chloride, $PbCl_2$, (h) titanium tetrachloride, (i) phosphorous pentachloride, (j) phosphorous pentoxide.

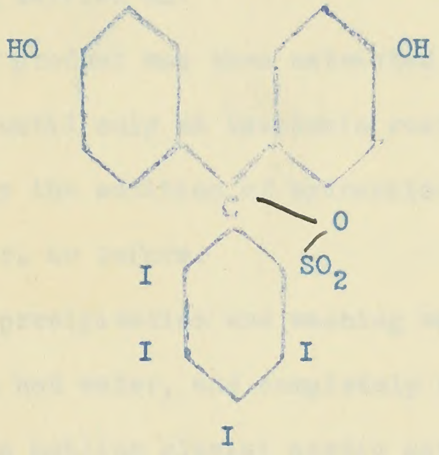
The test tubes were all immersed in the same oil bath, and heated to 170° for five hours. Then they were removed, and the contents dissolved in 20 c.c. of 5% sodium hydroxide. Then they were each made up to 2 L. with water, and compared with each other in the colorimeter. The greater the depth of color, the greater the amount of phthalein produced, assuming no colored by-products. This assumption is substantially correct, for none of the by-products of such condensations give the intense colors with alkali which characterize the phthaleins. Therefore from the readings obtained, it was possible to calculate the relative efficiency of the various condensing agents. The average, in round numbers, of several such experiments is given in the following table, where stannic chloride is rated at 100.

Condensing Agent	Efficiency
fuming stannic chloride	100
lead chloride	85
zinc chloride	50
sulphuric acid (concentrated)	35
sulphuric acid (fuming)	30
no condensing agent	25
aluminum chloride	0
titanium tetrachloride	0
phosphorous pentrachloride	0
phosphorous pentoxide	0

In addition to the evidence afforded by these figures, it was evident from the quality of the product that stannic chloride was immensely superior to all the others except lead chloride and zinc chloride. In the other cases, large amounts of gummy and tarry substances were produced which made the recrystallization of the phthalein practically impossible. Stannic chloride was used in all the condensations which follow.

3. PHENOL-TETRA-IODO-SULPHONE-PHTHALEIN

Preparation of phenol-tetra-iodo-sulphone-phthalein



The first member of the new series to be prepared was the above compound. The details of a typical preparation are given below. The quality of the reagents necessary and the proper conditions are discussed later.

80 g. of tetra-iodo-ortho-sulpho-benzoic anhydride were dissolved in 320 g. of melted phenol, 80 g. of fuming stannic chloride added, and the mixture heated in an oil bath to 170° for eight hours. The flask was kept closed by a ground-in glass stopper containing a tube ending in a calcium chloride tube, located at a lower level than the glass stopper, to avoid contamination of the reaction mixture by reflux of the contents of the calcium chloride tube, which are eventually liquefied. The reaction mixture gradually became a deep red, lost its transparency, and became much more viscous.

At the end of eight hours it was poured into about six liters of hot water, stirred, and washed by decantation with successive portions of hot water until the phenol had practically all been removed. This required about two days, during which time other preparations were being carried on.

This washed raw product was then extracted with 2% sodium hydroxide solution, until only an insoluble residue remained; filtered, and reprecipitated by the addition of hydrochloric acid. It was then washed with hot water, as before.

This alternate precipitation and washing was carried on until the product was solid in hot water, and completely soluble in alkali. It was then dissolved in boiling glacial acetic acid, filtered, concentrated, and allowed to stand. From this acetic acid solution small, reddish-brown crystals were deposited. They were filtered off, and recrystallized from more acetic acid, as at first.

Experiments were made to determine the optimum temperature for the above condensation. A solution of tetra-iodo-ortho-sulpho-benzoic anhydride in melted phenol, in the proportion of one gram of the anhydride to four of phenol, was prepared, three cubic centimeters of this pipetted into a test tube, and the several samples heated at different temperatures. At the end of five hours the contents of each tube were dissolved in twenty cubic centimeters of 5% sodium hydroxide, made up to two liters and compared with each other in the colorimeter. From the

readings obtained it was possible to calculate the relative yield at each temperature. The results are given in the following table, where the yield at the optimum temperature (170° C.) is arbitrarily set at 100.

Yields at various temperatures

Temperature	Yield
110°	10
120	10
130	30
140	50
150	70
160	90
170	100
180	90
190	70

In the above experiments the temperatures were maintained within 2 to 3 degrees by regulating the amount of gas admitted to a burner under a small oil bath. For actual preparations, a variation of 5° was found to be admissable, and the temperature was found to be maintained constant with this degree of exactness by the following device:

The flask in which the condensation was being carried out was immersed in an oil bath, which was placed on an ordinary laboratory electric stove. Also immersed in the bath was a mercury-filled thermostat, which when the temperature rose, closed a circuit actuating a

heavy relay which interrupted the current to the stove. This device performed satisfactorily without any attention throughout the present work.

The conditions determining the yield (and quality of product) are much more numerous than this brief discussion might indicate, and are not all independently variable, so that an exact determination of all the conditions optimal for this preparation would have involved an enormous amount of work, and was not attempted. For instance, up to a certain point, heating to a higher temperature for a shorter time was equivalent to heating to a lower temperature for a longer time. But partly as a result of consideration of the procedures of others in preparing analogous compounds, and as the result of experience gained in the numerous preparations involved in the present work, a set of conditions was arrived at which were, it was felt, a close approach to optimal conditions, and which were conformed to during all the later preparations. They may be summarized thus:

Preparation of phenol-tetra-iodo-sulphone-phthalein

Reagents

Freshly distilled colorless phenol.

Tetra-iodo-ortho-sulpho-benzoic anhydride, free from free iodine, and dry.

Pure fuming stannic chloride.

Proportions of Reagents

Reagent	Parts by Weight
Phenol	4
Anhydride	1
Stannic chloride	1

Temperature

165° - 175° C.

Time

5 - 8 hours.

Purification of phenol-tetra-iodo-sulphone-phthalein

Tetra-iodo-phthalic anhydride can be easily recrystallized from benzene, tetrachlorethane, phenol, or a mixture of alcohol and acetic acid, yielding well-formed crystals. In the author's experience, only the last mentioned solvent can be used for the recrystallization of tetra-iodo-ortho-sulpho-benzoic anhydride, and the resulting crystals are small and imperfect. There may be added to this observation the fact that the phthaleins derived from tetra-iodo-phthalic anhydride are much more difficult to recrystallize than the parent substance.

In view of these facts, it is not surprising that the greatest difficulty was experienced in purifying phenol-tetra-iodo-sulphone-phthalein. It is readily soluble in ethyl alcohol, methyl alcohol, acetone, ethyl acetate. It is soluble in benzene, carbon tetrachloride, chloroform, tetrachlorethane, ether. But it cannot be recrystallized from any of these solvents. If the substance, even in a pure state, is dissolved in any of these, and the solution concentrated, the phthalein does not separate until practically all of the solvent has evaporated, when it coats the sides of the beaker with a hard uniform layer, like red paint. Repeated solution in alkali and precipitation by acid, while useful as a means of preliminary purification, cannot be relied upon to effect sufficient purification so the compound can be used biologically, or for analytical purposes.

After many attempts, it was found that phenol-tetra-iodo-sulphone-phthalein could be recrystallized in the following manner. It was dissolved in boiling glacial acetic acid, filtered, and the solution concentrated. If it had not been concentrated too much, the solution, on standing, deposited small reddish-brown crystals of phenol-tetra-iodo-sulphone-phthalein.

Pratt and Shupp[1], in their paper already referred to, describe the use of bone-black in the purification of phenol-tetra-iodo-phthalein. This has often been used, and is certainly of value in the purification of phenolphthalein prepared by the use of sulphuric acid. After many trials of bone-black in connection with various solvents, it was eventually discarded as of no value in the present case.

The whole group of phthaleins with which the present study is concerned are exceedingly difficult to purify, and it is impossible to communicate through the medium of written language all of their peculiarities, a knowledge of which is necessary for working with them successfully. It can only be said briefly that during the preparation of phenol-tetra-iodo-sulphone-phthalein, there are formed a number of by-products, greater in amount than the phthalein. These are in large measure divisable into two classes; products insoluble in alkali, and products soluble in alkali but insoluble in acetic acid. Upon this the rationale of purification

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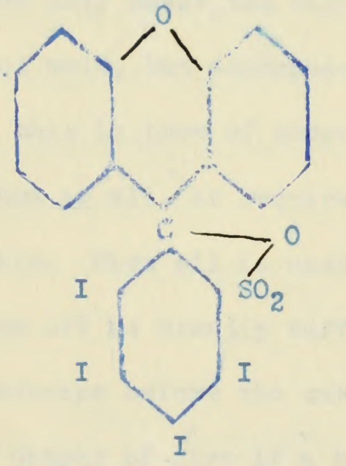
[1] Pratt and Shupp, JACS, 40, 257 (18)

largely depends. The nature of the by-products could not be more completely investigated in the time available for this study, but they include, as a relatively minor constituent, the analogue of fluorane:



Properties of phenol-tetra-iodo-sulphone-phthalein

(a) Physical

Pure phenol-tetra-iodo-sulphone-phthalein, obtained as described above, consists of small imperfect crystals, of a reddish-black color. Crystal faces can be seen only under the high power of the microscope.

