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The identification and properties of some naphthoquinone trimers

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The identification and properties of some naphthoquinone trimers

by

Therese Walter Michels

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Chemistry
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Approved:

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For the Graduate College

Iowa State University
Ames, Iowa

1977

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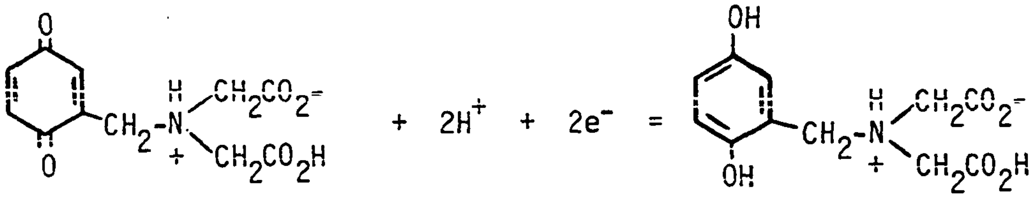
I. INTRODUCTION

> *The adjective "chelate," derived from the great claw or "chela" of the lobster and other crustaceans, is suggested for these caliper-like groups which function as two associating units and fasten on to the central metallic atom so as to produce heterocyclic rings — G.T. Morgan.*

The ultimate purpose of the chemical research described in this paper was to devise an oxidation-reduction system, the potential of which depends on the concentration of some particular metal ion in solution. The basic concept involved the introduction of a chelating group such as methyleneiminodiacetic acid, $-CH_2N(CH_2COOH)_2$, into organic molecules of the quinone-hydroquinone type. Basically then the system proposed embodies the features of both oxidation-reduction chemistry and chelate-ring chemistry.

Work on this general concept was begun at Iowa State University by Pietrzykowski (1) and extended by Contario (2, 3). Pietrzykowski succeeded only in synthesizing hydroquinonemethyleneiminodiacetic acid (II). Contario improved the preparation of hydroquinonemethylene-

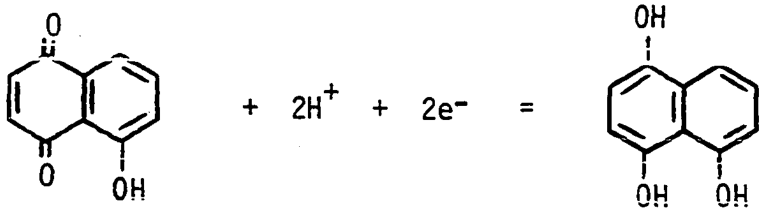


(I)
QMIDA

(II)
H_2QMIDA

iminodiacetic acid and succeeded in preparing quinonemethyleneiminodiacetic acid (I). For these compounds he adopted the abbreviations QMIDA and H_2QMIDA. He proved that these two compounds are interrelated, as are quinone and hydroquinone, by two hydrogen ions and two electrons, in accord with the chemical equation given above. The standard reduction potential of the new couple was $E^{o}_{Quin,\ H_2Quin} = +\ 0.699$ V vs the N.H.E. The potential of the new couple proved dependent on the concentration of metal ions present, and in particular, the response to aluminum was studied. Unfortunately, the union of the new compounds with metals reduced the stability of the quinone, ring opening occurred, and the potentials measured drifted. Thus, the system proved of no use for the purposes of chemical analysis.

The initial purpose of the present research was to enlarge the aromatic portion of the molecule in the hope of increasing the stability of the chelating derivatives. For this purpose I selected the known oxidation-reduction couple juglone-hydrojuglone:



(III)
juglone

(IV)
hydrojuglone

Although juglone is available from the commercial houses marketing fine organic chemicals, the price, \$12.00 per gram, was so high as to preclude purchase for the research proposed. The work described in the thesis I presented for the Master of Science degree was devoted almost entirely to the synthesis of juglone; a really satisfactory procedure was never found. During the work on the preparation of juglone, a dark green, very insoluble material was frequently obtained, often in considerable amount and exasperating in that it obviously reduced the already depressingly low yield of juglone. In physical appearance this dark green iridescent material strikingly resembles quinhydrone. Indeed, in quite analogous fashion to quinhydrone, the reduction potential of solutions saturated with this material is a function of pH, but with the surprising response of 40 millivolts per pH unit in contrast to the 60 millivolts per pH unit of quinhydrone. This finding merited attention and experiment, and the result of that still further experiment, and the result of that still further experiment. In the end, the entire research was devoted to this unusual material, to which I have given the common name *juglone green*. This happy bit of serendipity[a] and the diversion from the initial purpose and program has had a

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[a] The term serendipity is derived from Sanskrit mythology. The Oxford English Dictionary traces the first English usage to Horace Walpole:

> SERENDIPITY (serendi:piti). (f. Serendip, a former name for Ceylon + ITY.
> A word coined by Horace Walpole, who says (let. to Mann, 28 Jan. 1754) that he had formed it upon the title of the fairy-tale 'The Three Princes of Serendip', the heroes of which were always making discoveries, by accidents and sagacity, of things they were not in quest of'.)
> The faculty of making happy and unexpected discoveries by accident. 1754 H. Walpole Let. to Mann 28 Jan., This discovery, indeed, is almost of that kind which I call Serendipity. 1880 E. Solly Index Titles of Honour Pref. 5 The inquirer was at fault, and it was not till some weeks later, when by the aid of Serendipity, as Horace Walpole called it--that is looking for one thing and finding another--that the explanation was accidentally found.

pay-off for the science of chemistry. Juglone green joins the very limited group of stable free radicals; what can be done with it, I have barely hinted at in the following pages. The dissertation is organized in chapters roughly in the same order as the research progressed from point to point, the closing Chapter IX being used to draw the various lines of evidence into a picture of just what juglone green is.

The work actually done was not without bearing on the initial problem. The knowledge gained of the chemistry of juglone, juglone green, and compounds related to juglone make it evident that the choice of the juglone-hydrojuglone couple as a base for building in a chelating group was not a good one; that lawsone-hydrolawsone will probably prove far better. Lawsone is 2-hydroxy-1,4-naphthoquinone, an isomer of juglone, it is relatively inexpensive, its chemistry is simpler than that of juglone. That a chelating derivative of lawsone is being left for future work brings not regret; juglone green was a challenge and promises to provide other workers with interesting problems.

II. SYNTHESIS OF JUGLONE

Regardless of how extensive the reading has been, regardless of how much thinking has been done, and regardless of how many theories have been built up, the final contest between mind and matter must take place in the arena of the laboratory — E.E. Reid.

A. Review of Earlier Work

Juglone is available commercially from the chemical companies marketing fine organic chemicals, but the price, $12 per gram, is so high as to preclude the purchase of the significant quantities needed for the proposed development of an oxidation-reduction couple responsive to metal ions. A major part of the work carried out and reported in my M.S. thesis (4) was devoted to attempts to improve the methods of preparing juglone. Briefly, the findings reported in that thesis are:

1. The extraction of juglone from walnut shells (Combes (5)) is very troublesome and time consuming and is impractical for the preparation of juglone in more than milligram amounts.

2. The oxidation of 1,5-naphthalenediol with chromic acid (Bernthsen and Semper (6)) produced a great deal of tarry by-products and a yield of juglone of only 5 per cent. This is, however, the method preferred by recent workers (7, 8).

3. An attempt to oxidize 1,5-naphthalenediol to juglone by air in the presence of a silver oxide-cuprous oxide catalyst failed.

4. Oxidation of 1,5-naphthalenediol by lead dioxide (Wilstätter and Wheeler (9)) gave a yield of juglone of 30 per cent but the procedure was difficult and expensive.

5. Oxidation of 1,5-naphthalenediol with Fremy's salt, $K_2ON(SO_3)_2$, (Teuber and Götz (10)) gave a yield of 25 per cent. Fremy's salt is troublesome to prepare, decomposes spontaneously and must be used within 30 minutes, and is a good oxidizing agent only in very dilute solutions (0.016 M). The method is not satisfactory for the preparation of large amounts of juglone.

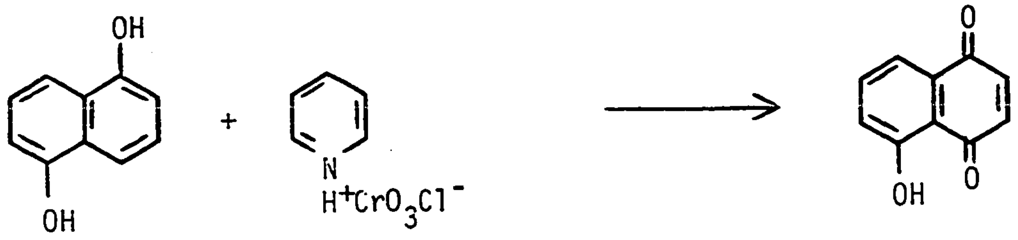
B. Further Studies on the preparation of Juglone

Two factors in the synthesis of juglone are responsible for the poor yields. First, in the synthesis from 1,5-naphthalenediol half of the naphthalene ring must be oxidized to the quinone with the other half left unchanged. The problem then lies in finding an oxidizing agent strong enough to effect such oxidation but not so strong that both rings are oxidized. Second, the recovery of juglone from the tarry materials which seem inevitable to be produced during the synthesis is cumbersome and much of the juglone is either not recovered or is decomposed during the clean-up process. Efforts to minimize the second problem will be discussed in Chapter III.

In the new work reported here, a new oxidizing agent, pyridinium chlorochromate is used to oxidize 1,5-naphthalenediol; this procedure was fairly successful. In a second study, an attempt was made to build up the juglone molecule by the condensation of maleic anhydride and phenol; this procedure failed, although as shown in Chapter VIII of this thesis, the procedure proved successful in synthesizing substituted juglones.

1. Oxidation of 1,5-naphthalenediol with pyridinium chlorochromate

An efficient reagent for the oxidation of a wide variety of alcohols to carbonyl compounds was reported recently, by Corey and Suggs (11). The reagent, pyridinium chlorochromate, offers the advantages of being stable, easily prepared, and the rapid oxidation of primary and secondary alcohols to aldehydes and ketones. No mention was made of any attempts to oxidize aromatic alcohols to quinones. In the present work, pyridinium chlorochromate was used successfully to oxidize 1,5-naphthalenediol to juglone:



Although the yields obtained were low, the method lends itself to large scale preparation.

a. Preparation of pyridinium chlorochromate To 92 ml. of concentrated (12 M) hydrochloric acid was added 92 ml of deionized water. The solution was stirred with a magnetically driven stirring bar and 100 g (1 mole) of chromium trioxide was added rapidly. After five minutes, the homogeneous solution was placed in an ice bath and cooled to 0°. To this solution was carefully added 79.1 g (1 mole) of pyridine, the addition being made from a dropping funnel over an interval of ten minutes. The reaction mixture was again cooled to 0°. The yellow-orange solid which had formed was filtered on a sintered glass funnel and dried in a vacuum oven at 65^0 for one hour. The yield obtained was 79 per cent or 170 g.

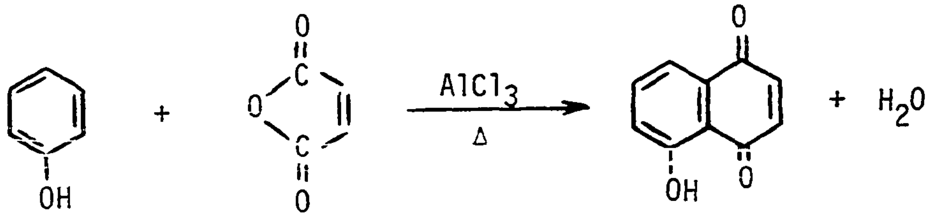
b. Preparation of juglone In a 2-liter round-bottomed flask fitted with a reflux condenser, mechanical stirrer and thermometer was suspended 108 g (0.5 mole) of pyridinium chlorochromate in 600 ml of anhydrous methylene chloride. An amount of 26.72 g (0.167 mole) of 1,5-naphthalenediol in 200 ml of methylene chloride was added to the stirred solution. The reaction mixture darkened and became warm to the touch. After 1.5 hours, 200 ml of anhydrous diethyl ether was added. The solution was agitated and the yellow supernatant layer was decanted from the black gummy residue. The insoluble residue was washed several times with 50 ml portions of anhydrous ether. The combined organic solutions were passed through a column packed with Florisil. The solvent was removed by distillation. The amount of juglone obtained was 2 g, a 7 per cent yield.

c. Results and discussion The synthesis of pyridinium chlorochromate is easy and inexpensive. The compound was found to be stable upon prolonged exposure to air and not appreciably hygroscopic. The chlorochromate ion is stable because chromium(VI), though a powerful oxidizing agent, is slow in oxidizing chloride to free chlorine (12).

Chromium(VI), in the form of the pyridinium salt, is milder in action than potassium dichromate in sulfuric acid solution. Unfortunately, as in the potassium dichromate-sulfuric acid oxidation, the yields were low and tarry material was produced. The small increase in yield, 2 per cent, is not significant enough in itself to recommend pyridinium chlorochromate as a reagent over potassium dichromate-sulfuric acid, but it is more suited to the large scale preparation of juglone. The molar concentration of 1,5-naphthalenediol in the pyridinium chlorochromate oxidation is 0.21 M whereas, in the potassium dichromate-sulfuric acid oxidation the concentration of the phenol is usually less than 0.1 M. Less time is required too, 1.5 hours in contrast to four hours or longer (13). In both methods, the recovery process is similar and involves the same steps of filtration and extraction. For the preparation of large quantities (in excess of 5 g) of juglone, the pyridinium chlorochromate oxidation is the most satisfactory available.

2. Synthesis of juglone from phenol and maleic anhydride

The route proposed is the condensation of phenol with maleic anhydride:



Such a method was used by Cooke and Dowd (14) for the synthesis of 7-methyl-8-chlorojuglone from 4-chloro-3-methylphenol and maleic anhydride, aluminum chloride being used as the condensing agent.

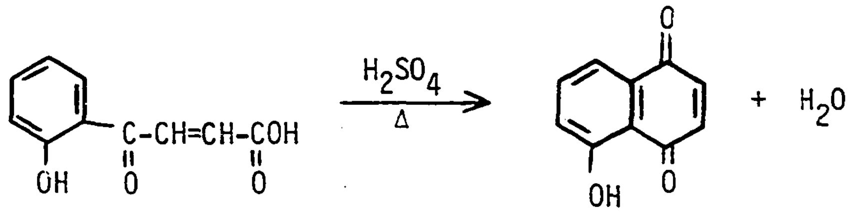
a. Condensation in an aluminum chloride melt Using a mortar and pestle, 24.0 g of maleic anhydride, 8 g of phenol, 50 g of sodium chloride and 240 g of anhydrous aluminum chloride were thoroughly blended. The mixture was heated with stirring in a 1-liter beaker on a hot plate until a melt was obtained. The reaction was heated for an additional thirty minutes and was then quenched with a hydrochloric acid ice mixture. The precipitate produced was filtered off, dried and placed in a Soxhlet extractor with Skelly A as the solvent. No juglone was extracted.

b. Condensation in carbon disulfide A mixture of 50 g of sodium chloride and 240 g of anhydrous aluminum chloride was thoroughly blended and dissolved in 100 ml of carbon disulfide. To this solution 8 g of phenol and 4 g of maleic anhydride was carefully added. The reaction was heated for several hours on a steam bath until most of the excess solvent was evaporated. The solution was treated with concentrated sulfuric acid and boiled for one hour, adding more sulfuric acid as needed. No product could be isolated from the reaction either by precipitation or by extraction.

c. Condensation of maleic anhydride with p-cresol A mixture of 8 g of maleic anhydride and 4.6 g of p-cresol was carefully added to a melt consisting of 240 g of anhydrous aluminum chloride and 50 g of sodium chloride. Upon addition, the reaction temperature reached 180-200^{0} and was cooled with stirring. The reaction was quenched by the addition of a hydrochloric acid and ice mixture. After standing several hours, the precipitate was filtered and placed in a Soxhlet extractor and extracted with Skelly A. No product was obtained.

d. Results and discussion The synthesis of juglone using the maleic anhydride condensation with phenol failed regardless of the experimental conditions used.

In the condensation using only aluminum chloride, a temperature of at least 120^0 was reached before the reagents formed a melt. The reaction was very exothermic and often the temperature reached 180^0. No juglone was produced, perhaps, simply because at such high temperatures juglone is decomposed; decomposition at 150^0 is observed when taking a melting point of pure juglone. To overcome this difficulty carbon disulfide was used as an inert solvent, all the reactants being soluble in it. Under these milder conditions no juglone was again produced, but it was thought the free acid may have formed. Concentrated sulfuric acid was added as a dehydrating agent and the contents of the flask heated to boiling in order to remove a molecule of water and close the ring:



No juglone was formed.

The maleic anhydride condensation proceeds well with chlorophenols, but no product is formed when phenol is used. Initially the problem was though to be due to the absence of a chloro group. However, Bogert and Ritter (15) reported that maleic anhydride and benzene condensed in an anhydrous aluminum chloride melt to give trans-benzoylacrylic acid. The compound did not further condense to give the naphthoquinone but rather dehydrated to the lactone.

Another problem with the phenol condensation is that in phenol the para position is not blocked and substitution can occur in both the ortho and para positions. If the para position is preferentially substituted, no juglone of course will form. To overcome this difficulty the condensation was carried out using p-cresol, the methyl group blocking the position para to the phenolic group. Again, no product was isolated. E. Gomez (16) has reported that no maleic anhydride condensation occurs with phenol, p-cresol or hydroquinone.

The synthesis of juglone from phenol and maleic anhydride for the production of large amounts is attractive. Currently the author is of the opinion that if the proper catalyst were known, the procedure might be successful. Some initial work has been tried using various metal catalysts, but to date no success has been achieved.

III. FORMATION OF JUGLONE GREEN

> *. . . more is owing to what we call chance, that is, philosophically speaking, to the observation of events arising from unknown causes, than to any proper design, or pre-conceived theory —*
>
> *Joseph Priestley.*

A. Initial Observations

The isolation of juglone from the tarry material produced during the oxidation of 1,5-naphthalenediol is cumbersome. The tarry material was filtered and dried, then placed in a Soxhlet extractor, and the juglone extracted using light petroleum ether, Skelly A. Approximately 24 hours is required for 3 g of solid material. The solvent is then removed by evaporation and the crude juglone purified by steam distillation or sublimation.

In the course of such recovery, or "clean-up", operations, a green insoluble substance was observed on numerous occasions. It was formed in the boiling flask of the Soxhlet extractor, on the walls of the flask above the boiling liquid, presumably where the glass was heated somewhat above the temperature of the solvent. It was formed also during steam distillation of juglone, appearing first after approximately half of the juglone had been distilled over. The green substance obviously came from the juglone as no further juglone came over with the steam. An appreciable amount of the green substance also formed during the sublimation of juglone, which sublimes readily at 70^{0}.

This green material, which bears a striking resemblance to quinhydrone, was obtained repeatedly during the course of the work for my M.S. thesis and in my thesis (4) given the common name *juglone green.* The resemblance of juglone green to quinhydrone proved to be more than superficial, in fact the more juglone green was examined, the more intriguing the compound became, and in the end this entire Ph.D. dissertation was devoted to it.

B. The Background. Survey of the Literature

A search of the literature showed that the green material which I have named juglone green had been observed and investigated by Phipson (17) as long ago as 1869. It was examined in some detail by Mylius (18) in 1885. It was observed again, and commented on briefly, in 1923 by Wheeler, Dawson and McEwen (19).

The work of Mylius is particularly important as use is made of some of his observations in the present thesis. A translation of that portion of his paper (18) which deals with juglone green is given herewith. The translated material occurs in a section of the paper devoted to the oxidation of juglone in neutral or acid mixtures. After describing the unusual resistance of juglone to attack by powerful oxidizing agents, he proceeds:

Translation of Mylius (18, pages 474-475)

> Phipson (Compt. rend. 69, 1372) states that Juglone, which he designates as Reagianin: "It is so slightly stable, that within a few hours extensive decomposition occurs, during which a new substance of acid properties is produced, the exact composition of which is expressed by the formula $C_6H_6O_7$," (Regianic acid). Recent research indicates that spontaneous decomposition of juglone

does not occur; juglone can be stored for years without change. A decomposition does occur, however, as Bernthsen and Semper have confirmed, if juglone is boiled for a time with water. Juglone suspended in water is volatilized in part with the steam, but gradually darkens and finally yields an amorphous green-brown mass which is completely insoluble in water and almost all other inert solvents. This product has neither a melting point nor can it be converted easily into a crystalline product. There is thus, no criterion to prove that the material is a true compound; on the other hand there is no basis for assuming that it is a mixture, particularly since a product of the same external appearance is obtained on repeated preparation. It appeared to me that the substance is identical with the Regianic acid of Phipson, and that an analysis should be made to establish the degree of correctness of the formula proposed by Phipson. The substance was filtered off and freed of a small amount of residual juglone by extraction with ether, then dried at 100° and burned. There was obtained

	I	II
C	65.07	65.63
H	3.14	3.21

Because the substance in any case still contains the nucleus of juglone and assuming that the material is a single compound, on the basis of the analysis, the formula is $C_{20}H_{10}O_7$, which requires the following values; the figures corresponding to the Phipson formula are also given:

$C_{20}H_{10}O_7$			$C_6H_6O_7$		
C_{20}	240	66.29	C_6	72	37.89
H_{10}	10	2.77	H_6	6	3.16
O_7	112	30.94	O_7	112	58.95

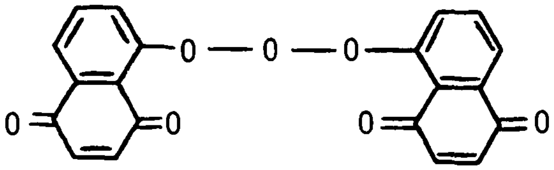
It follows from these numbers, that the amorphous substance formed by the spontaneous decomposition of juglone on boiling with water does not have the composition assigned it by Phipson; also that his formula is irrational because it expresses no relationship to juglone. Inasmuch as the substance may be considered to be $C_{20}H_{10}O_7$, it must be thought of as having been produced by the union of two molecules of juglone with the expulsion of two atoms of hydrogen and the uptake of one atom of oxygen.

$$2C_{10}H_6O_3 + 2O = C_{20}H_{10}O_7 + H_2O$$

The properties of the substance are the following: It forms a green-brown powder without odor or taste, which on strong heating, without melting carbonizes and moreover shows the properties of a phenol. It dissolves in alkaline solutions forming a deep violet color; the color is not changed by air as is that of juglone. The compound with alkali may be obtained as a violet amorphous powder by precipitation with alcohol. The material obtained this way and dries at 100° yielded on analysis 15.35 per cent sodium, calculated for the compound $C_{20}H_{10}O_7Na_3$ 16.35 per cent. Therefore, it appears that the substance carries three hydroxyls. I might add further that the substance dissolves in concentrated sulfuric acid forming a red color and that the violet solution in alkali is decolorized by stannous chloride, a property which characterizes the substance as a quinone.

In the same journal and only a few pages in advance of the Mylius paper containing the section on juglone green for which translation is given above, is the paper of Bernsthen and Semper (20, 21) referred to by Mylius. Bernthsen and Semper observed juglone green on several occasions while working with juglone. They simply comment that juglone is decomposed in hot water, hot alcohol, in wet ether, and on steam distillation and sublimation with the formation of dirty green-brown material. Dissolution in hot, concentrated acids, particularly hydrochloric acid, also caused the formation of this "decomposition product".

The report of Wheeler, Dawson and McEwen (19) on juglone green is brief. In the course of a study of the chlorination of juglone by the action of chlorine on juglone dissolved in glacial acetic acid, in a single instance juglone green was formed. They commented on their surprise but went little further. They were aware of the work of Mylius, adopted his empirical formula, and attributed to Mylius the peroxide structure for juglone green.



Although I found only one other mention of juglone green in the literature, and that one casual and incidental to other work, it is very probable that juglone green has been obtained on numerous occasions by workers with juglone and simply discarded as a nuisance.

C. The Precise Conditions Under which Juglone Green is Formed

It was apparent from the work of Mylius and my own initial observations that juglone green was formed by some change in juglone, possibly a disproportionation. In a series of experiments, the conditions under which the transformation occurs were established more firmly. The results are given in the following statements; appropriate information is given with each statement to indicate how each individual experiment was carried out.

1. Once juglone green was formed during a steam distillation of juglone, no further juglone is carried over by the steam, indicating that juglone and juglone green are not components of some simple equilibrium system.

2. Oxygen is not involved in the transformation. Purging water and vessel prior to steam distillation with nitrogen did not prevent the formation of juglone green.

3. The rate of conversion of juglone to juglone green during steam distillation depends on the pH of the solution, being inhibited by

decreasing pH but very rapid above pH 8. This was determined by preparing a series of McIlvaine buffers (chosen because they are all composed of the same materials), pH 3 to 8, adding 30 mg. of pure, sublimed juglone to each, steam distilling each solution for the same length of time, and weighing the juglone green produced and the juglone remaining. A plot of the results is given in Figure 1.

4. During sublimation, juglone is converted in part to juglone green. This occurs both in the presence and the absence of air. Water is not involved in the conversion.

In one experiment, the juglone was placed in a large evaporating dish resting in an electric heating mantel. The material was covered with a large perforated filter paper and an inverted glass funnel placed over the contents. A thermometer was suspended through the stem of the funnel and a coil of lead tubing was wrapped around the outside of the funnel. A stream of cold water was passed through the tubing and the temperature of the material in the dish was raised to 70^{0}. The sublimed juglone was collected on the funnel. During the sublimation, juglone green formed in the evaporating dish. No moisture condensed on the inverted funnel during the sublimation.