The compound does not melt, but decomposes about 210°. It may be said now that in general this is true of compounds of this group. If it is possible to melt them at all, it requires such a high temperature as to be hardly practicable. When oil is used in the melting point apparatus, the smoke given off is usually sufficient to obscure the major features of the landscape before the substance can be said to be liquid. (There is little danger of fire if a heavy transparent oil such as Nujol is used instead of the very inflamable cotton-seed oil usually recommended.) The smoke produced when sulphuric acid is used in the apparatus is equally dense, and even less soothing to the respiratory tract. Attempts were made to take a melting point using a bath of Rose's metal in the apparatus instead of oil or acid, but it was found that the melting point of many of the substances, assuming they could be melted at all, lay above the range of a mercury-filled thermometer. And in the case of the others, the so-called melting point really consisted in a gradual charring and decomposition. So it was concluded that, just as White found in the case of the mercurochromes[1], the melting point could not be used as a critical constant for this group of compounds.

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[1] White, JAMA, 73, 1483, (19)

The crystals obtained as outlined evidently contained moisture or acetic acid absorbed on the surface, or possibly in part as liquid of crystallization, for a sample of the air-dried substance lost weight on being heated to 180° for eight hours. A sample which weighed .5208 g. lost .0459 g., or 8.8%. The crystals were unaltered in appearance microscopically after this treatment.

Before analysis, the compound was heated to 180° for ten hours. The analyses were done by the method of Baubigny and Chavanne, as the same difficulties were experienced with other methods as in the case of the parent substance, tetra-iodo-ortho-sulpho-benzoic anhydride. Analysis gave the following figures:

	Found	Calculated
I_2	59.0%	59.3%
	58.8	
	59.1	

The solubilities of the compound have already been mentioned in discussing its purification. It is readily soluble in methyl alcohol, ethyl alcohol, ethyl acetate, acetone, acetic acid. It is somewhat soluble in benzene, carbon tetrachloride, tetrachlorethane, chloroform, ether. It is only very slightly soluble in neutral water, but does dissolve to a sufficient extent to dye cloth immersed in the water. This will be referred to again more in detail.

The compound dissolves readily in dilute sodium hydroxide solution, forming the disodium salt. By dissolving the purified phthalein in the stoichiometric amount of standard sodium hydroxide solution, and evaporating to dryness, the disodium salt may be obtained as a dark reddish-black mass, with a bluish tinge. It is very difficult to free this from moisture. The solution of the disodium salt in water is an intense purple, quite similar to the color in alkali of phenol-tetra-iodo-phthalein.

The phthalein dissolves, apparently without change, in concentrated sulphuric acid, with the formation of the same purple color which characterizes its solution in alkali. This rather strange phenomenon has been noticed with most of the phthaleins which have been reported in the literature (see the introduction to this thesis), but has never received a completely satisfactory explanation.

The phthalein is very stable, and will resist heating to temperatures of 200° in air for an indefinite period, will withstand the action of concentrated cold sulphuric or hydrochloric acid.

In order to determine the pH range of the substance, it was necessary to prepare a series of buffer solutions of standard pH. In order that the same set might serve for work in the course in physiological chemistry in which the author was instructing, it was decided to make up the set desdribed in Folin[1], and supplement them in the most alkaline range with the glycine mixtures of Sørensen. The solutions were prepared from standard stock solutions made up according to the directions of Clark and Lubs[2]. These

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[1] Folin, "Laboratory Manual of Biological Chemistry", Appleton & Co., N.Y. 1925, pp.49-53

[2] Clark, "The Determination of Hydrogen Ions", Williams and Wilkins, Baltimore, 1928, pp. 192-220

solutions were the following.

M/5 acid potassium phosphate (KH_2PO_4) solution

Merck blue label acid potassium phosphate was recrystallized three times from distilled water, and dried to constant weight at 110-115°C. 27.232 g. were dissolved in 1 L. of carbonate-free water, obtained by boiling distilled water, and allowing it to cool while protected by a soda-lime trap.

M/5 boric acid (H_3BO_3) M/5 potassium chloride (KCl) solution.

Boric acid was recrystallized three times from distilled water, and air dried in thin layers between filter paper for a week, then the constancy of weight checked by drying small samples in a desiccator over calcium chloride. The potassium chloride was Merck blue label potassium chloride, and was recrystallized three times from distilled w water, and dried in the electric oven at 120°C. for two days. The solution contained 12.4048 g. of boric acid and 14.912 g. of potassium chloride in 1 L., made up with the carbonate-free water, which was used in all this work.

M/5 sodium hydroxide (NaOH) solution

This solution was prepared from an amalgam obtained electrolytically. A deep layer of redistilled mercury was placed in a conical separatory funnel. Over this was placed a saturated solution of recrystallized Merck blue label sodium chloride. It was found possible to

use the regular 110 v. D.C. current for this electrolysis, by cutting down the voltage by the following simple device. The 110 v. line was connected to a bank consisting of a 100 w. and a 50 w. lamp in series. Then there was connected in parallel with the 50 c. lamp a circuit consisting of a 250 w. lamp, and the electrolytic cell, connected in series. The negative pole of this circuit entered the mercury in the electrolytic cell through a glass-protected platinum wire, and the positive pole entered the sodium chloride solution through a platinum electrode. The distance from this electrode to the surface of the mercury was about 6 cm., and the area of the mercury surface was about 150 sq. cm. Under these conditions the fall in potential across the cell was found to be 6 volts, and the current passing through it 0.65 amperes. The current was allowed to run continuously for about four days.

At the end of this time the amalgam was separated off, washed several times with distilled water, and reacted with carbonate-free water in a paraffined flask, protected from the CO_2 of the air. When the solution had reached the desired strength, it was siphoned off into a paraffined container containing CO_2-free air, which was connected to an automatically filling burette, likewise protected from the atmospheric CO_2. It was standardized, and diluted to exactly 0.2 N (M/5).

The hydrochloric acid against which this sodium chloride solution was standardized was prepared as follows: 6 L. of approximately 0.15 N. hydrochloric acid were made up from C.P. hydrochloric acid, and mixed

NOTE. The above preparation of c.p. sodium hydroxide solution follows in general the directions given in Clark (loc. cit.). The details are in part modelled after procedures observed at the Rockefeller, and are in part original with the author.