To confirm that juglone green is formed in the absence of oxygen, juglone was heated in an evacuated test tube. Initially, the juglone formed yellow crystals on the upper walls of the tube. Then a green vapor was observed in the tube and the juglone was converted to juglone green. The juglone green particles collected at the bottom of

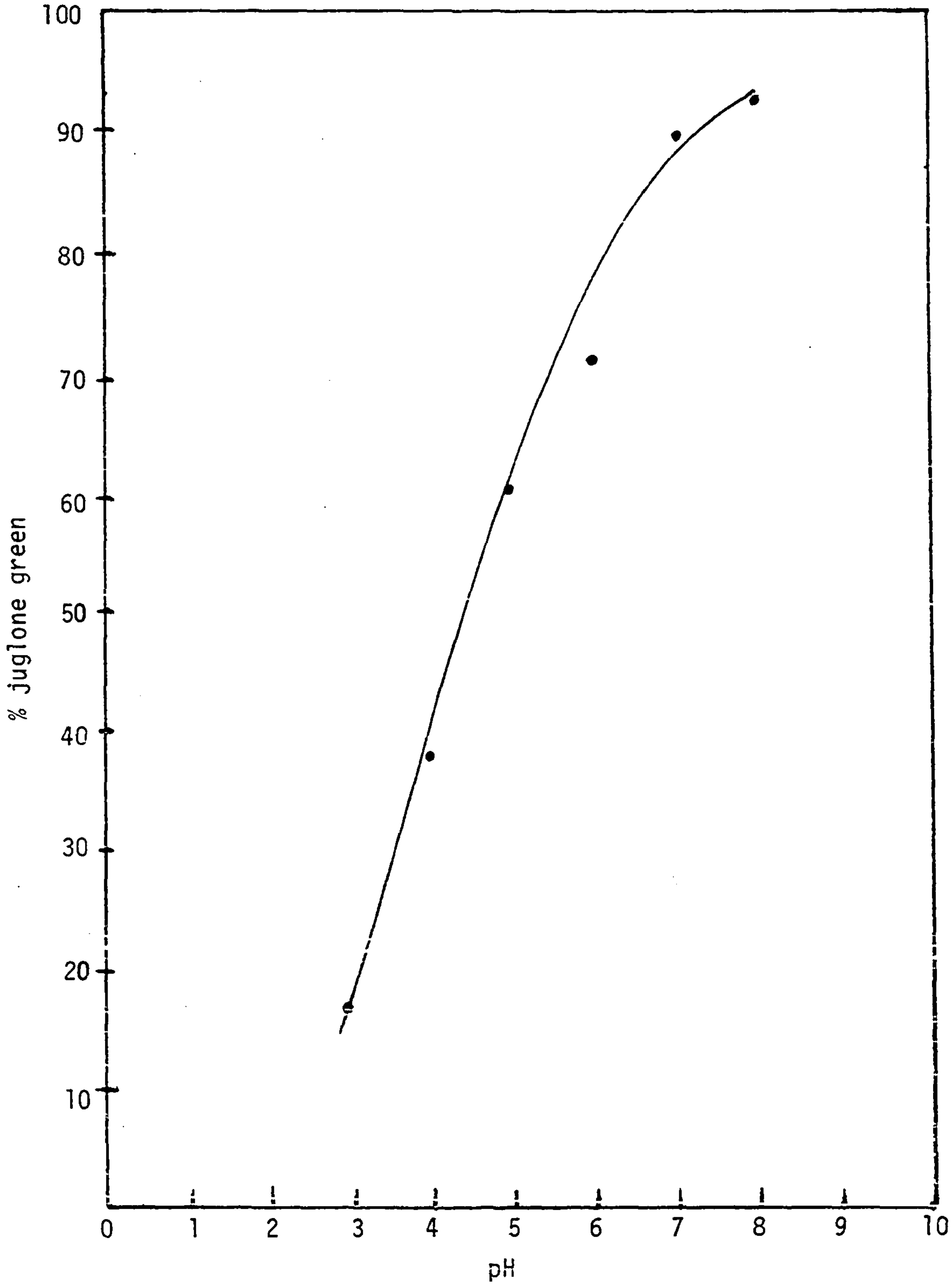


Figure 1. Effect of pH in the conversion of juglone to juglone green by steam distillation

the tube, no crystals were observed. No moisture condensed on the cold upper walls of the condenser during this experiment.

5. In the controlled anodic oxidation of 1,5-naphthalenediol to juglone, juglone green was produced.

The synthesis of juglone was attempted by oxidizing 1,5-naphthalenediol at a platinum anode. Papouchado et al. (22) reported the hydroxylation of 1,5-naphthalenediol under voltammetric conditions. The oxidation was carried out in 2 M perchloric acid at a carbon paste electrode. The oxidation peak occurred at 0.54 V vs the s.c.e. In this work, 1,5-naphthalenediol was found not to be sufficiently soluble in perchloric acid. Methanol was used as the solvent with sodium perchlorate as supporting electrolyte. In order for sufficient current to pass in the methanol solution, the potential was adjusted and held at 1.0 V vs the n.c.e. and the oxidation was carried out at a platinum anode. The product of the electrolytic oxidation was primarily juglone green.

D. Composition and Empirical Formula of Juglone Green

A sample of juglone green was submitted to a commercial analytical firm for a determination of the carbon and hydrogen content. The results are given in Table 1; the values obtained by Mylius are also given. If the assumption is made that the juglone nucleus remains, the number of carbon atoms must be C_{10}, C_{20}, or C_{30}. As will be shown later, the molecular weight by the Rast method and by mass spectroscopy indicate that the material is a trimer. The empirical formula calculated on the Chemalytics data is $C_{30}H_{17.24}O_{9.46}$ or $C_{28.53}H_{16.41}O_{9}$, not

Table 1. Composition of juglone green.

	Found by Mylius	Found by Chemalytics	$C_{20}H_{10}O_7$ Mol. Wt. 362	$C_{30}H_{18}O_9$ Mol. Wt. 522.5	$C_{30}H_{17}O_9$ Mol. Wt. 521.5	$C_{30}H_{16}O_9$ Mol. Wt. 520.5
C	65.35	67.95, 68.28 Average 68.115	66.30	68.97	69.10	69.23
H	3.18	3.36, 3.21 Average 3.285	2.76	3.47	3.29	3.10
O[a]	31.47	28.69, 28.51 Average 28.60	33.15	27.56	27.61	27.67

[a] By difference.

sufficiently clean-cut to make a positive choice between $C_{30}H_{18}O_9$, $C_{30}H_{17}O_9$ and $C_{30}H_{16}O_9$.

As stated by Mylius and confirmed by me, juglone green does not melt and carbonizes above 300°. The difficulty of burning carbon is well known and incomplete combustion quite probably occurred during the Mylius analysis in which the combustion tube was undoubtedly heated by a gas flame, and quite likely also in the Chemalytics determination which was probably carried out at a fixed and too rapid rate by the mechanical movement of an electric heating element past the combustion zone of the combustion tube.

The hydrogen values of both Mylius and Chemalytics are probably very reliable. They agree best with $C_{30}H_{17}O_9$. On the other hand, the titration data (reported later in this dissertation) indicates a molecular weight of 520 and the stoichiometry postulated for the disproportionation of juglone and other evidence is best explained by $C_{30}H_{16}O_9$.

IV. ELECTROCHEMICAL BEHAVIOR OF JUGLONE GREEN

Oxidations and their reversal, reductions, are fundamental and most important reactions in all branches of chemistry, but especially in biochemistry — L. Michaelis.

A. Introduction

Physically, juglone green is a dark-green, iridescent solid; it is insoluble in all of the common solvents. In these respects, it has an astonishing resemblance to quinhydrone. The unique color of these compounds provides a key to their constitution. The dark-green color of quinhydrone is radically different from the colorless hydroquinone and the yellow colored quinone, one molecule of each which combine to form the "molecular compound", quinhydrone. Because of the dark-green iridescent appearance, it appeared likely that juglone green also was a molecular compound, formed by the union of oxidized and reduced forms produced by the disproportionation of juglone.

The formation of dark and intense colors by the union of the oxidized and reduced forms of an oxidation-reduction couple is not unique to quinone-hydroquinone couples; Diehl observed examples among inorganic compounds. Cuprous chloride in concentrated hydrochloric acid is colorless, cupric chloride in the same solvent is light green; when mixed, a dark brown to black solution results (23). Similarly, a solution of antimony trichloride in hydrochloric acid is colorless, antimony pentachloride in the same solvent is pale yellow. On mixing the two an intense red-brown color develops (24). The iron oxides are another example. Hematite, the ferric oxide (Fe_2O_3) is red, ferrous oxide (FeO) gray, magnetite, a mixture of the two having the chemical composition Fe_3O_4, (the iron being in two valence states in the compound) is black.

The response of the electrode indicates the reduction potential of a solution saturated with it to pH. The quinhydrone electrode, of course, has an honorable place in the history of chemistry, for it was used for three decades in just this manner as the preferred method of measuring pH, having only been displaced finally by the superior glass electrode. A direct check as to whether juglone green is an intermediate "molecular compound" analogous to quinhydrone is to measure the reduction potential of a solution saturated with it to pH. The result indicated the reduction potentials of solutions saturated with juglone green do depend on pH, but in a manner so different from quinhydrone as to excite still further research.

B. Experimental Work

That juglone green did function like quinhydrone was shown by direct experiment, the success and surprising results of which dictated further experiments of increasing sophistication. Parallel experiments were made with quinhydrone as checks.

1. The reduction potential of juglone green as a function of pH

A series of Clark and Lubs buffers were prepared covering the pH range 3 to 10. A buffer of pH 11 was also prepared using disodium hydrogen phosphate plus sodium hydroxide. All measurements of hydrogen ion concentrations were made with a Beckman Zeromatic SS-3 pH meter equipped with a Beckman glass electrode and a saturated calomel reference electrode (s.c.e.). The meter was calibrated against two of the standard buffers of the National Bureau of Standards: potassium acid phthalate, pH 4.01; and, potassium dihydrogen phosphate plus disodium hydrogen phosphate, pH 6.86.

The buffer solutions were saturated with juglone green and allowed to equilibrate for 24 hours. The potential of each solution was measured with a bright platinum foil electrode and an s.c.e. This same experiment was repeated using quinhydrone. The results are shown in Figures 2 and 3.

2. Reduction potential in the presence and absence of oxygen

It became of interest to learn if the reduction potential was affected by oxygen. A buffer of pH 7 was saturated with juglone green and the reduction potential measured. Nitrogen gas was then bubbled for 15 minutes through the solution. A shift in the reduction potential of 50 mV in the negative direction resulted. Next, air was bubbled through the solution; this caused the potential to return to the initial value. Oxygen gas was then bubbled through the solution for 15 minutes; the potential now being shifted 30 mV in the positive direction.

3. Reduction potential in buffers of constant ionic strength

In another experiment, measurements with juglone green and quinhydrone were repeated with a series of buffers so prepared as to be of constant ionic strength. McIlvaine buffers were used because, although they cover a wide range of pH, they are composed of the

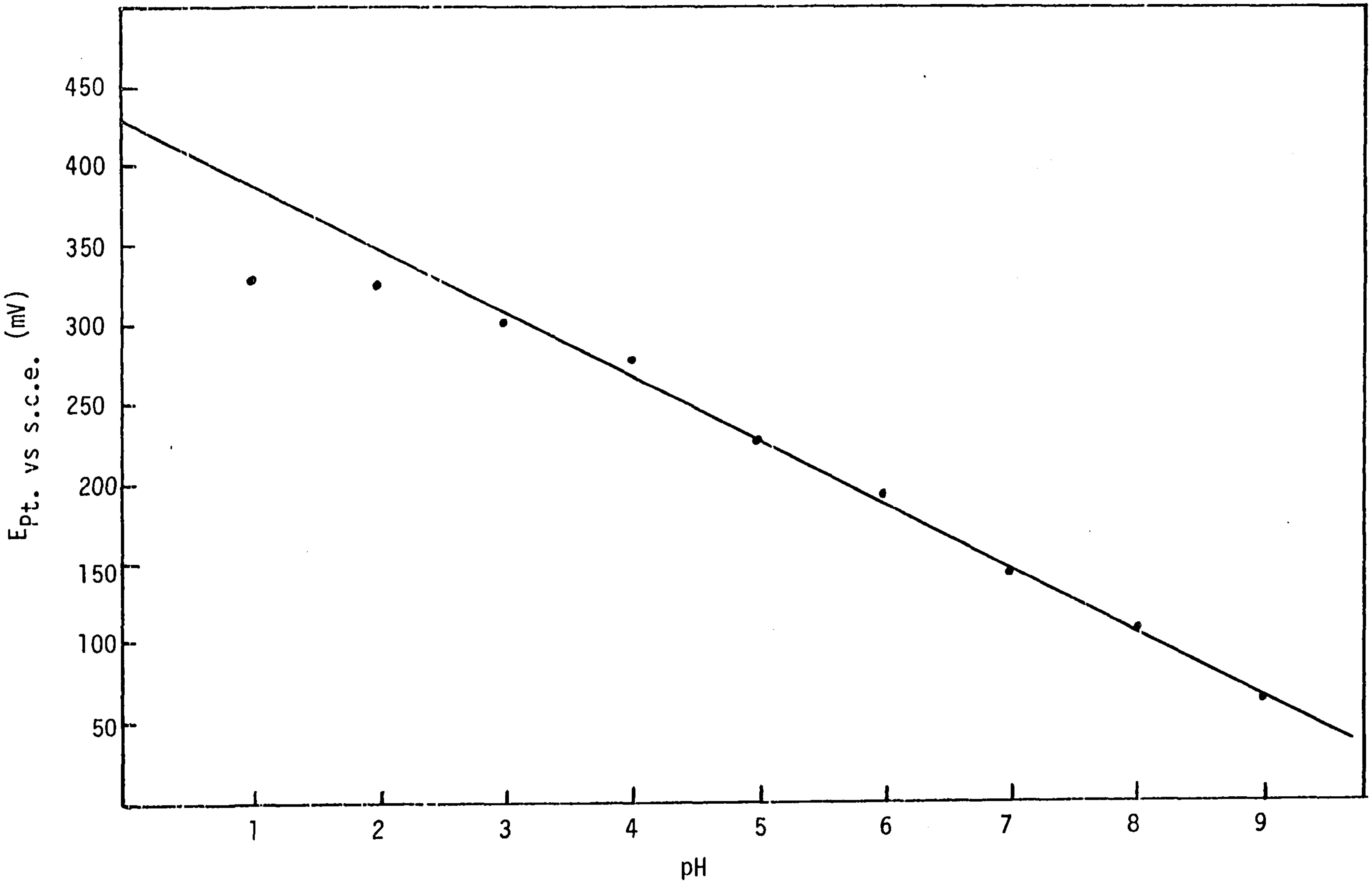


Figure 2. Reduction potential of buffer solutions saturated with juglone green and measured as a function of pH.

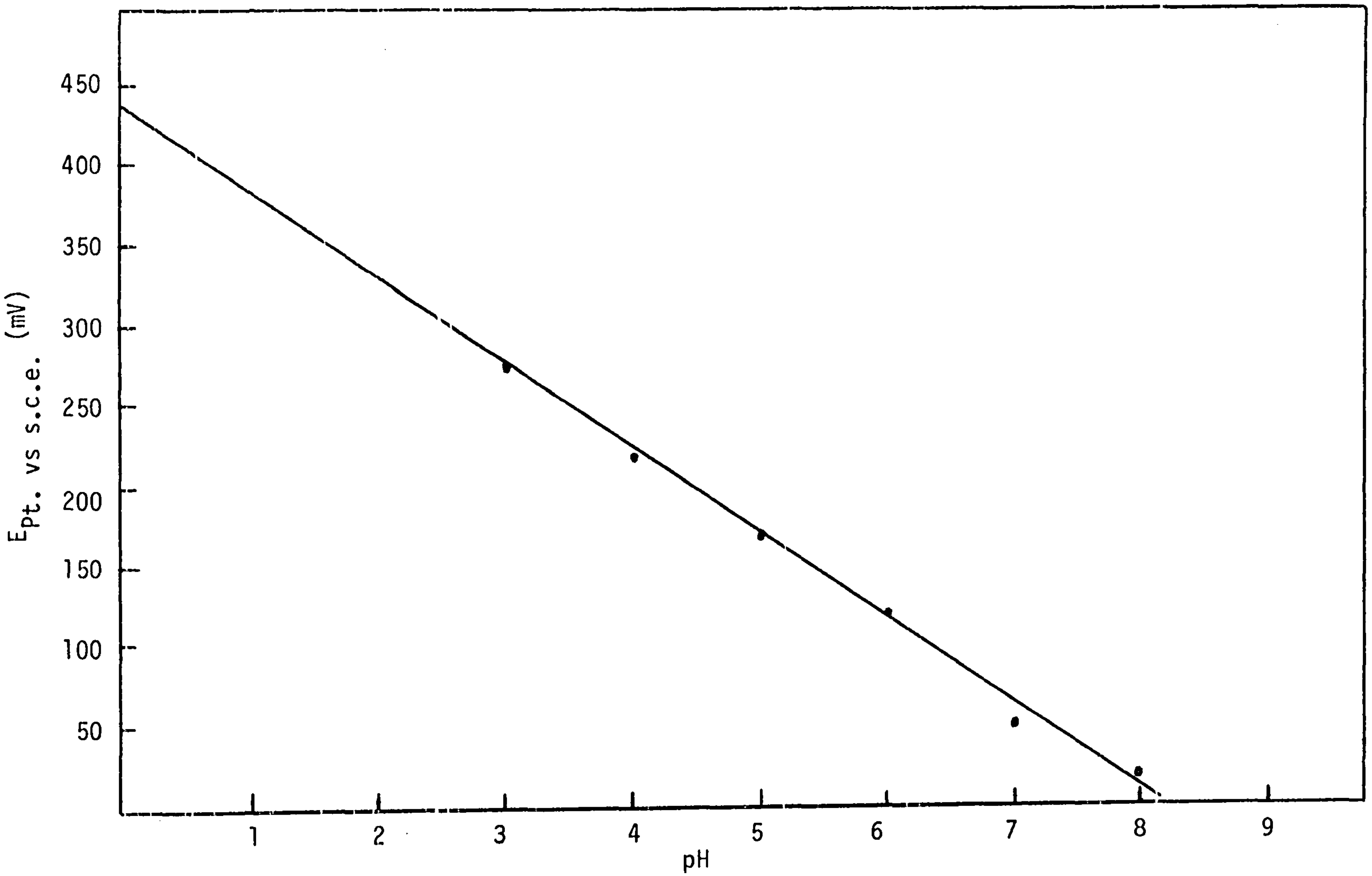


Figure 3. Reduction potential of buffer solutions saturated with quinhydrone and measured as a function of pH.

same two constituents, disodium hydrogen phosphate and citric acid. The buffers were prepared according to Elving et al. (25); the ionic strength was 1.0 M. The buffers were purged with nitrogen gas for 5 minutes, juglone green (or quinhydrone) was added, the mixture was allowed to equilibrate while under a nitrogen atmosphere, and the potential was then measured.

4. Reduction potential measured with iodized platinum foil

In still another experiment, a modified platinum foil electrode was used, the experiment being identical in other respects to that in the preceding paragraph. Before use, the platinum foil electrode was cleaned and then dipped into a solution of sodium iodide. The measurements were made with both juglone green and quinhydrone. The results are shown in Figures 4 and 5.

C. Results and Discussion

A review of the theory of the quinhydrone electrode is first presented inasmuch as a knowledge of it is essential to an interpretation of the measurements just described.

1. Theory of the quinhydrone electrode

The quinhydrone electrode, used for the measurement of pH, is based on the reversible, oxidation-reduction couple, quinone-hydroquinone:

$$\underset{(Q)}{C_6H_4O_2} + 2H^+ + 2e^- = \underset{(H_2Q)}{C_6H_6O_2} \qquad (1)$$

The electrode potential based on the Nernst equation is:

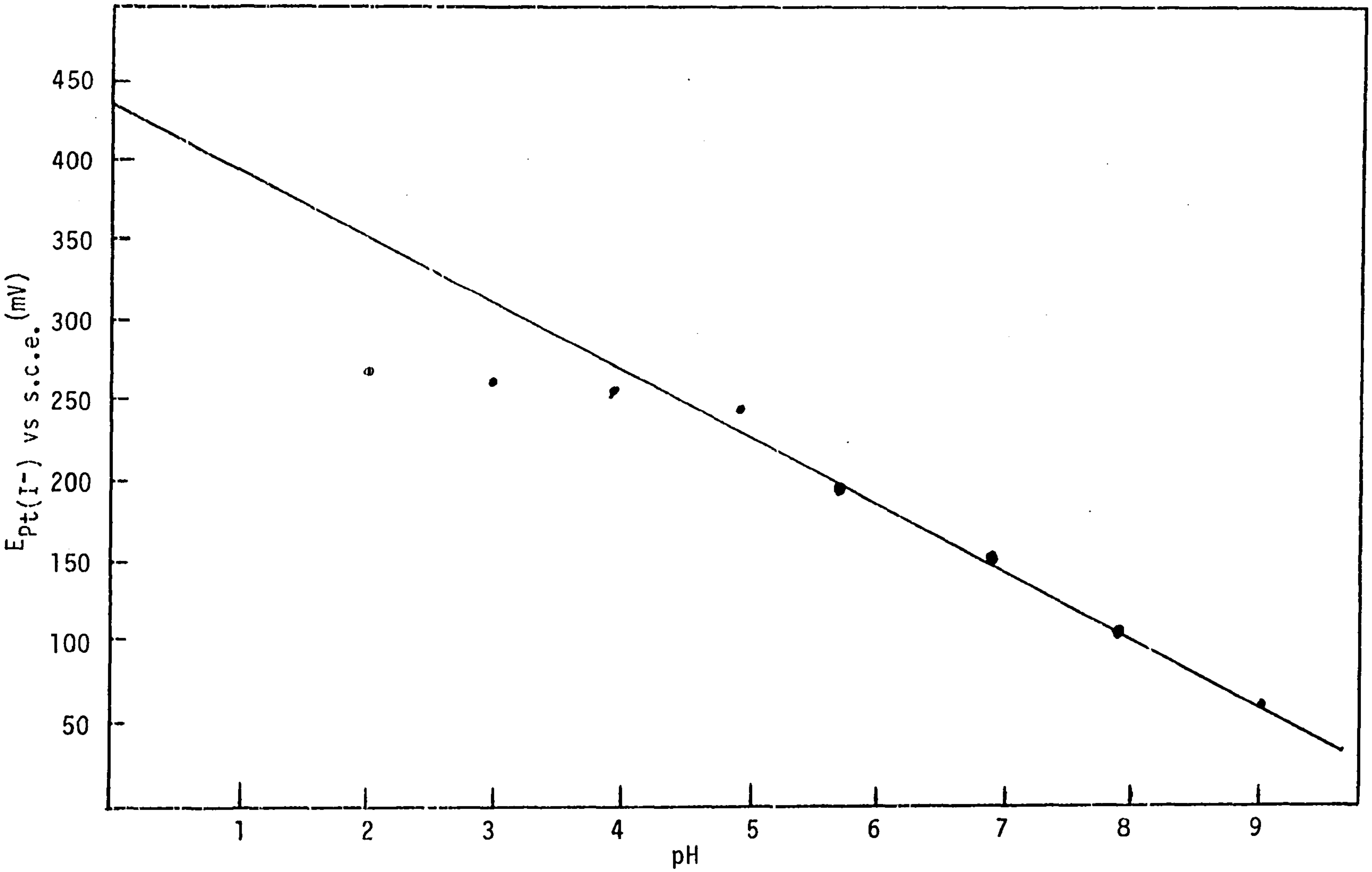


Figure 4. Reduction potential of buffer solutions of constant ionic strength saturated with juglone green and measured as a function of pH using an iodide coated platinum electrode.

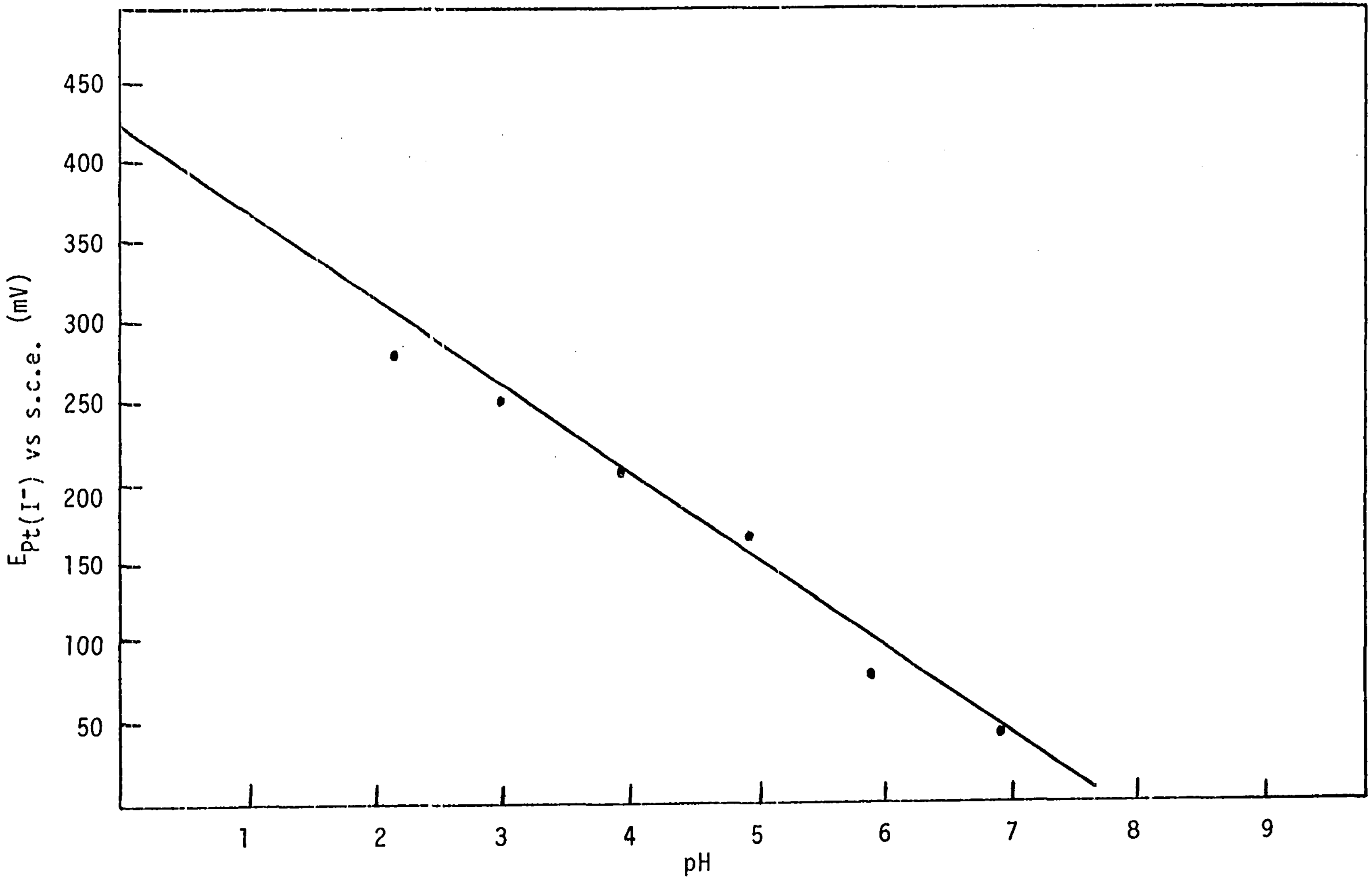


Figure 5. Reduction potential of buffer solutions of constant ionic strength saturated with quinhydrone and measured as a function of pH using an iodide coated platinum electrode.