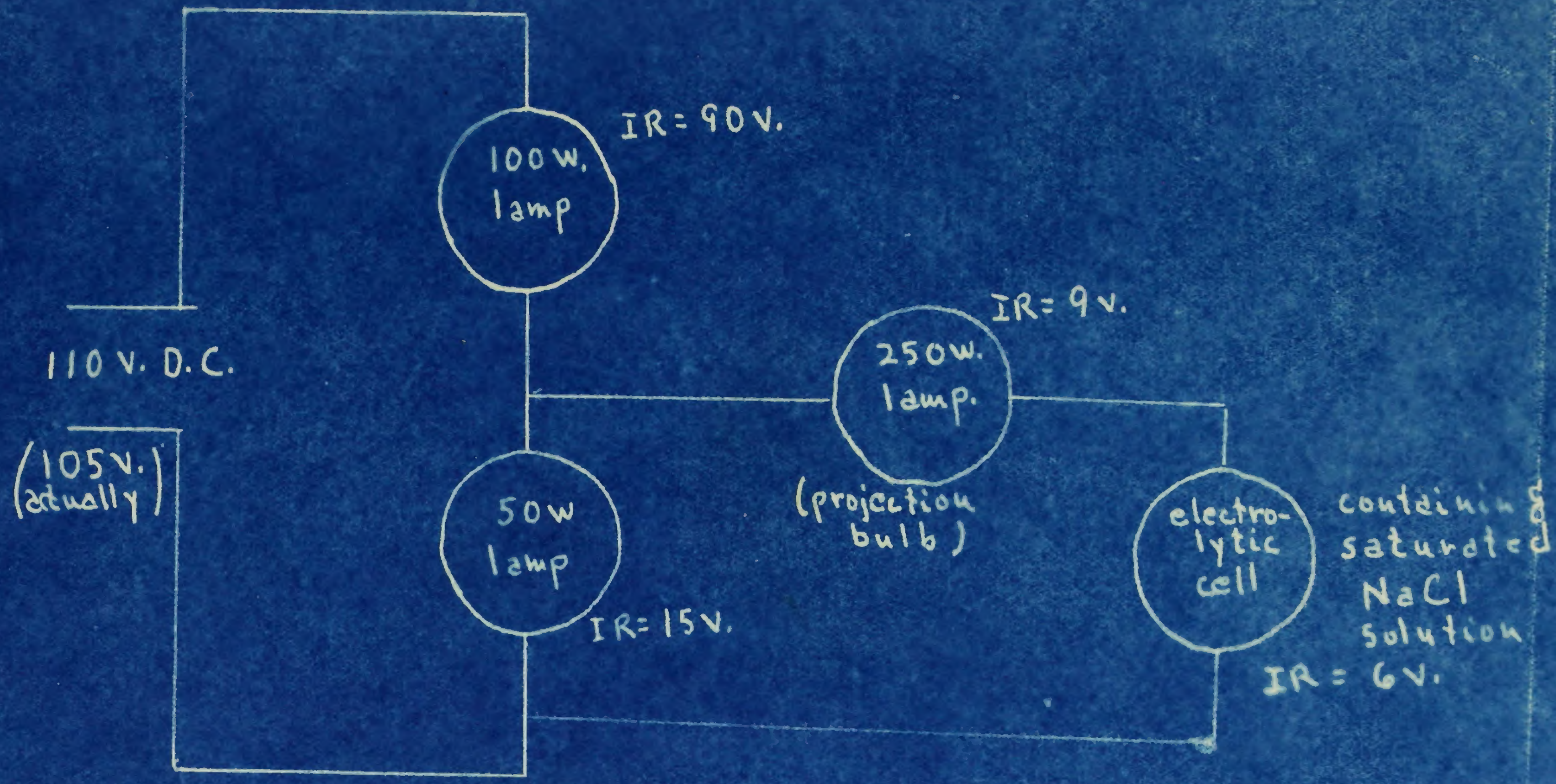


DIAGRAM OF CIRCUIT
FOR ELECTROLYSIS

thoroughly. After the mixture had stood for a day, samples of it were taken with a standardized pipette and precipitated by the addition of a slight excess of silver nitrate solution. The precipitates were allowed to stand in the dark until clear, then filtered off on weighed Gooch crucibles containing a fine mat. The precipitate was thoroughly washed on the Gooch and dried at 120°C. in the electric oven, cooled in a desiccator, and weighed. From the weights so obtained (which checked to one part in one thousand) the normality of the acid was calculated.

M/5 acetic acid (CH_3COOH) solution

C.P. acetic acid was distilled under reduced pressure, a solution somewhat stronger than .2 N made up, standardized against the standard sodium hydroxide solution, diluted to exactly 0.2 N, and checked.

Glycine (CH_2NH_2COOH) sodium chloride (NaCl) solution

7.505 g. Pfanstiehl glycine and 5.85 g. of sodium chloride, recrystallized three times, were dissolved in 1 L. of CO_2-free water.

The standard buffer mixtures were then prepared from these stock solutions by diluting to 200 c.c. the proper amounts, as shown in the following table.

Composition of Standard Buffer Mixtures

pH	c.c. 0.2 M CH_3COOH	c.c. 0.2 M HaOH
4.6	50	23.0
4.8	50	29.0
5.0	50	34.5
5.2	50	38.5
5.4	50	42.5
5.6	50	45.0
	c.c. 0.2 M KH_2PO_4	c.c. 0.2 M NaOH
5.8	50	3.66
6.0	50	5.64
6.2	50	8.55
6.4	50	12.60
6.6	50	17.74
6.8	50	23.60
7.0	50	29.54
7.2	50	34.90
7.4	50	39.34
7.6	50	42.74
7.8	50	45.17
8.0	50	46.85

pH	c.c. 0.2 M H_3BO_3KCl	c.c. 0.2 M NaOH
7.8	50	2.65
8.0	50	4.00
8.2	50	5.90
8.4	50	8.55
8.6	50	12.00
8.8	50	16.40
9.0	50	21.40
9.2	50	26.70
9.4	50	32.00
9.6	50	36.85
9.8	50	40.80
10.0	50	43.90

Sørensen's glycine mixtures

pH	Parts Glycine Solution	Parts 0.1N NaOH (carbonte-free)
10.42	5.5	4.5
11.01	5.1	4.9
11.25	5.0	5.0
11.51	4.9	5.1
12.04	4.5	5.5
12.33	4.0	6.0
12.60	3.0	7.0
12.79	2.0	8.0
12.97	1.0	9.0

These mixtures were kept in bottles of paraffin. The pH range of the indicators was determined in the following manner.

In a clean test tube there were placed, from a dropping pipette, four drops of the buffer mixture, and five c.c. of carbonate-free water. Then there was added one to three drops, (depending on the phthalein being tested) of an alcoholic solution of the phthalein. Results were usually checked by using also a solution in a mixture of water and alcohol, containing enough alkali to form the sodium salt of the phthalein. In every case the two methods gave results which, for the purposes of the present work, were identical.

The phthalein was tested with solutions of varying pH, such as pH 5, 6, 7, 8, and 9, and the portion of the scale covered by its range approximately determined. Then the intervening steps in this range, and a few on either side, were tried, and the useful range of the phthalein as an indicator determined. On the whole this group of phthaleins was found to be rather poor as indicators.

pH range of phenol-tetra-iodo-sulphone-phthalein

Phenol-tetra-iodo-sulphone-phthalein has a useful pH range of 7.0 to 8.2. Its color changes in this interval from a brownish-yellow to a wine-purple.

Properties of phenol-tetra-iodo-sulphone-phthalein as a dyestuff

Like many of the phthaleins[1], this substance will dye silk and wool fast. This will be discussed more fully later. Phenol-tetra-iodo-sulphone-phthalein dyes silk a color which varies from brownish-yellow to reddish-walnut, depending on the method employed. It does not give an especially attractive shade, and would be too expensive to have commercial application, in any case.

X-ray permeability of phenol-tetra-iodo-sulphone-phthalein

As is well known[2], permeability to X-rays is practically free from such unpredictable variations as is permeability to electromagnetic radiation of visible wave lengths. The amount of mass interposed between the source and the observer determines the intensity of the transmitted X-rays, and the effect is practically independent of the form of chemical union in which the matter may be.

So it was expected that a 10% solution of phenol-tetra-iodo-sulphone-phthalein would offer the same resistance to the passage of X-rays as the same thickness of a 10% solution of potassium iodide, and this was verified experimentally. The same is of course true of the other phthaleins, and the matter will not be referred to again until the end of this discussion.

[1] Cain and Thorpe "The Synthetic Dyestuffs", Charles Griffin & Co. London, 1920, pp. 100 - 113

[2] M. de Broglie-"X-Rays", Methuen & Co., London, 1925, p.1, p.53

Kaye - "X-Rays", Longmans, Green & Co., London, 1923, p.105

(b) Biological properties of phenol-tetra-iodo-sulphone-phthalein

Toxicity. The toxicity of this substance has not been completely worked out. A dose of .12 g. per kilogram of body weight has been injected intravenously into several rabbits without producing any permanent damage that could be detected. The substance is made up for injection by dissolving a weighed amount in the calculated amount of sodium hydroxide solution, making up to some convenient volume, with isotonic saline if calculation shows the resulting solution would be hypotonic, and sterilizing. The last animal experiment involved the injection of 0.50 g. of the phthalein into a 2 Kg. rabbit. The animal died almost at once, probably of thrombosis, as a large clot was found in the heart on autopsy. The author has taken doses of 0.2 g. by mouth without any detectable effects.

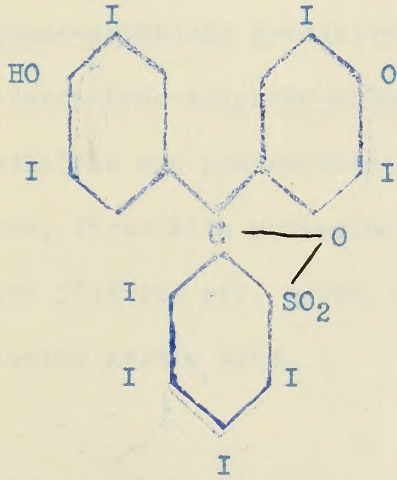
Fate in the body. In the autopsy mentioned above, on an animal which had died within a few minutes of injection, the dye was easily demonstrated in the liver substance and the kidney, but could not be demonstrated in the blood.