$$E = E^o_{Q,H_2Q} + \frac{2.303RT}{2F} \log \frac{[Q][H^+]^2}{[H_2Q]} \qquad (2)$$

in which E^o_{Q,H_2Q} is the standard reduction potential of the quinone-hydroquinone couple, R is the gas constant, T is the temperature and F is the faraday. Inserting the numerical values for R and F and $22^o + 273^o$ for T, the temperature at which the experiment is usually performed, gives for equation (1)

$$E = E^o_{Q,H_2Q} + \frac{0.057}{2} \log \frac{[H^+]^2[Q]}{[H_2Q]} \qquad (3)$$

Separating out the H^+ term yields

$$E = E^o_{Q,H_2Q} + \frac{0.057}{2} \log \frac{[Q]}{[H_2Q]} + \frac{0.057}{2} \log [H^+]^2 \qquad (4)$$

Quinydrone is a "molecular compound" consisting on one molecule of hydroquinone and one molecule of quinone. If the solution is saturated with quinhydrone, the solution will contain fixed and equal concentrations of quinone and hydroquinone, the second term of equation (4) thus becomes zero and the constant term is designated as the standard reduction potential of the quinhydrone electrode:

$$E = E^o_{Quinhydrone} + (0.057) \log [H^+] \qquad (5)$$

Rewriting this equation in terms of pH:

$$E = E^o_{Quinhydrone} - (0.057)pH \qquad (6)$$

As can be seen from equation (6), the potential developed depends only on the hydrogen ion concentration, and the quinhydrone electrode is thus an indicator electrode for hydrogen ions. The electrode consists of a platinum foil dipping into a solution to which has been added a bit of solid quinhydrone. For the measurement of pH a reference electrode is inserted and the potential between the platinum foil and the reference electrode is measured. For an s.c.e. the potential of the cell and the pH are related by:

$$E_{\text{Cell}} = E_{\text{Quinhydrone}} - E_{\text{s.c.e.}} \tag{7}$$

$$E_{\text{Cell}} = E^{o}_{\text{Quinhydrone}} - 0.057\text{pH} - E_{\text{s.c.e.}} \tag{8}$$

Referring to Figure 3 where E_{Cell} is plotted versus pH, the slope was calculated to be -54 mV. The intercept obtained by extrapolation is 0.442 V versus the s.c.e., the literature value being 0.453 V versus the s.c.e. (26).

2. The juglone green electrode

As shown in Figure 2, the reduction potential of buffer solutions saturated with juglone green, as measured by the potential difference between a bright platinum foil and an s.c.e. is linear with pH over the region pH 4 to 10. The slope of this line however, is -40 mV per pH unit (as found by least squares treatment of the data), quite different from the -54 mV per pH unit of quinhydrone (Figure 3). This value is surprising, inasmuch as the slope of the reduction potentials of oxidation-reduction couples in general is given usually by -(2.303) RT/nF, n being 1, 2, or 3, the numerical values at 25^o being respectively -0.05916, -0.02958 and -0.01972 mV per pH unit.

The concentration of the hydrogen ion, of course, enters and as observed in the discussion of the quinhydrone electrode given above, two hydrogen ions and two electrons enter the chemical reaction and in the theoretical treatment the numbers cancel (equation (4)). To explain the slope found for juglone green, it is necessary to postulate that three electrons are involved for each two hydrogen ions:

$$Ox + 2H^+ + 3e^- = Red \qquad (9)$$

Writing this expression in terms of the Nernst equation at 25^o:

$$E = E^o_{Ox,\ Red} + \frac{0.0591}{3} \log \frac{[OX]}{[Red]} + \frac{0.0591}{3} \log [H^+]^2 \qquad (10)$$

Because the solution is saturated, the concentrations of the oxidized and the reduced species remain constant. Equation (10) would simplify to:

$$E = E^{o'}_{Ox,Red} + \frac{0.0591}{3} \log [H^+]^2 \qquad (11)$$

$$\text{where} \quad E^{o'}_{Ox,Red} = E^o_{Ox,Red} + \frac{0.0591}{3} \log \frac{[Ox]}{[Red]} \qquad (12)$$

Rewriting equation (11) in terms of pH:

$$E = E^{o'}_{Ox,Red} - (39.4)pH \qquad (13)$$

Thus, the slope of the plot of reduction potential versus pH should be -39 mV per pH unit.

Equation (9) was actually, of course, deduced from equation (13) by reasoning in reverse of the order in which just presented. Use is made of this deduction in Chapter IX in establishing the constitution of juglone green, that is, that the oxidized and reduced species present in the molecule of juglone green are interrelated by

$$(\text{Juglone Green, Ox}) + 2H^+ + 3e^- = (\text{Juglone Green, Red}) \qquad (14)$$

As will be seen later, this relationship posed some difficult problems in working out the stoichiometry of the disproportionation of juglone.

The standard reduction potential of the juglone green electrode, obtained by extrapolating the line of Figure 2 back to pH = 0, is

$$E^o_{\text{Juglone Green}} = 0.429 \text{ V vs s.c.e.} \qquad (15)$$

$$E^o_{\text{Juglone Green}} = 0.675 \text{ V vs n.h.e.} \qquad (16)$$

3. Further observations

During the measurements of the potentials of buffers saturated with juglone green, a slow drift in the potential was frequently observed. Experiment No. 2 described above indicated that oxygen is involved but not responsible for all of the drift experienced.

The shift in the reduction potential of the juglone green electrode on the removal of oxygen and in the positive direction on the addition of oxygen prompted experiments to learn if juglone green functioned as an oxygen electrode. Long equilibration times were required and no reproducible data were obtained.

Recent research (27) has shown that platinum metal is not as inert as once believed and that electrode reactions at a platinum surface are often affected by the presence on the surface of platinum oxide and layers of adsorbed anions. In Experiment 4, above, a layer of iodide was placed on the electrode and the reduction potentials of the quinhydrone and juglone green electrodes in a series of buffers measured. The results are shown in Figures 4 and 5. The potential for juglone green was again linear with pH but only over a limited region, pH 6 to 9. The slope was calculated to be -37 mV with an intercept obtained by extrapolation of 0.435 V vs the s.c.e. or 0.681 V vs the n.h.e. For quinhydrone a slope of -58 mV was calculated with an intercept of 0.440 V vs the s.c.e.; 0.686 V vs the n.h.e.

The results were identical with those obtained with the untreated electrode, Experiment 3, the scatter in the data being slightly greater with the treated electrode, although equilibration times were shortened somewhat using the treated electrode.

As will be shown later in this dissertation, juglone green is a radical, probably a biradical, and titration of the unpaired electrons with standard acid has been effected. The drift in the reduction potential of the juglone green electrode below pH 6 is probably caused by the dissolution of the juglone green by attack by the hydrogen ion.

V. THE MOLECULAR WEIGHT AND CERTAIN CHEMICAL BEHAVIOR OF JUGLONE GREEN

> *"No data," Holmes answered. "It is a capital mistake to theorize before you have all the evidence. It biases the judgement —*
>
> *A. Conan Doyle.*

The molecular weight of juglone green was determined by three different methods: mass spectrometry, depression of the freezing point of camphor, and calculation from the equivalent weight obtained by titration with standard acid.

A. Molecular Weight by Mass Spectrometry. Fragmentation of a "Molecular Compound"

In the modern practice of organic chemistry, molecular weight is commonly determined from the mass spectrum, the accuracy of the value obtained depending on the quality and calibration of the instrument used; but in any case being better by far than the approximate values obtained by methods based on colligative properties.

Because of the very low volatility of juglone green (no melting point prior to decomposition by carbonization, about 300^{0}), the mass spectrum was obtained only with great difficulty. A satisfactory spectrum was obtained by the so-called "direct insertion probe technique".

The mass spectrum of juglone green was obtained on an Atlas CH-4 mass spectrometer. The spectrum was obtained at 70 eV and 18 eV and at various temperatures of the ion source. For purposes of comparison, the mass spectrum of quinhydrone was also obtained under the same operating conditions.

Assignments of masses in the mass spectrum are reliable to ± 2 mass units.

In the mass spectrum of juglone green prominent peaks were observed at m/e 174, 175 and 176. A significant peak was also observed at m/e 345. Below 90 units of heat the highest mass in the spectrum occurred at m/e 345. To determine if this peak was the molecular ion, the energy of the electron beam was lowered from 70 eV to 18 eV. At the lower energy, the m/e 345 peak did not increase in intensity relative to the other peaks in the spectrum. The m/e 345 ion was not the molecular ion.

When the sample was warmed above 90 units of heat a new peak not previously observed appeared at m/e 520; the relative abundance being 1 per cent. When the energy of the electron beam was lowered from 70 eV to 18 eV this peak had no noticeable change in intensity. The other peaks in the spectrum did decrease in intensity on lowering the energy of the electron beam. The increase in intensity of a peak relative to the other peaks in a spectrum when the energy of the electron beam is lowered is characteristic of a molecular ion. The peak at m/e 520 was interpreted as the molecular ion.

A very small peak was observed at m/e 538 when the ion source was above 90 units of heat. This peak had a relative intensity of less than 1 per cent and did not increase when the energy of the electron beam decreased. The peak appeared in the various spectra of juglone green sporadically. This peak was interpreted as being an artifact probably due to an oil impurity from the instrument since it was only present a few times and only when the ion source temperature was above 200^0.

The parent peak or molecular weight of juglone green was interpreted as being 520 ± 2. Because the molecular ion is the precursor of all other ions in the spectrum it requires the least energy for formation. Therefore, if the energy of the electron beam is lowered from 70 eV to 18 eV the parent peak should be the last one to disappear. The intensity of the m/e 520 peak increased relative to the intensity of the other peaks when the spectrum was run at 18 eV.

The spectrum between the m/e 520 ion and m/e 345 ion shows only a few peaks in low relative abundances (less than 1 per cent). Prominent peaks are observed at m/e 174, 175 and 176. Below m/e 150 many intense peaks are observed.

In conjunction with the data obtained from the carbon hydrogen analysis the peak at m/e 345 is interpreted as being a dimer of the juglone nucleus. The peaks at m/e 174, 175 and 176 can be assigned to cleavage of the 345 dimer and to hydrojuglone (m/e 176). The dimer and hydrojuglone are assumed to be bound together in juglone green, presumably by hydrogen bonding, as in quinhydrone.

To determine if such a hydrogen bonded species should give a parent peak, the mass spectrum of quinhydrone was obtained. A weak parent peak for quinhydrone, less than 1 per cent relative intensity, was observed at m/e 218. Prominent peaks were observed at m/e 110 and 108, corresponding to hydroquinone and quinone respectively. As in the mass spectrum of juglone green, the spectrum of quinhydrone between the parent peak and the base peak (m/e 110) had relatively few peaks, and the peaks which were observed had very low relative abundances.

Thus, the parent peaks of both juglone and quinhydrone are of low intensity and only observed at high ion source temperatures. In both spectra, very few, and those barely discernible, peaks appear between the parent peak and the prominent peaks corresponding to the component parts of the molecular compound. Or reasoning more properly, from the evidence, the general character of the mass spectra of juglone green and quinhydrone makes it likely that juglone green is a molecular compound composed of two units of masses 345 and 175.

B. Molecular Weight by the Rast Method

The Rast method is based on the depression of the melting point of natural d-camphor, a solvent having an unusually large cryoscopic constant, 39.7 per gram molecular weight (28).

1. Procedure

The weight of a small pyrex test tube (8 x 50 mm) was determined. Approximately 35 mg of juglone green was placed in the tube and weighed accurately. To this was added 0.25 g of camphor and the tube was again weighed. The contents of the tube were quickly melted and the solution stirred to insure homogeneity. The liquid solution was poured out on a watch glass where it immediately solidified. The material was then ground in a mortar and pestle and the powder placed in a small pyrex tube. A thermometer was inserted in the tube and the assembly was then placed in an oil bath and gently heated until all the solid had melted. The melting point of the mixture was recorded. The melting point of camphor was determined by filling another tube with pure camphor and heating in an oil bath until all the solid melted. The temperature at which the solution became clear of solid particles was recorded.

2. Results

The molecular weight was calculated by the formula:

$$M = \frac{(40.6)(w)(1000)}{(\Delta)(W)}$$

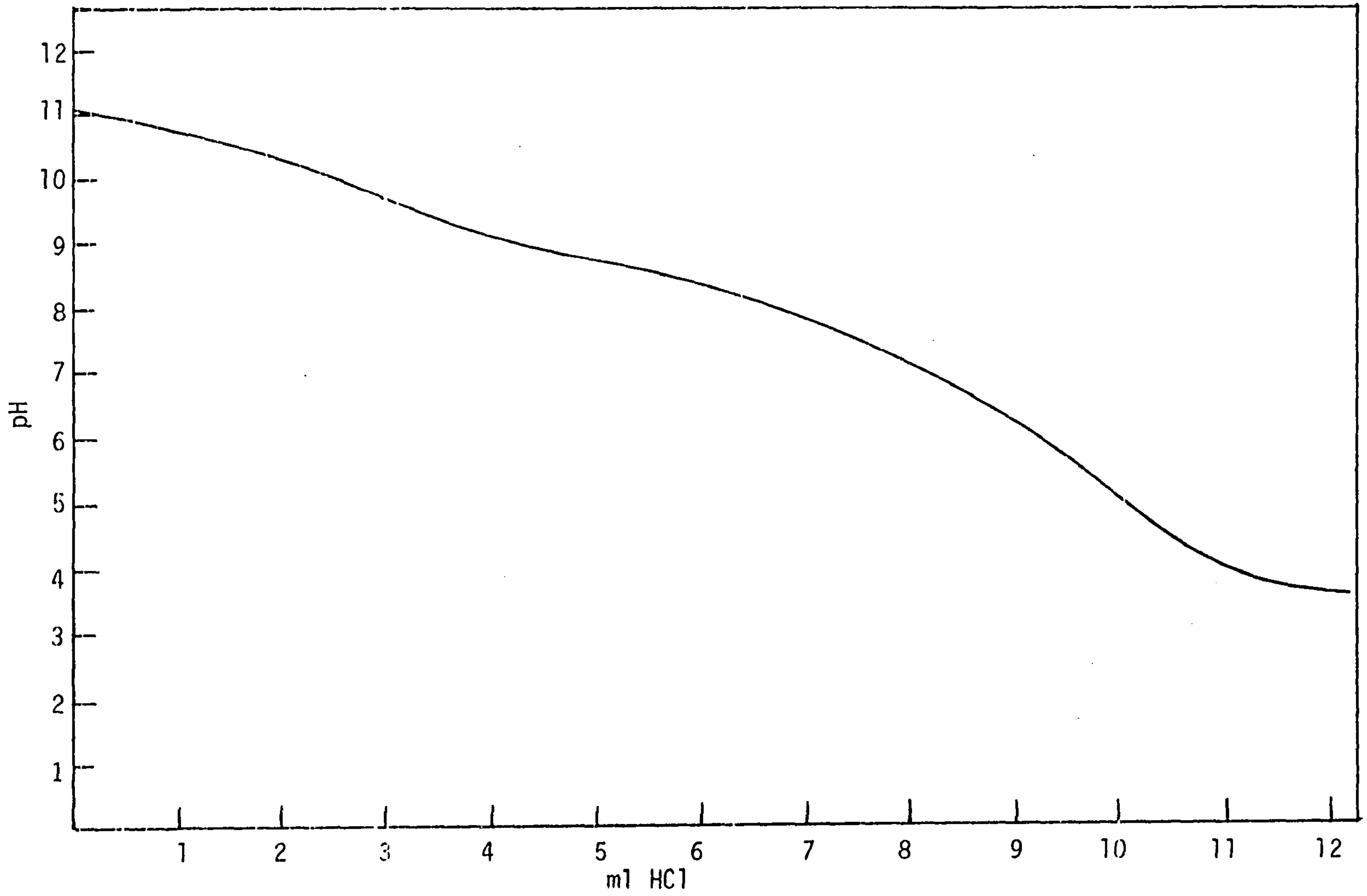
in which M = molecular weight, w = weight of juglone green, W = weight of camphor and Δ = depression in the melting point. The molal depression constant of 40.6 was used instead of 39.7 inasmuch as the molality of juglone green was 0.15. The cryoscopic constant of 39.7 holds only for molalities of 0.2 - 0.5 m. More dilute solutions increase the constant as high as 50 (29). The results of the analysis indicated a molecular weight of 471. From this it appears likely that juglone green is a trimer (molecular weight 520) rather than a dimer (molecular weight 348).

C. Titration of Juglone Green with Standard Acid

Once the molecular weight of a compound has been established approximately by a physical method, an exact value for the molecular weight can sometimes be established by a titration, taking advantage of some acid-base or oxidation-reduction property of the compound and assuming the number of protons or electrons involved in the titration is the obvious integral number.

Juglone green is insoluble in the common solvents, but does dissolve in alkaline solutions. This made it possible to dissolve a weighted sample in an excess of standard alkali and back titrate with standard acid. In carrying out this analysis, the standard sodium hydroxide was first degassed with nitrogen for thirty minutes. The excess sodium hydroxide was back titrated with standard hydrochloric acid as rapidly as possible. The end-point was determined potentiometrically using a glass indicating electrode and a saturated calomel reference electrode. The titration was carried out in a closed vessel with four inlet holes, two for the electrodes, one for the titrant and one for the nitrogen gas. The nitrogen was first passed through a drying tube containing anhydrous calcium chloride and then through a U-tube containing ascarite. A flow of gas in the solution during the titration served to stir the solution. The titration curve obtained is shown in Figure 6.

The titration described above was carried out in the absence of oxygen to preclude any oxidation of the juglone green which is presumed to have one or more phenolic groups in the molecule.



Weight of juglone green: 0.0504 g
Added 10.00 ml of 0.1152 N sodium hydroxide
Normality of hydrochloric acid = 0.0981 N

Figure 6. Titration of juglone green with standard acid after dissolution in excess standard alkali.

The first point of inflection in the curve shown in Figure 6 corresponds to the neutralization of the excess alkali. The second point of inflection corresponds to the end-point in the neutralization of the sodium salt of juglone green. The points of inflection, as determined by independent observers, were located with sufficient precision to provide a value for the equivalent weight 260, with an uncertainty of 1 per cent. The curve indicates that a disodium salt of juglone green was formed and that the molecular weight of juglone green is 520.

The pH at the mid-point of the titration curve is 7.7. This would be an average value for the dissociation constant of two acidic groups, $pK_{A,average} = 7.7$, surprisingly strong for phenolic groups; the uncertainty in this average is 0.5.

D. Oxidation-Reduction Titrations of Juglone Green

Inasmuch as juglone green is soluble in alkali, it appeared feasible to perform oxidation-reduction titrations on it with the standard oxidizing and reducing agents which operate in alkaline solution. Sodium hydrosulfite and potassium ferricyanide were selected as the titrants.

The titration was carried out in 2 M sodium hydroxide, juglone green was added to potassium ferricyanide and the excess potassium ferricyanide was titrated with sodium hydrosulfite (30). The equations describing the reactions are:

$$Fe(CN)_6^{3-} + J.G. = J.G.' + Fe(CN)_6^{2-}$$

$$\text{XS}\quad 2\text{Fe(CN)}_6^{3-} + \text{S}_2\text{O}_4^{2-} + 4\text{OH}^- = 2\text{SO}_3^{2-} + 2\text{Fe(CN)}_6^{4-} + 2\text{H}_2\text{O}$$

The titration proved unsatisfactory. Equilibrium in the back titration was attained very slowly, probably owing to the gradual decomposition of the hydrosulphite ion by air, although the sodium hydrosulfite was delivered from a Machlett burette to minimize this decomposition. Decomposition might have occurred during the interval between the time the sodium hydrosulfite was delivered from the burette and before completely reacting with the potassium ferricyanide.

An attempt was made to determine the equivalent weight of juglone green by oxidation using potassium molybdicyanide (31). The potassium molybdicyanide was prepared according to Kratochvil from potassium molybdocyanide (32). The solution was standardized using primary standard cobaltous sulfate previously synthesized by Diehl and Butler (33). Juglone green was added to the potassium molybdicyanide solution and the excess potassium molybdicyanide was back titrated with cobaltous sulfate. The titration was carried out in 50 per cent ammonia and 50 per cent ammonium citrate. The end-point was determined potentiometrically using a platinum foil indicating electrode and a saturated calomel reference electrode.

The titrations failed since juglone green was not sufficiently soluble in the ammoniacal medium.

VI. PROPERTIES OF JUGLONE GREEN AS FOUND BY VARIOUS FORMS OF SPECTROPHOTOMETRY

And God said, Let there be light — Genesis.

Use was made of several techniques, mostly spectrophotometric in nature, to establish the properties of juglone green. In this chapter, the results are reported individually by the particular method employed, specifically, the infrared spectrum, the proton magnetic resonance spectrum, the carbon-13 nuclear magnetic resonance spectrum, the electron spin resonance spectrum, the ultraviolet absorption spectrum, and the arc-emission spectrum. The evidence obtained from these various lines of study is drawn together in Chapter IX.

A. The Infrared Absorption Spectrum of Juglone Green

The infrared spectrum of juglone green and also that of quinhydrone was reported in my M.S. thesis (ref. 4, pp. 34, 35, and 42). These spectra, obtained by the nujol-mull technique, are not repeated here but a listing of the positions of the absorption bands is given in Table 2, inasmuch as an assignment of the various bands has now been made. The infrared spectrum of juglone has now been obtained by the potassium bromide technique (Figure 7). For comparison, the infrared spectrum of juglone green in potassium bromide is again reported (Figure 7). The spectra of quinone, hydroquinone and quinhydrone are shown in Figure 8. Assignments of prominent frequencies are given in Table 4. The spectra are presented to visually illustrate how the spectrum of quinhydrone

Table 2. Assignments of prominent absorption bands in the infrared spectrum of juglone green and quinhydrone in nujol.

Assignment	Juglone Green Band Pos. cm^{-1}	Int.	Quinhydrone Band Pos. cm^{-1}	Int.
v(OH)	3420	W	3230	W
v(C=O)	1640	S	1639	S
v(C=O)	1600	S	----	-
v(C-O·)	1590	M	----	-
v(C=C)	1580	M	1580	W
v(C-O)	1292	M	1258	M
v(C-O)	1210	W	1215	M
δ(C-H)	1165	W	----	-
δ'(C-H)	835	W	869	M
δ'(C-H)	822	W	824	W
δ'(C-H)	----	-	752	W
δ'(ring)	----	-	650	B & W

v = stretch

δ = in-plane bending

δ' = out-of-plane bending

Band pos. = Band position

Int. = Intensity

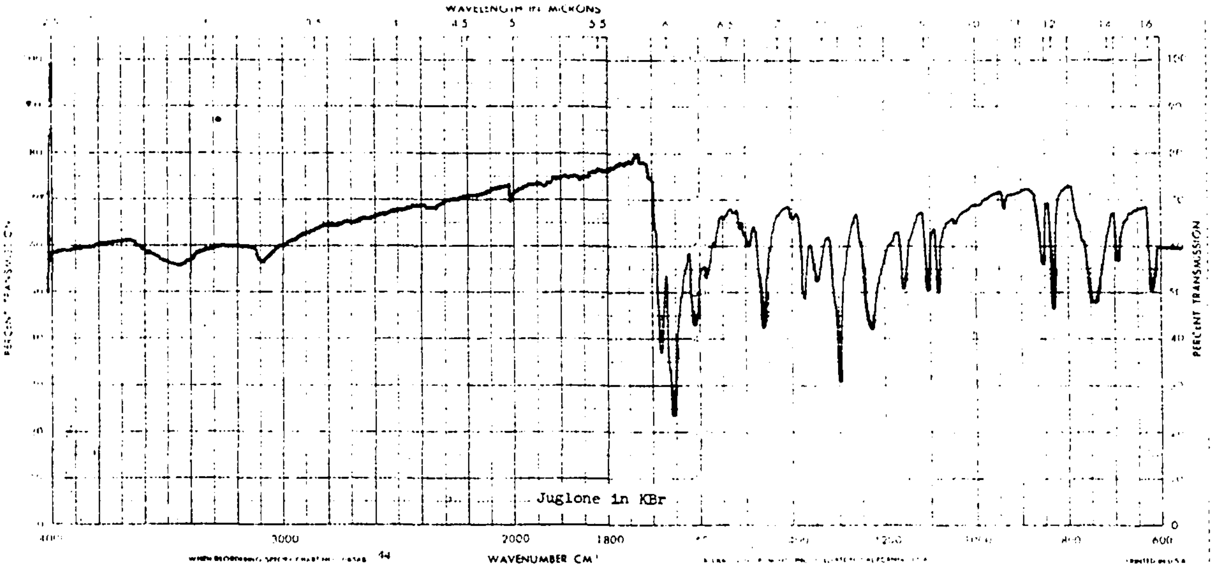
S = strong

M = medium

W = weak

B = broad

Figure 7. Infrared absorption spectra of juglone (top) and juglone green (bottom) in potassium bromide.