In one experiment, 0.24 g. of the phthalein was injected intravenously into a rabbit weighing 2.1 Kg., the urine collected for 24 hours, made alkaline, made up to 200 c.c., and compared in the colorimeter with a standard containing a known amount of the dye per c.c. From the reading thus obtained, and the total volume of the urine, the amount of the dye excreted in the urine could be calculated. It was found that about 20% of the amount injected had been eliminated in the urine. Thus the substance is, at any rate in part, eliminated through the kidney, as was predicted before it was made.

Three of the experimental animals were X-rayed after injection, but in no case was the kidney more visible than might have been expected normally. More of this work will be necessary before a definite decision can be made, but progress is very slow, owing to the congestion in the X-ray department here.

Derivatives of phenol-tetra-iodo-sulphone-phthalein

(a) Tetra-iodo-phenol-tetra-iodo-sulphone-phthalein



To prepare this compound, 4 g. of phenol-tetra-iodo-sulphone-phthalein was dissolved in glacial acetic acid, and 3 g. of iodine added. The solution was refluxed for an hour, poured into water, washed, recrystallized from glacial acetic acid. The substance gave a green color in alkali, and in all respects agreed in properties with the tetra-iodo-phenol-tetra-iodo-sulphone-phthalein prepared by another method, but when it was attempted to repeat this preparation by this method no trace of the substance was ever again obtained, although in all half a dozen attempts were made. Fate had played one of the little tricks which

she reserves for chemists, where some trace of impurity, or some un-noticed variation in the conditions, is the essential, and un-reproducible factor. In this case, however, there is another method of making the substance.

30 g. of phenol-tetra-iodo-sulphone-phthalein were dissolved in 600 c.c. of 5% sodium hydroxide solution, cooled by ice, stirred mechanically, while a solution of 80 g. of iodine in 100 g. of potassium iodide in 200 c.c. of water was added during the course of half an hour. The mixture was kept alkaline by the addition of small quantities of 30% sodium hydroxide solution. The deep purple of the phenol-tetra-iodo-sulphone-phthalein gradually gave way to the green of the tetra-iodo-phenol-tetra-iodo-sulphone-phthalein. After one and one-half hours, the phthalein was precipitated by the addition of hydrochloric acid, and washed, first with potassium iodide solution, then with water. It was then filtered off, dried, washed with cold chloroform, recrystallized from glacial acetic acid.

Properties of tetra-iodo-phenol-tetra-iodo-sulphone-phthalein

The compound, purified as described above, consists of small, irregular crystals, of a reddish brown color. No melting point could be determined, and the phthalein is very stable.

Analysis after drying at 180° for 10 hours gave the following figures:

	Found	Calculated
I_2	74.4	74.6%
	74.0	
	74.2	

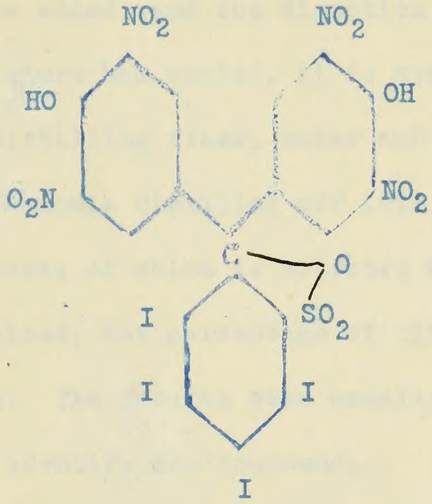
The iodine method of Baubigny and Chavanne was used, as before.

The compound is soluble in alcohol, methyl alcohol, ethyl acetate, acetone, acetic, but in all to a less extent than phenol-tetra-iodo-sulphone-phthalein. It is practically insoluble in chloroform, ether, tetrachlorethane, and water. It can be dyed on silk or wool from a water bath containing a little acetic acid, giving a greenish-brown color.

The most disappointing property was its slight solubility in alkali. (In other words, the disodium salt is not very soluble in water.) This is so marked that a greater amount of organically bound iodine can be got into solution by using the disodium salt of phenol-tetra-iodo-sulphone-phthalein than by using this compound. This was so discouraging that such time as was available for biological experiments was devoted to other compounds, instead of this, which had seemed so promising before it was made.

The pH range of this phthalein is from 6.2 to 7. The color ranges from one shade of green to another, and it is hardly possible that the compound could be of any value as an indicator.

(b) Tetra-nitro-phenol-tetra-iodo-sulphone-phthalein



This compound was prepared by adding to 1 g. of phenol-tetra-iodo-sulphone-phthalein a mixture of 10 c.c. concentrated nitric acid and 5 c.c. fuming nitric acid, and warming almost to boiling on the electric stove. After about half an hour the mixture was poured into water, washed, and recrystallized from ethyl alcohol.

The compound crystallizes in small brown crystals, melting with decomposition at 190° C. It dyes silk (and the experimenter's hands) a peculiar greenish-black. This color can be used as a test for protein, but is no more sensitive than some of the tests already in use.

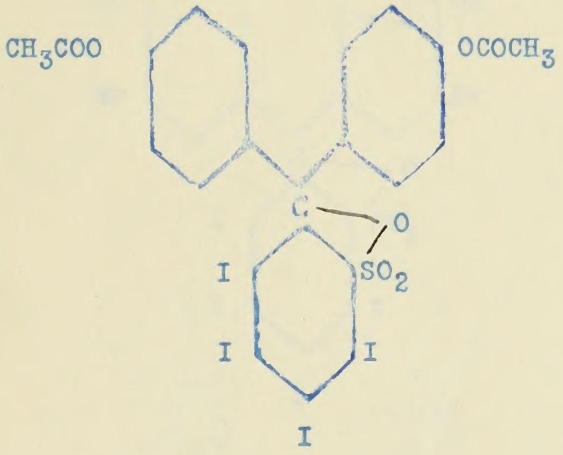
It may be analyzed for nitrogen by the Kjeldahl method by the following modified procedure:

To a weighed sample of the substance in a Kjeldahl digestion flask is added 20 c.c. of sulphuric acid. Then about 1 g. of zinc dust is gradually added, and the mixture gradually heated. When charring begins, a few crystals of copper sulphate and about 10 g. of potassium sulphate are added, and the digestion continued in the usual way. After the mixture has cooled, it is transferred quantitatively to a Kjeldahl distilling flask, water and 30% sodium hydroxide solution added, and the ammonia distilled off into a measured amount of standard acid, the excess of which is titrated with standard alkali. From the figures so obtained, the percentage of nitrogen in the substance can be calculated. The results were usually low, but good enough, it was felt, to identify the compound.

	Found	Calculated
N_2	5.20	5.39
	5.23	

This compound is quite unsuitable as an indicator, and difficulty was experienced in determining its pH range. It probably lies between pH 6 and pH 7. The color in alkali is purple.

(c) Di-acetyl-phenol-tetra-iodo-sulphone-phthalein



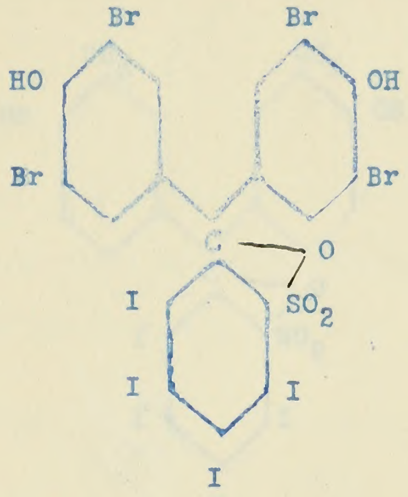
Di-acetyl-phenol-tetra-iodo-sulphone-phthalein was prepared by boiling together 5 g. of phenol-tetra-iodo-sulphone-phthalein, 3 g. of freshly fused sodium acetate, and 15 c.c. of acetic anhydride for 1¼ hours. The mixture was poured onto ice. It was at first gummy, but solidified on standing over night. It was filtered off, washed with water, recrystallized from 95% ethyl alcohol.

The substance consists of light brown crystals, soluble in ethyl alcohol, methyl alcohol, acetic acid, acetone, practically insoluble in water. The melting point was 136° C.

Analysis

	Found	Calculated
I_2	56.4	56.6%

(d) Tetra-brom-phenol-tetra-iodo-sulphone-phthalein.

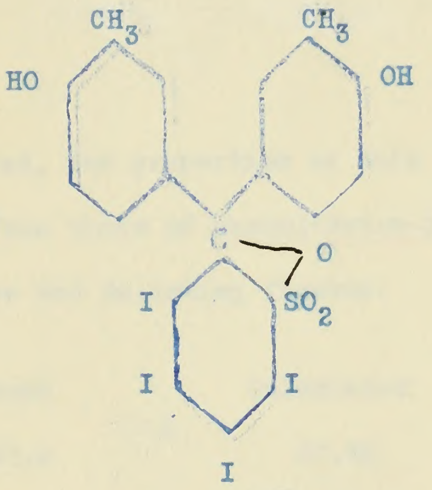


This compound was made by dissolving 1 g. of phenol-tetra-iodo-sulphone-phthalein in about 20 c.c. of glacial acetic acid, and adding, while boiling, 1 g. of bromine, and boiling for half an hour. The compound was poured into water, washed, and recrystallized from glacial acetic acid.