Juglone in KBr

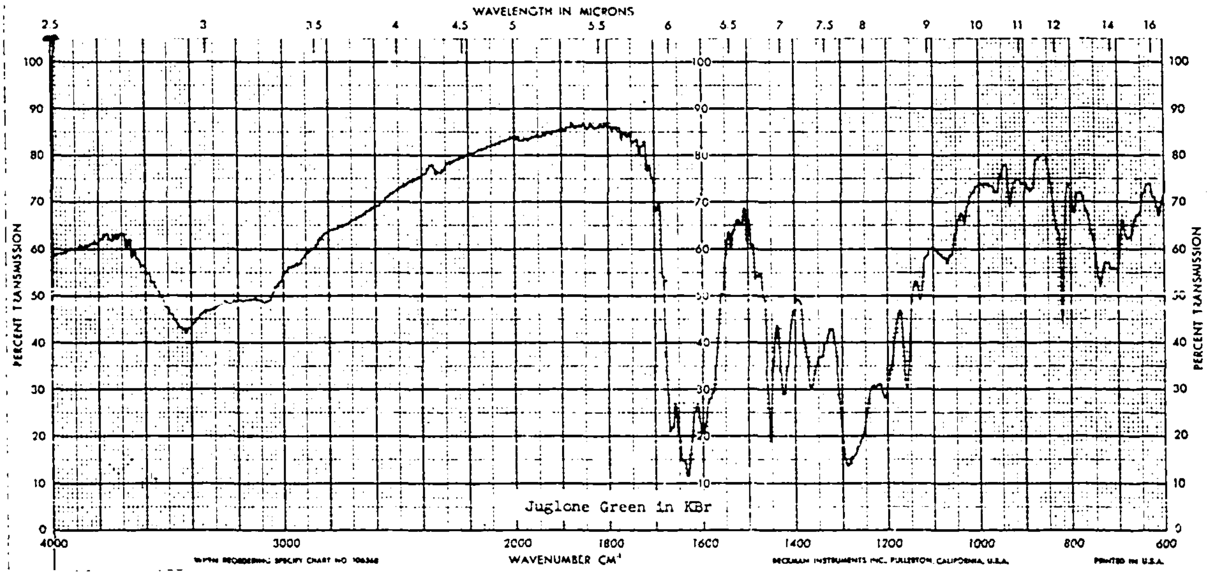

Juglone Green in KBr

Table 3. Assignments of prominent absorption bands in the infrared spectrum of juglone and juglone green in potassium bromide.

Assignment	Juglone Band Pos. cm-1	Int.	Juglone Green Band Pos. cm-1	Int.
v(OH)	3440	W	3420	W
v(C-H)	3090	W	3090	W
v(C=O)	1685	S	1670	S
v(C=O)	1655	S	1650	S
v(C=C)	1610	S	1600	S
v(C-O·)			1590	Sh&S
v(C=C)	1460	S	1455	S
δ(OH)	1372	M	1425	M
v(C-O)	1300	S	1365	S
v(C-O)	1230	S	1345	Sh&W
v(C-O)			1290	B&S
v(C-O)	1160	S	1160	S
δ(C-H)	1105	S	1130	W
δ(C-H)	1080	S	1070	W
δ(C-H)	1045	W	1030	W
δ'(C-H)	855	S	820	S
δ'(C-H)	830	S	740	M
δ'(C-H)	745	B&M	710	B&M
δ'(C-H)	695	M	675	B&W
δ'(ring)	620	M	605	W

v = stretch

δ = in-plane bending

δ' = out-of-plane bending

Band Pos. = Band Position

Int. = Intensity

S = strong

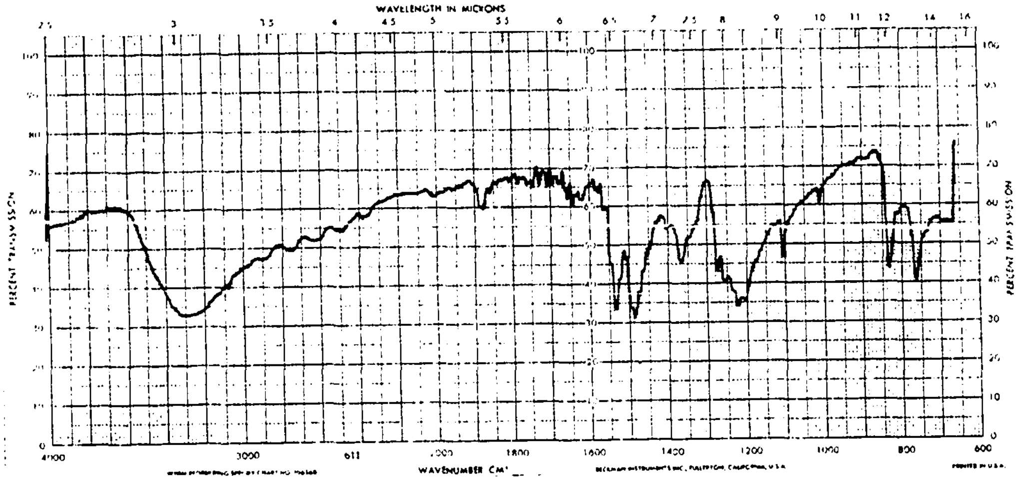
M = medium

W = weak

B = broad

Sh = shoulder

Figure 8. Infrared absorption spectra of hydroquinone (top), quinone (middle), quinhydrone (bottom) in potassium bromide.


WAVELENGTH IN MICRONS
PERCENT TRANSMISSION
WAVENUMBER CM⁻¹
4000
3000
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1800
1600
1400
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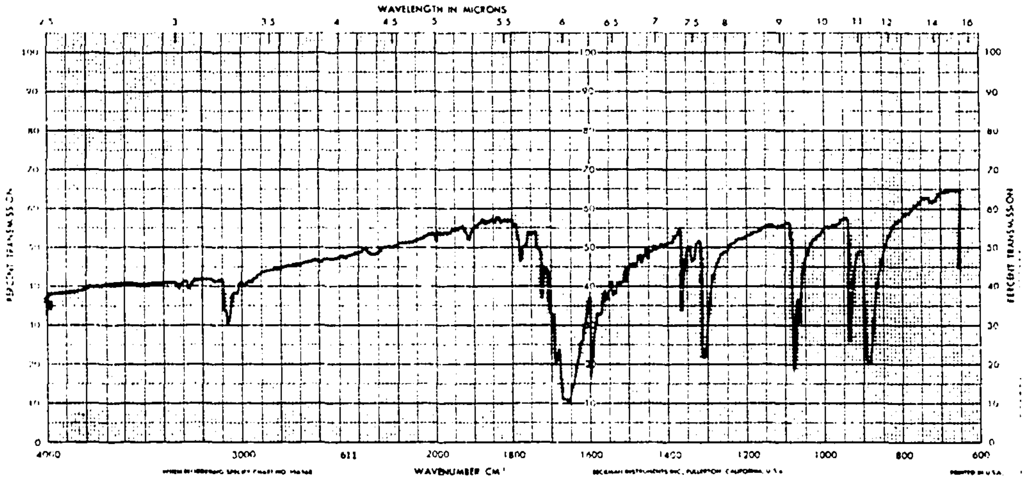
600


WAVELENGTH IN MICRONS
PERCENT TRANSMISSION
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4000
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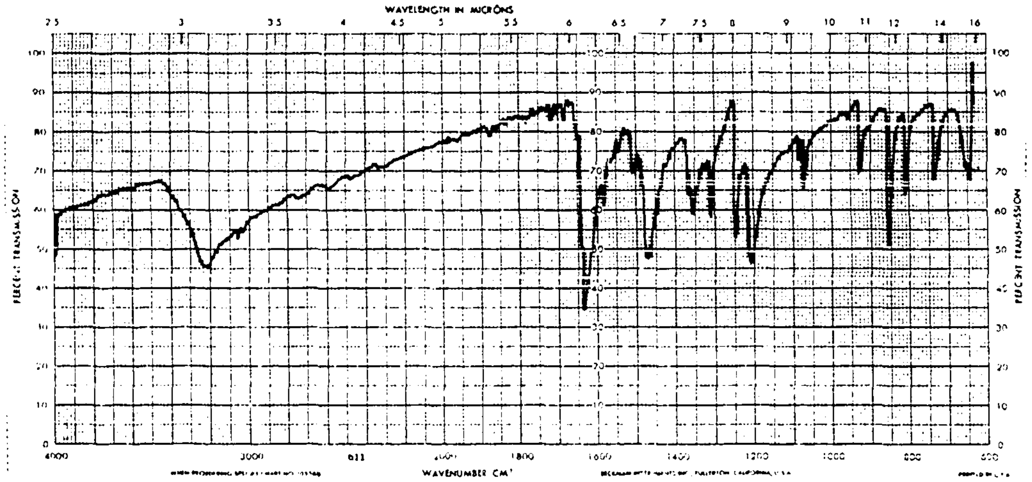

WAVELENGTH IN MICRONS
PERCENT TRANSMISSION
WAVENUMBER CM⁻¹
4000
3000
2000
1800
1600
1400
1200
1000
800
600

Table 4. Assignments of prominent absorption bands in the infrared spectrum of hydroquinone, quinone and quinhydrone in potassium bromide.

Assignment	Hydro-quinone Band Pos. cm^{-1}	Int.	Quinone Band Pos. cm^{-1}	Int.	Quinhydrone Band Pos. cm^{-1}	Int.
v(O-H)	3300	S	----	-	3230	M
v(=C-H)	3080	W	3070	W	3030	W
v(=C-H)	----	-	----	-	3015	W
v(C=O)	----	-	1660	S	1635	S
v(C=C)	1535	S	1600	M	1580	W
v(C=C)	1487	S	----	-	1505	S
v(C=C)	----	-	----	-	1470	M
v(C-O)	1255	M	----	-	1250	M
v(C-O)	1205	M	----	-	1205	S
δ(C-H)	1105	W	1080	S	1075	M
δ(C-H)	1010	W	1065	W	----	-
δ'(C-H)	835	M	935	S	928	M
δ'(C-H)	765	M	890	S	855	S
δ'(C-H)	---	-	---	-	810	S
δ'(C-H)	---	-	---	-	135	S

v = stretch

δ = in-plane bending

δ' = out-of-plane bending

Band Pos. = Band Position

Int. = Intensity

S = strong

M = medium

W = weak

compares to the spectra of quinone and hydroquinone. All spectra were obtained on a Beckman IR-33 spectrometer.

Assignments of the peaks in the infrared spectra of juglone green and quinhydrone are listed in Table 2. The wavelength scale was calibrated using the wavelength of the two carbon hydrogen bending modes of nujol, 1468 cm^{-1} and 1379 cm^{-1}. The primary bands of interest are the C=0 and 0-H stretching frequencies. The values assigned these bands for quinhydrone are in good agreement with published data by Davies (34), Kotera (35), and Brockmann and Franck (36).

The infrared data indicate the presence of hydrogen bonded phenolic groups in juglone green. Hydrogen bonding, of course, was unknown at the time of the work on juglone green of Mylius in 1885 and of Wheeler, Dawson and McEwen in 1923 and of course does not appear in the structural formula for juglone green postulated by the latter workers (see Chapter III, p. 17).

In general, the C=0 stretch of quinones lies in the region 1690 cm^{-1}-1630 cm^{-1}. The low frequencies observed for juglone green (1640 cm^{-1}) and for quinhydrone (1639 cm^{-1}) are caused by conjugation and hydrogen bonding effects. Conjugation results in delocalization of the pi electrons of the unsaturated groups; delocalization of the pi electrons of the C=0 group reduces the double bond character of the carbon to oxygen bond resulting in absorption at longer wavelengths.

Hydrogen bonding to the carbonyl group also contributes to the decrease in the double bond character of the carbon to oxygen bond, C-0---H-0, resulting in absorption at lower frequencies.

The position of the oxygen-hydrogen stretching band of quinhydrone, 3230 cm^{-1}, and of juglone green, 3420 cm^{-1}, indicates that hydrogen bonding to an electron-rich atom or group is occurring. The lower frequency for quinhydrone implies a stronger hydrogen bond has been formed than in juglone green, that is, the O-H bond is weaker and absorbs at a longer wavelength in juglone green.

The infrared spectra of juglone and juglone green are shown in Figure 7. The assignments of the absorption frequencies are given in Table 3. Assignments of these bands and of the bands in Table 2 are based on the general regions in which the functional groups absorb. These regions were demarcated using information from Conley (37) and from Silverstein and Bassler (38). Where several absorptions can occur in the same region, assignments were made by referring to other published data.

As will be seen by examination of Figure 7, the absorption spectra of juglone and juglone green are similar but with a few important exceptions. Like juglone green, juglone has a broad peak at 3440 cm^{-1} indicating the phenolic group is hydrogen bonded. As a result of this hydrogen bond in juglone, the two carbonyl groups are not identical. Two dinstinct absorption bands are present in the juglone spectra in the carbonyl stretch region, at 1655 cm^{-1} and 1685 cm^{-1}. The weaker band, present at 1610 cm^{-1}, is probably the aromatic C=C skeletal vibration.

The spectrum of juglone green in the absorption region of the carbonyl group is more complicated than that of juglone in that the two major peaks in this region are broader and each have shoulders.

Absorption in this region more closely resembles the corresponding absorption in the spectrum of quinhydrone. In quinhydrone the bands also have shoulders in this region and the bands are not as well-resolved into sharp peaks as in the juglone spectrum. These additional shoulders in quinhydrone and juglone green could be the result of the effect of the hydrogen bonding in these molecular compounds on the carbonyl groups.