It consisted of brown crystals, giving a green color in alkali, but its sodium salt was practically as insoluble in water as that of tetra-iodo-phenol-tetra-iodo-sulphone-phthalein, so it was not investigated further.

It dyes silk a greenish-brown color, similar to that of tetra-iodo-phenol-tetra-iodo-sulphone-phthalein, but greener.

4. ORTHO-CRESOL-TETRA-IODO-SULPHONE-PHTHALEIN



Preparation

The preparation of this compound does not differ in any essential particular from the preparation of phenol-tetra-iodo-sulphone-phthalein.

30 g. of ortho-cresol, which had been freshly distilled under reduced pressure, 9 g. of tetra-iodo-ortho-sulpho-benzoic anhydride, and 15 g. of fuming stannic chloride were heated to 160 - 165°C. for ten hours, the reaction mixture being protected from the moisture of the atmosphere as before. At the end of this time, the mixture was poured into about 1 L. of water, and washed repeatedly with hot water, being finally boiled with water, to remove the cresol. (The cresol is much more difficult to remove than the phenol from the preceding preparation, due to its relative insolubility in water, but the above procedure is preferable to any other which was tried, such as distillation under reduced pressure.)

When as much of the cresol had been removed as possible, the raw product was dissolved in about 2% sodium hydroxide solution, filtered, and reprecipitated with hydrochloric acid. This was repeated three or four times, the product washed with water, and recrystallized from glacial acetic acid.

Properties

As would be expected, the properties of this substance are not essentially different from those of phenol-tetra-iodo-sulphone-phthalein. Analysis for iodine gave the following figures:

	Found	Calculated
I_2	57.0	57.4%
	57.2	

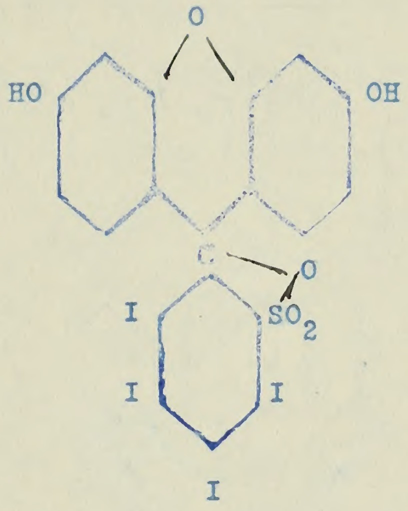
The sodium salt was found to be purple. It is not as soluble as the sodium salt of phenol-tetra-iodo-sulphone-phthalein, and the compound contains a smaller percentage of iodine, so no biological experiments were carried out with it.

Ortho-cresol-tetra-iodo-sulphone-phthalein has a pH range of pH 8.8 to 9.6, passing through various not very different shades of pink. It would probably not have any especial usefulness as an indicator.

The compound dyes silk shades similar to, and in fact practically indistinguishable from, those produced by phenol-tetra-iodo-sulphone-phthalein, ranging from a golden yellow to a walnut brown, depending on the method of application. But other conditions being the same, the shades produced by it are a trifle darker, as would be expected.

5. RESORCIN-TETRA-IODO-SULPHONE-PHTHALEIN

(Tetra-iodo-sulphone-fluorescein)



Preparation. This analogue of fluorescein and eosin was prepared in the following manner:

180 g. of resorcin, freshly distilled under reduced pressure, was melted, and 40 g. of tetra-iodo-ortho-sulpho-benzoic anhydride dissolved in it, 80 g. of fuming stannic chloride added, and the mixture, protected from the moisture of the air, heated to about 165°C. for ten hours. It was then poured into about 1.5 L. of water, and washed by decantation, finally being boiled with water. The boiling was stopped at intervals, the water poured off, the mass allowed to solidify, pulverized, washed by decantation, and boiled again with water. After as much purification as possible had been accomplished in this way (and it is important that

this treatment should be very thorough), the residue was dissolved in 2% sodium hydroxide, filtered, precipitated by the addition of hydrochloric acid, washed with water, boiled with water, and the solution in alkali repeated. After a sufficient amount of such preliminary treatment (which cannot be replaced by later treatment with bone-black), the substance can be recrystallized from glacial acetic.

The crystals come out practically black, and when they are filtered off and spread out to dry a curious phenomenon occurs. The crystals, which could be seen to have definite crystalline form, and felt to be firm, which may be sucked perfectly dry on the filter, and even washed with water or ether, when spread out to dry slowly melt down into a viscous liquid, which smells of acetic acid, and which eventually hardens into a black mass like deKhotinsky cement or Bakelite. This finally becomes hard enough to be ground to a powder, and it was such preparations that were used for the further study of this compound. Repeated recrystallization apparently did not improve the compound in this respect in the slightest.

Properties of resorcin-tetra-iodo-sulphone-phthalein.

This compound is soluble in the usual solvents; acetone, ethyl alcohol, methyl alcohol, ethyl acetate, chloroform, glacial acetic acid. It is slightly soluble in ether, and dissolves in water to a sufficient extent to form a dye bath. Its appearance, prepared as described above, was that of a black solid, with a greenish sheen. When dissolved in alkali it gives a red solution with a splendid greenish fluorescence.

Its pH range is about pH 8 - pH 10, but it would be quite useless as an indicator, as the change in color is not great, and takes place over a wide range.

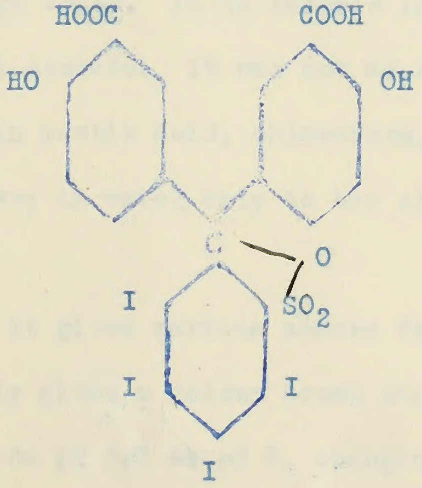
The compound dyes silk a rather handsome golden reddish-brown, with a faint fluorescence.

The solubility of its sodium salt is less than that of phenol-tetra-iodo-sulphone-phthalein, and it consequently has less promise as a possible means of visualizing the kidney.

Analysis. Analysis gave the following figures:

	Found	Calculated
I_2	58.4%	58.4%

6. SALICYLIC-ACID-TETRA-IODO-SULPHONE-PHTHALEIN



Salicylic -acid-sulphone-phthalein was probably made for the first time in 1898 by Sohon[1], but has been recently rediscovered[2], like many

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[1] Am. Ch. J. 20, 269, (1898)

[2] JACS 49, 3139 ('27)

of the phthaleins. The two acid groups seemed to make it an interesting member of the series to include, so salicylic acid-tetra-iodo-sulphone-phthalein was made.

Preparation. 70 g. of pure, white salicylic acid was melted and 15 g. of tetra-iodo-ortho-sulpho-benzoic anhydride dissolved in it. Then 55 g. of fuming stannic chloride were added, and the mixture heated, protected from the moisture of the air, to 165° - 170°C. for twenty hours. It was treated like the crude resorcin-tetra-iodo-sulphone-phthalein with water, but although there was a larger proportion of impurity present, it was easier to purify. It proved easiest to recrystallize it from a mixture of alcohol and acetic acid, as it did not seem to be as soluble in pure acetic acid as resorcin- or phenol-tetra-iodo-sulphone-phthalein.

Properties. The product so obtained consists of small, imperfect crystals of a rose-beige color. It is soluble in ethyl alcohol, methyl alcohol, acetone, ethyl acetate. It was not as soluble as the other members of this group in acetic acid, chloroform, and ether. As in the other cases, it dissolves in water only to the slight extent necessary to form a dye bath.

When dyed on silk it gives various shades depending on the mode of application, but usually gives a golden brown which is quite attractive.

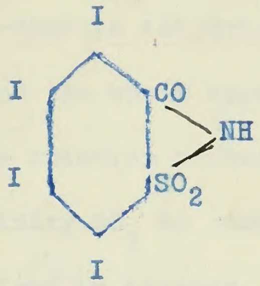
Its pH range is from pH 7.2 to pH 8, changing from a yellowish-brown to a pinkish-purple. It would probably prove a moderately satisfactory indicator.

It was thought that the compound might have a special interest as a precipitant for calcium, but such work as was done did not give any promise of a lead toward a satisfactory micro-calcium method, as the magnesium salt was also quite insoluble.

Analysis

	Found	Calculated
I_2	54.6	54.7%

7. TETRA-IODO-SACCHARIN



This compound is not really a member of the present series, but sacchareins[1] (compounds analogous to the phthaleins, but having a NH group instead of the lactone O of the phthalein) have been prepared from saccharin, as have also phthaleins themselves[1]. Therefore, since saccharin was known to be eliminated through the kidneys[2], it seemed to be of considerable interest to synthesize tetra-iodo-saccharin.

[1] C 1899, I 718

Monnet and Cattier, Bl. (3) 17 690 (C 97)

Sisley, C 1897, II 847

Remsen, Am. Ch. J. 6, 180 (84), 11, 73 (89)

Sohon, Am. Ch. J. 20, 257 (98)

Dutt, Soc. 121, 2389 (22)

[2] Sollmann, "A Manual of Pharmacology", W. B. Saunders Co., Phila., 1926. p. 67.

Preparation. 25 g. of pure saccharin (Merck), 100 c.c. of fuming sulphuric acid containing 50% free SO_3, and 70 g. of pure, powdered iodine, were heated to 120° C. for about four hours, then heated to 175° C. for twenty minutes. The same apparatus described for tetra-iodo-ortho-sulpho-benzoic anhydride was used. The source of heat was then removed, and the whole apparatus allowed to cool to room temperature, and the reaction mixture was poured into water and washed with water containing SO_2 to remove the excess iodine. The crude product was dissolved in acetone, filtered, and concentrated. The compound crystallized easily from this concentrate, giving silky yellow crystals with a greenish tinge.

Properties. The silky greenish-yellow crystals of tetra-iodo-saccharin are somewhat soluble in acetone, and practically insoluble in everything else, including sodium hydroxide solution. The sodium salt is so insoluble that no attempts were made to use this compound biologically. It has a faint, bitter taste.

The crystals did not melt or otherwise alter even at 345° C. They contained carbon, iodine, sulphur, and nitrogen, as demonstrated by the usual tests. Analysis for nitrogen was carried out by the Kjeldahl method, a rather long time being required for digestion.

	Found	Calculated
N_2	1.99	2.03%

Attempts to condense this substance with phenolic substances were made, using as condensing agents fuming stannic chloride, zinc chloride, and concentrated sulphuric acid. They were all failures, no substance having the properties of an indicator ever being produced. The steric hindrance caused by the four massive iodine atoms has completely wiped out the reactivity of the benzoic-imide group. One might be tempted to say that here we have a compound containing carbon, nitrogen, hydrogen, sulphur, oxygen, and iodine, arranged in the most stable configuration possible, but though it is improbable, new classes of simple organic compounds may remain to be discovered, and the statement might be a rash one to make.

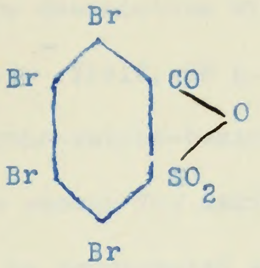
8. THYMOL-TETRA-IODO-SULPHONE-PHTHALEIN
9. ANTHRAQUINONE-TETRA-IODO-SULPHONE-PHTHALEIN
10. PYROGALLOL-TETRA-IODO-SULPHONE-PHTHALEIN

Attempts were made to prepare these compounds from tetra-iodo-ortho-sulpho-benzoic-anhydride, using various condensing agents, but the attempts were all failures, no substance having the properties of a phthalein ever being produced in any of them. It is easier to understand why these substances could not be made than it is to explain it in words. But in general, as the phenolic molecule becomes more complex, condensation with anhydrides, and especially the heavy tetra-iodo-ortho-sulpho-benzoic anhydride molecule, where steric hindrance must be considerable, becomes excessively difficult.

It is not intended to imply that the above compounds can not be prepared, and indeed, the author believes that, had occasion demanded, he could have made them a part of the present study, preparing them from more active parent substances, such as tetra-iodo-ortho-sulpho-benzoic anhydride di-chloride.

B. BROMINATED SULPHONE-PHTHALEINS

1. TETRA-BROM-ORTHO-SULPHO-BENZOIC ANHYDRIDE



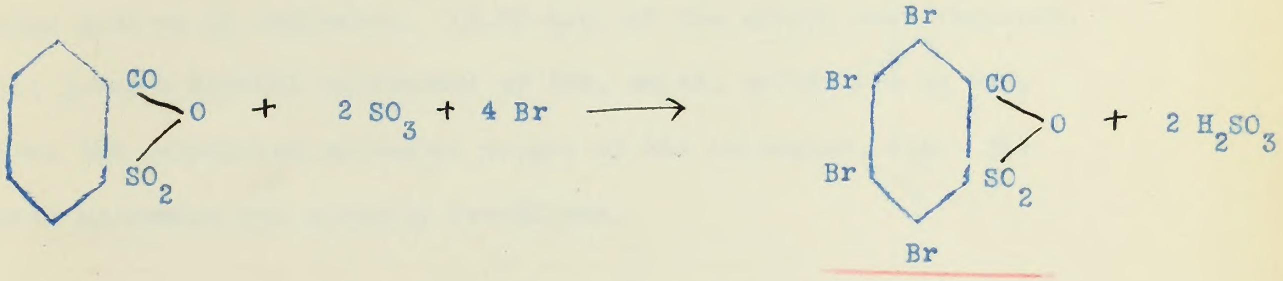
Preparation. 25 g. of ortho-sulpho-benzoic anhydride (Eastman, practical grade), 1 g. of iodine, and 125 c.c. of fuming sulphuric acid containing 50% free SO_3 were mixed together in a long necked flask of about 300 c.c. capacity. The same set-up was used as in the preparation of tetra-iodo-ortho-sulpho-benzoic anhydride. About 39 c.c. of bromine was measured out into a flask, and added as described.

About two-thirds of the bromine was added, and the mixture heated to 80°C. in an oil bath until the bromine was all absorbed. The apparatus was then allowed to cool to room temperature, when about two-thirds of the remaining bromine was added, and the mixture heated to 100°C. until the bromine was absorbed, as before. The apparatus was again allowed to cool, two-thirds of the remaining bromine added, the mixture heated to 130° until the absorption of the bromine took place, and again allowed to cool. The next time all of the remaining bromine was added, and the mixture heated to 170° C. for thirty minutes, and finally allowed to cool.

When cold the reaction mixture was poured into water, and the solid repeatedly washed by decantation with water containing a little sulphurous acid, and dried. Yield, 56 g. = 81%, calculated on the basis of the amount of ortho-sulpho-benzoic anhydride used. The compound so obtained is pure enough for making the phthaleins.

The preparation may be represented as follows. The iodine plays the part of a "carrier", but has not been included in the equation.

(a)



(b) $H_2SO_3 + SO_3 \longrightarrow H_2SO_4 + SO_2 \uparrow$

Purification. The substance is much more tractable than the corresponding iodine compound, and may be easily recrystallized from glacial acetic acid. The addition of alcohol is not necessary, as it is sufficiently soluble in the acid alone.

Properties

Color. The crystals obtained after washing and drying the crude preparation have a faint brownish color, which deepens on exposure to the light; but the pure compound, (recrystallized three times from glacial acetic acid) is pure white. The crystals are easily seen under the low power of the microscope as small, imperfect, hexagonal prisms.

Melting Point After the recrystallized product had been dried in a desiccator to remove traces of acetic acid (which experience had already shown to be quite difficult to remove), the melting point of the compound was 219° C. (uncorrected).

Molecular weight The molecular weight was determined by the neutral equivalent method. 0.7344 g. of the material was weighed out and titrated with 0.1450 N. hydrochloric acid, phenolphthalein being used as an indicator. 19.59 c.c. of the alkali were required. This gives a neutral equivalent of 258, which, multiplied by two, gives the calculated molecular weight of the substance, 516. The exact agreement was probably fortuitous.

Analysis. The compound was analyzed for bromine by the method of Carius.

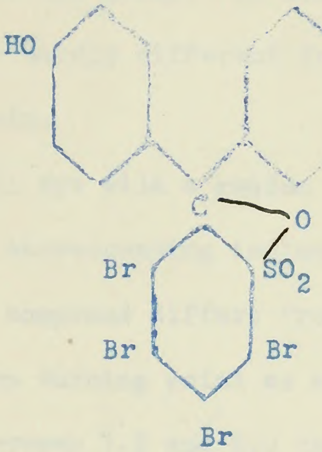
	Found	Calculated
Br_2	61.8	61.9%
	61.8	

Solubility. The substance is soluble in alcohol, acetone, glacial acetic acid, and to some extent in water.

Condensation of tetra-brom-ortho-sulpho-benzoic anhydride with phenolic substances.

For the purpose which primarily held the attention of the investigator, the brominated compounds did not offer the same chances of success as did the iodinated compounds, and though easier to study, were not worked out with the same thoroughness as the iodinated group. Therefore, for example, fuming stannic chloride was employed for all the condensations without making a series of tests with various condensing agents, as was done in the case of tetra-iodo-ortho-sulpho-benzoic anhydride.

2. PHENOL-TETRA-BROM-SULPHONE-PHTHALEIN



Preparation. 80 g. of phenol, which had been freshly distilled under reduced pressure, 20 g. of tetra-brom-ortho-sulpho-benzoic anhydride, and 20 g. of fuming stannic chloride were heated to 160°C. for five hours. The mixture was then poured into water, washed repeatedly by decantation and finally boiled with water, dissolved in about 2% sodium hydroxide, reprecipitated by the addition of hydrochloric acid, and this purification treatment repeated. Finally the crude product was dissolved in glacial acetic acid, filtered, concentrated, and allowed to stand. Reddish-black crystals were slowly deposited.

Properties. The recrystallized product consists of very small reddish-black crystals of high, but indefinite, melting point.

It is soluble in methyl alcohol, ethyl alcohol, acetone, ethyl acetate, chloroform, glacial acetic acid. It is practically insoluble in water, like all the group. It dissolves in alkaline solutions giving a purple color hardly different from that given by phenol-tetra-iodo-sulphone-phthalein.

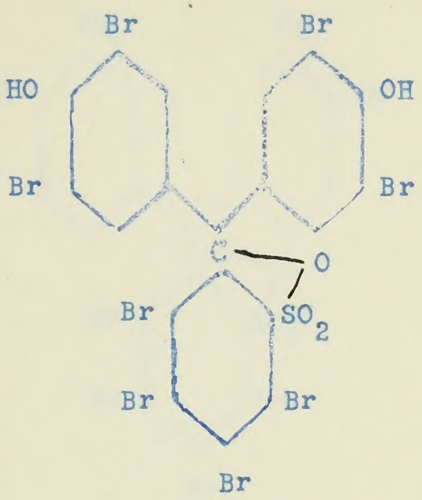
The compound will dye silk a golden brown shade very similar to that produced by the corresponding iodine compound.

This particular compound differs from all the others investigated in having a very sharp turning point as an indicator, or in other words, a short pH range. Between 7.2 and 8.0 the color changes from a light brown to a plum-purple, so abruptly that its turning point might almost be fixed at pH 7.4. It was felt that this substance might be of some value in titrations where an end point in this region is desired, but no work was done to ascertain how free it might be from the so-called protein and salt errors.

Analysis.

	Found	Calculated
Br_2	47.5	47.7

Tetra-brom-phenol-tetra-brom-sulphone-phthalein



Preparation. This compound was prepared by dissolving 5 g. of phenol-tetra-brom-sulphone-phthalein in 100 c.c. of glacial acetic acid, adding 5 g. of bromine, and boiling the mixture for twenty minutes. It was then poured into water, washed, and recrystallized from glacial acetic acid.

Properties. The color of the compound so obtained is not very different from that of the parent compound. It is somewhat less soluble, especially in ether and chloroform.

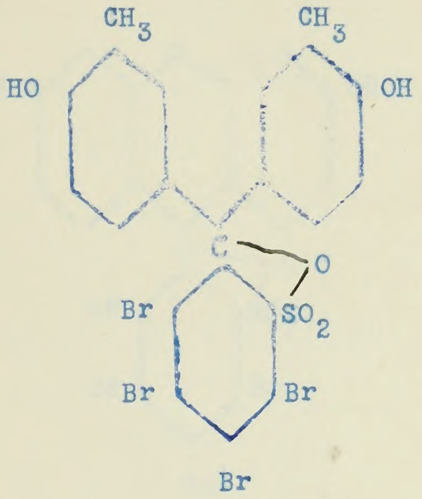
It has a pH range of pH 5.6 - 7.0, but the color is some shade of bluish-green at all the hydrogen ion concentrations within this range, so its usefulness as an indicator would be practically zero.

It dyes silk a bluish green.

Analysis

	Found	Calculated
Br_2	65.3	65.2%

3. ORTHO-CRESOL-TETRA-BROM-SULPHONE-PHTHALEIN



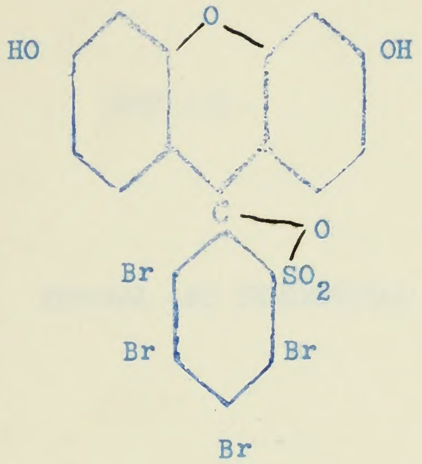
Preparation. 100 g. of o-cresol, which had been freshly distilled under diminished pressure, 25 g. of tetra-brom-ortho-sulphobenzoic anhydride, and 25 g. of fuming stannic chloride were heated to 165°C. for nine hours. The crude mass was purified with water, then by solution and reprecipitation with alkali and acid as usual, then recrystallized from glacial acetic acid.

Properties. So far as they were investigated, they did not seem to be notably different from those of phenol-tetra-brom-sulphone-phthalein.

Analysis

	Found	Calculated
Br_2	45.6	45.7%

4. RESORCIN-TETRA-BROM-SULPHONE-PHTHALEIN



Preparation. 100 g. of freshly distilled resorcin, 30 g. of tetra-brom-ortho-sulpho-benzoic anhydride, and 30 g. of fuming stannic chloride were heated to 165°C. for ten hours. Treated with water, alkali and acid, and glacial acetic acid as usual.

Properties. The substance was a black solid, with a greenish sheen. It was soluble in the usual solvents, dissolving in alkali to give a red solution with a magnificent greenish fluorescence.

The substance dyed silk a light reddish brown which was rather attractive.

The substance was red in solution between the hydrogen ion concentrations of 10^{-8} and 10^{-10}, but did not posses any useful pH range, as the change was too gradual, and the color too complex.

Analysis.

	Found	Calculated
Br_2	46.5	46.7%

PART III

GENERAL AND THEORETICAL

A. COMPARISON WITH THE CORRESPONDING HALOGENATED PHTHALEINS

It is natural to compare these compounds with the corresponding compounds prepared by Pratt[1]. They differ from them only in that a $\overset{|}{\underset{|}{S}}O_2$ group has been substituted (by roundabout methods, certainly) for a $\overset{|}{\underset{|}{C}}O$ group.

In the first place, the sulpho-compounds are throughout much harder to prepare. The author prepared for this work by repeating a large part of the work of Pratt, and makes this statement with confidence, even with feeling. And having been prepared, they are much harder to purify. This is due partly to a large number of by-products being formed, but principally to the fact that the compounds, perhaps because of greater asymmetry in the molecule, are naturally reluctant to form crystals.

The crystals of tetra-iodo-phthalic anhydride and of tetra-bromphthalic anhydride are larger and better formed than those of the corresponding sulpho-compounds. These substances have a lighter, more fluffy feel than the rather sticky, amorphous sulpho-compounds.

It is proposed in the following pages to compare the two classes of compounds point by point.

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[1] Pratt and Perkins, JACS 40, 198 (18)

Pratt and Shupp, JACS 40, 257 (18)

Pratt JACS 40, 1416 (18)

41, 1290 (19)

Color. Tetra-iodo-ortho-sulpho-benzoic anhydride is about the same color (yellow) as tetra-iodo-phthalic anhydride, and the tetra-brom compounds are both colorless when pure. But the sulphone-phthaleins derived from tetra-iodo-ortho-sulpho-benzoic anhydride and from tetra-brom-ortho-sulpho-benzoic anhydride are dark red, almost black, in color, while the corresponding phthaleins are light yellow. It is possible that there is normally enough of the quinoid form present in the (otherwise pure) sulphone-phthalein to account for this difference. In that case, the sulpho-group would have produced a shift in the lactoid-quinoid equilibrium, in favor of the quinoid form. Support for this idea is found in the data presented under the next two points.

Color of the sodium salts. (color in alkali) The color of the phthaleins in alkali is practically indistinguishable from that of the corresponding sulphone-phthalein, so that the difference introduced in this respect is slight. But the alkali color is developed at a lower pH. Pratt does not report pH ranges in his papers, but the author of this thesis has determined the pH ranges of two of his compounds. They are herewith compared with the corresponding compounds of the present series.

Compound	pH Range	Color
(phenol-tetra-iodo-phthalein	9.0 - 9.6	Purple
(		
(phenol-tetra-iodo-sulphone-phthalein	7.0 - 8.2	"
(tetra-iodo-phenol-tetra-iodo-phthalein	8.4 - 9.2	Blue
(		
(tetra-iodo-phenol-tetra-iodo-sulphone-phthalein	6.2 - 7.0	"

Color the substances dye silk. This is associated with the shift in turning point. The two phthaleins above mentioned, when applied to silk from a 5% acetic acid bath, leave it to casual inspection unchanged, though the application of alkali shows that the phthalein is attached to the silk, but in its colorless state. The corresponding sulphone-phthaleins, on the other hand, color silk, respectively golden yellow and brownish-green. It seems logical to suppose that the phthalein or sulphone-phthalein attached to the silk has the color which it would display in a solution of pH corresponding to the isoelectric point of the proteins of the silk. If this theory is true, the iso-electric point of silk proteins can be determined, from the above observations only, to lie between pH 7 and pH 8, probably at pH 7.2. This checks with the colors on silk given by the other sulphone-phthaleins. A summary of pH ranges, and of colors, is given at the end of this discussion.

Toxicity. It has been difficult to find data on the toxicity of these compounds. Phenol-tetra-iodo-phthalein is ordinarily administered intravenously in doses of about 40 mg. per Kg. of body weight in cholecystography, and while not dangerous, sometimes gives the patient a bad afternoon. Doses of as much as 120 mg. per Kg. of body weight of phenol-tetra-iodo-sulphone-phthalein have been injected intravenously into rabbits without any apparent damage either temporary or permanent, but of course the rabbit was not asked how he felt. It is probable, in view of such work as has been done, that the sulphone-phthaleins are certainly not more toxic than the corresponding phthaleins.

Fate in the body. The phthaleins are said to be excreted in the bile[1], and the sulphone-phthaleins, in part at any rate, in the urine[2]. This is borne out by the author's work, described under phenol-tetra-iodo-sulphone-phthalein.

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[1] Vámossy, Ther. Gegenw. 43, 203 (02)
Rosenthal and White, J. Pharm. 24, 265, (24)

[2] Abel and Rowntree, J. Pharm. and Exp. Ther. 1, 231 (09)

B. SUMMARY OF PROPERTIES OF THE NEW SERIES

pH Ranges

Compound	pH Range
phenol-tetra-iodo-sulphone-phthalein	7.0 - 8.2
o-cresol-tetra-iodo-sulphone-phthalein	8. - 9.
resorcin-tetra-iodo-sulphone-phthalein	8. -10.
tetra-nitro-phenol-tetra-iodo-sulphone-phthalein	6. - 7.
tetra-iodo-phenol-tetra-iodo-sulphone-phthalein	6.2 - 7.0
salicylic-acid-tetra-iodo-sulphone-phthalein	7.2 - 8.6
phenol-tetra-brom-sulphone-phthalein	7.2 - 8.0
tetra-brom-phenol-tetra-brom-sulphone-phthalein	5.6 - 7.0
resorcin-tetra-brom-sulphone-phthalein	8. -10.

Color on silk The compounds were dyed on silk from a 5% acetic acid bath, usually, but sometimes from pure acetic acid. Where the turning point of the alkali was near the (assumed) iso-electric point of the silk proteins, it was sometimes possible to obtain a different shade by dyeing the compound on from a weak alkaline bath containing a large amount of sodium chloride. Samples of the cloth are given on the next page.

The author is of course not skilled in the application of dyestuffs, and much better results could have been obtained by a worker with practical experience, without any doubt.

Black marks on the bits of cloth are not due to the dye, but are marks made to identify the dye used, before the cloth was pasted into the typescript.

phenol-tetra-iodo-sulphone-phthalein

(from 5% acetic acid)

o-cresol-tetra-iodo-sulphone-phthalein

(5% acetic acid)

resorcin-tetra-iodo-sulphone-phthalein

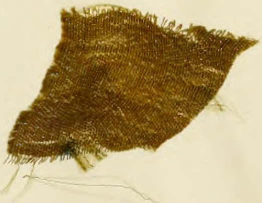
(5% acetic acid)

salicylic-acid-tetra-iodo-sulphone-phthalein

(5% acetic acid)

tetra-nitro-phenol-tetra-iodo-sulphone-phthalein

(5% acetic acid)



tetra-brom-phenol-tetra-iodo-sulphone-phthalein

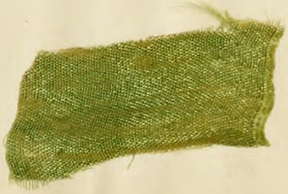
(5% acetic acid)

tetra-iodo-phenol-tetra-iodo-sulphone-phthalein

(5% acetic acid)

tetra-iodo-phenol-tetra-iodo-sulphone-phthalein

(sodium chloride and weak alkali)



phenol-tetra-brom-sulphone-phthalein

(5% acetic acid)

tetra-brom-phenol-tetra-brom-sulphone-phthalein

(5% acetic acid)

resorcin-tetra-brom-sulphone-phthalein

(5% acetic acid)

Pratt's compounds on silk

For comparison, a few examples are given of other phthaleins, including two of the group prepared by Pratt, dyed on silk under similar conditions.

phenol-tetra-iodo-phthalein
(5% acetic acid)

tetra-iodo-phenol-tetra-
iodo-phthalein
(weak alkali and sodium chloride)

tetra-iodo-resorcin-phthalein
(5% acetic acid)

Color in alkaline solutions.

No attempt is made to make the names of the colors more than merely suggestive.

Compound	Color
phenol-tetra-iodo-sulphone-phthalein	purple
o-cresol-tetra-iodo-sulphone-phthalein	purple
resorcin-tetra-iodo-sulphone-phthalein	red with strong greenish fluorescence.
salicylic-acid-tetra-iodo-sulphone-phthalein	pink
tetra-brom-phenol-tetra-iodo-sulphone-phthalein	bluish-green
tetra-iodo-phenol-tetra-iodo-sulphone-phthalein	green
phenol-tetra-brom-sulphone-phthalein	purple
o-cresol-tetra-brom-sulphone-phthalein	purple
resorcin-tetra-brom-sulphone-phthalein	red with strong greenish fluorescence
tetra-brom-phenol-tetra-brom-sulphone-phthalein	blue

Effect of substitution in the sulphone-phthaleins

Color. It is well known that the general effect of substitution of heavy atoms or groups in a compound is to shift the absorption bands in the direction of decreasing frequency. (See the references to Pratt's work, or the numerous references given to work on absorption spectra in the introduction to this thesis) Thus a colorless (apparently) substance may be colored after heavy groups have been substituted in it, because the absorption-bands have been shifted from the ultra violet into the visible spectrum. These ideas find qualitative support in the progression of the colors in alkali of phenol-tetra-iodo-sulphone-phthalein (purple or violet), tetra-brom-phenol-tetra-brom-sulphone-phthalein (blue), tetra-brom-phenol-tetra-iodo-sulphone-phthalein (bluish-green), and tetra-iodo-phenol-tetra-iodo-sulphone-phthalein (green).

More than this apparently cannot be said, and the writer has been assured by one of the workers in the field of the new quantum mechanics that the quantitative explanation of the color of organic compounds might be impossible, and at any rate could not be affected in the present state of our knowledge.

The light reflected by an organic compound is of course very complex, and only analysis of it by a photospectrometer would be even approximately objective.

Toxicity. Apparently the substitution in the phthalein molecules of any groups which are firmly bound does not greatly increase the toxicity of the compound. 0.5 g. of phenolphthalein is a large dose, but 6 to 7 g. of tetra-iodo-phenol-phthalein or phenol-tetra-iodo-phthalein can be administered. Even if the weight of the phthalein skeleton only be considered, there is still a much greater tolerance for the iodine-substituted-compound. Exact figures are lacking, but similar relations obtain in the sulphone-phthalein-series.

Fate in the body. There is some evidence that although phenol-sulphone-phthalein is practically completely excreted in the urine[1], the highly substituted phenol-sulphone-phthaleins, due probably to masking of the sulphone-group, which is kidney-specific, if the use of such a term may be permitted, are not so completely eliminated in the urine. This agrees with the observations of the present writer. See data under phenol-tetra-iodo-sulphone-phthalein.

Solubility The solubility of the sulphone-phthaleins themselves in organic solvents and of their alkali salts in water, is greatly diminished by halogen substitution.

Opacity to X-rays. As mentioned **before**, opacity to X-rays is merely a matter of mass, so the substitution of the halogen enormously increases the opacity to X-rays exhibited by the compounds.

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[1] Abel and Rowntree, J. Phar. and Exp. Ther. 1, 231 (09)

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