The absorption bands in the spectrum of juglone green appearing at 1630 cm^{-1}, and 1670 cm^{-1} are in the region of the carbonyl stretching frequency and indicate that carbonyl groups are present in juglone green. In addition, in the juglone green spectrum an intense shoulder appears at 1590 cm^{-1}; this peak is either not observed or barely detectable in the juglone and quinhydrone spectra.

Nearly all stable aroxyl radicals, that is, a phenolic group carrying an unpaired electron owing to the loss of the hydrogen atom, show a strong band at 1590 cm^{-1} in the solid state and in solution. Cook, Kuhn, and Fianu (39) and Müller and Ley (40) have assigned this absorption band to the C-O· group. The intense peak at 1590 cm^{-1} in the juglone green spectrum has been assigned to this aroxyl C-O· group. This implies juglone green is a free radical in the solid state. Further evidence for this is presented in Section E, in which the electron spin resonance absorption spectrum of juglone green is reported.

Aroxyl groups have also been observed to exhibit a doublet in the region 1681 cm^{-1} to 1613 cm^{-1}. Uncertainty exists, whether these peaks should be ascribed to an aroxyl vibration or ascribed to the presence of dimeric (polymeric) molecules (Forrester, Hay, and Thomson (41)). In the spectrum of juglone green, shoulders are present on the two major peaks in this region. The shape of these peaks could be interpreted as doublets, but the actual absorption can result from one of several possible functional groups, not necessarily the aroxyl group.

The difficulty in making absolute band assignments in the infrared is that more than one type of functional group can absorb in a particular region. Conjugation, chemical environment, symmetric and asymmetric stretching frequencies all contribute to complicating the spectrum.

The two major peaks in the 1600 cm^{-1} to 1700 cm^{-1} region of the juglone green spectrum can be attributed to the two different carbonyl groups present. Interpretation of the shoulders is uncertain, aroxyl vibrations, presence of polymeric molecules, carbonyl absorptions in different chemical environments, aromatic overtones or quite possibly some combination of these effects may produce them. The band at 1590 cm^{-1} has been assigned the C-O· vibration, characteristic of aroxyl groups.

Summarizing, the infrared absorption spectrum indicates that there is present in the juglone green molecule: 1) hydrogen bonded phenolic groups, 2) an aroxyl group, 3) two or more carbonyl groups.

B. The Proton Magnetic Resonance Spectrum of Juglone Green

The proton magnetic resonance (PMR) spectrum of juglone green and quinhydrone were obtained on a Varian A-60 spectrometer in hexadeuterated dimethylsulfoxide (D_6-DMSO). The spectra were also recorded in deuterated sodium hydroxide (NaOD). The NaOD was prepared by dissolving small pieces of sodium metal in commercially available D_2O.

No peaks were observed in the PMR spectrum of juglone green in D_6-DMSO. Initially this was thought to result from the low concentration of juglone green in the DMSO, about 10 mg in 1.5 ml of solvent. The PMR spectrum of quinhydrone was obtained in D_6-DMSO at approximately the same concentration; two well defined peaks were present as expected.

The PMR spectrum of juglone green was obtained in NaOD at a higher concentration, 3 mg per ml of solvent. Again no peaks were observed. The absence of peaks in the spectrum were rationalized as follows. Either the molecule contained a paramagnetic impurity such as iron or else the compound itself contains an unpaired electron. The electron spin magnetic moment is about one thousand times greater than a typical nuclear magnetic moment. If an unpaired electron is present in a molecule any absorptions by protons is suppressed. In short, the peaks in the PMR spectrum become very broad or are eliminated.

C. Carbon-13 Nuclear Magnetic Resonance Spectrum of Juglone Green

Approximately 20 ml of juglone green was dissolved in 2 ml of D_6-DMSO. A carbon-13 spectrum was obtained on this saturated solution using a Bruker HX-90 FT-NMR spectrometer.

As found in the PMR spectrum of juglone green, the carbon-13 magnetic resonance spectrum showed no peaks.

D. Arc-Emission Spectrum of Juglone Green

The arc-emission spectrum of juglone green was obtained to determine if any metal impurities were present in the molecule. Such impurities could explain the absence of peaks in the proton and carbon-13 nuclear magnetic resonance spectra. The spectrum was obtained on an Applied Research Lab (ARL) Model 26000-1 Spectrographic Analyzer. Carbon electrodes were used for the sample and iron electrodes were used for the reference spectrum. The spectrograph was recorded on film.

Only cyanogen bands were present in the spectrum; these are due to carbon interacting with nitrogen in the atmosphere when the sample is arced. No emission lines due to metals were observed.

E. Electron Spin Resonance Spectrum of Juglone Green

The electron spin resonance (e.s.r.) spectrum of juglone green was obtained on a Varian Spectrometer, Model E3, equipped with a 3-inch magnet. The spectrum was recorded using a solution of juglone green in degassed DMSO. The DMSO was placed in the cell, frozen with liquid nitrogen and then melted by warming, the gases being pumped away by a vacuum pump. This process was repeated three times. Juglone green was synthesized in the absence of oxygen by heating sublimed juglone in a sealed evacuated test tube in an oil bath; the juglone decomposed to juglone green at 80^0. Using a dry box, the sample was then transferred to the cell containing the degassed DMSO. The e.s.r. spectrum was then recorded and the cell was then briefly exposed to air, and the spectrum again recorded. The spectrum is shown in Figure 9.

The e.s.r. spectrum showed a strong broad signal indicating the presence of a free electron. The signal was not resolved sufficiently to permit a determination of hyperfine splitting constants. Brief exposure to the oxygen in air did not change the signal significantly.

Little work on the e.s.r. spectra of dimeric naphthoxyl radicals has been reported. Stone and Waters (42) obtained spectra on alpha- and beta-naphthoxyl radicals but were unable to determine hyperfine splitting constants.

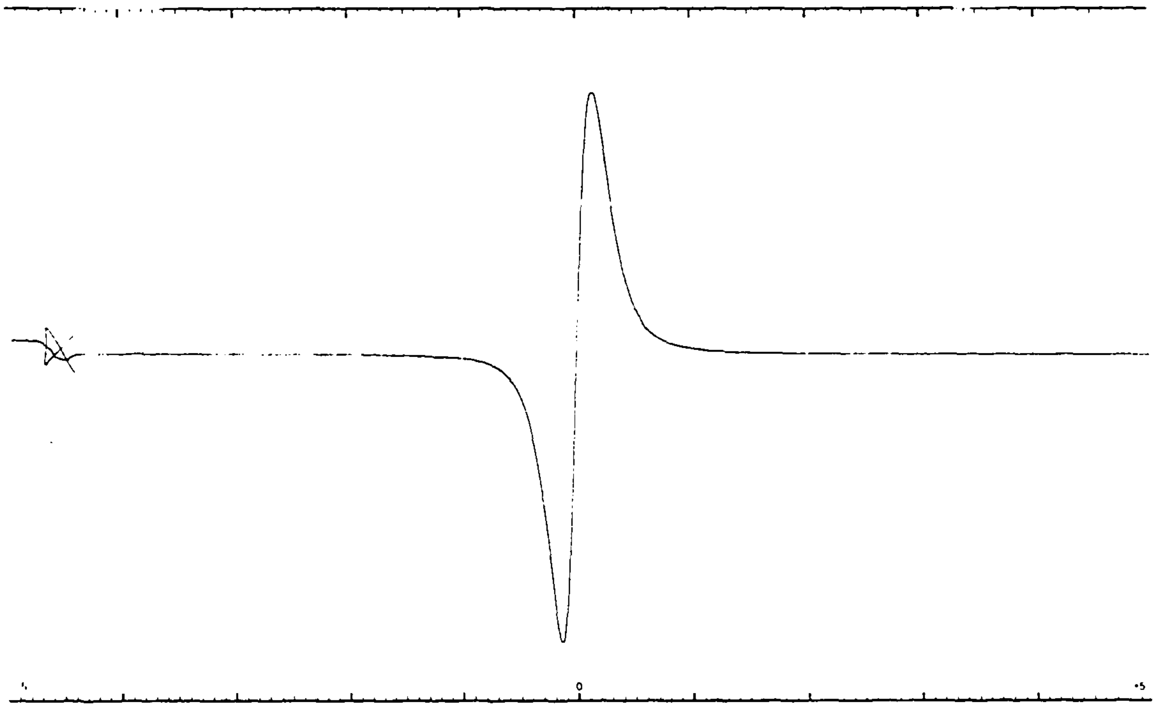
The e.s.r. spectrum of solid juglone green was also obtained. A very intense signal was observed indicating the molecule contains an unpaired electron in the solid state. The spectrum is shown in Figure 9.

The e.s.r. spectrum confirms that juglone green is a free radical both in the solid state as well as in solution. Unfortunately no information can be obtained using this technique to determine the number of unpaired electrons in the molecule. If more than one unpaired electron is present, the arrangement in the molecule must be such that they do not interact.

F. The Ultraviolet Absorption Spectrum of Juglone Green Dissolved in Alkaline Solution

Absorption in the ultraviolet was used as a tool to investigate some additional properties of juglone green. Experiments were

Figure 9. E.s.r. spectrum of juglone green in the solid state (top) and in DMSO (bottom).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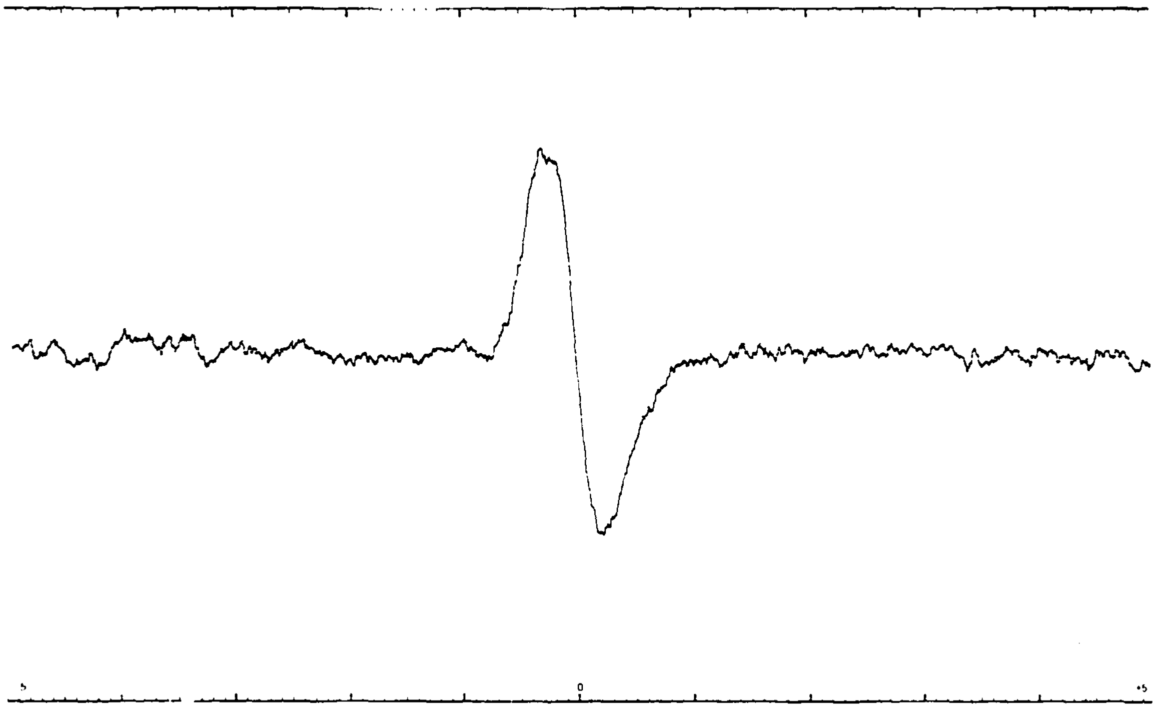
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0
+5

designed first to establish conformity to Beer's law. Once this was established, an attempt was made to evaluate the dissociation constant of juglone green as an acid. This experiment was unsuccessful. A final experiment was devised to determine the stability of juglone green in alkali. The stability was compared to juglone dissolved in alkaline solution.

1. Visible absorption spectra of juglone and juglone green

The visible absorption spectrum of juglone green was obtained as follows. Approximately 1 mg of juglone green was dissolved in 25 ml of 1 M sodium hydroxide and scanned from 600 to 350 nm using a Carey 14 Recording Spectrophotometer, 1-cm. silica cells, and 1 M sodium hydroxide solution as a reference. The spectrum of juglone under identical conditions was also recorded.

The visible absorption spectra of juglone green and juglone showed a peak with a maximum at 520 nm. Both solutions are purple in alkali.

2. Conformity to Beer's law

To determine if juglone green in alkali conformed to Beer's law, a study of absorbance versus concentration was made. To 22.1 mg of juglone green was added sufficient 1 M sodium hydroxide to dissolve the compound. The solution was diluted to a volume of 50 ml with deionized water. Using a pipet, 2 ml of this stock solution was added to various amounts of deionized water. The absorbance of each solution was measured using a Bausch & Lomb Spectronic 20.

The results of the analysis are shown in Figure 10, where a plot of absorbance versus concentration is given. As can be seen from the

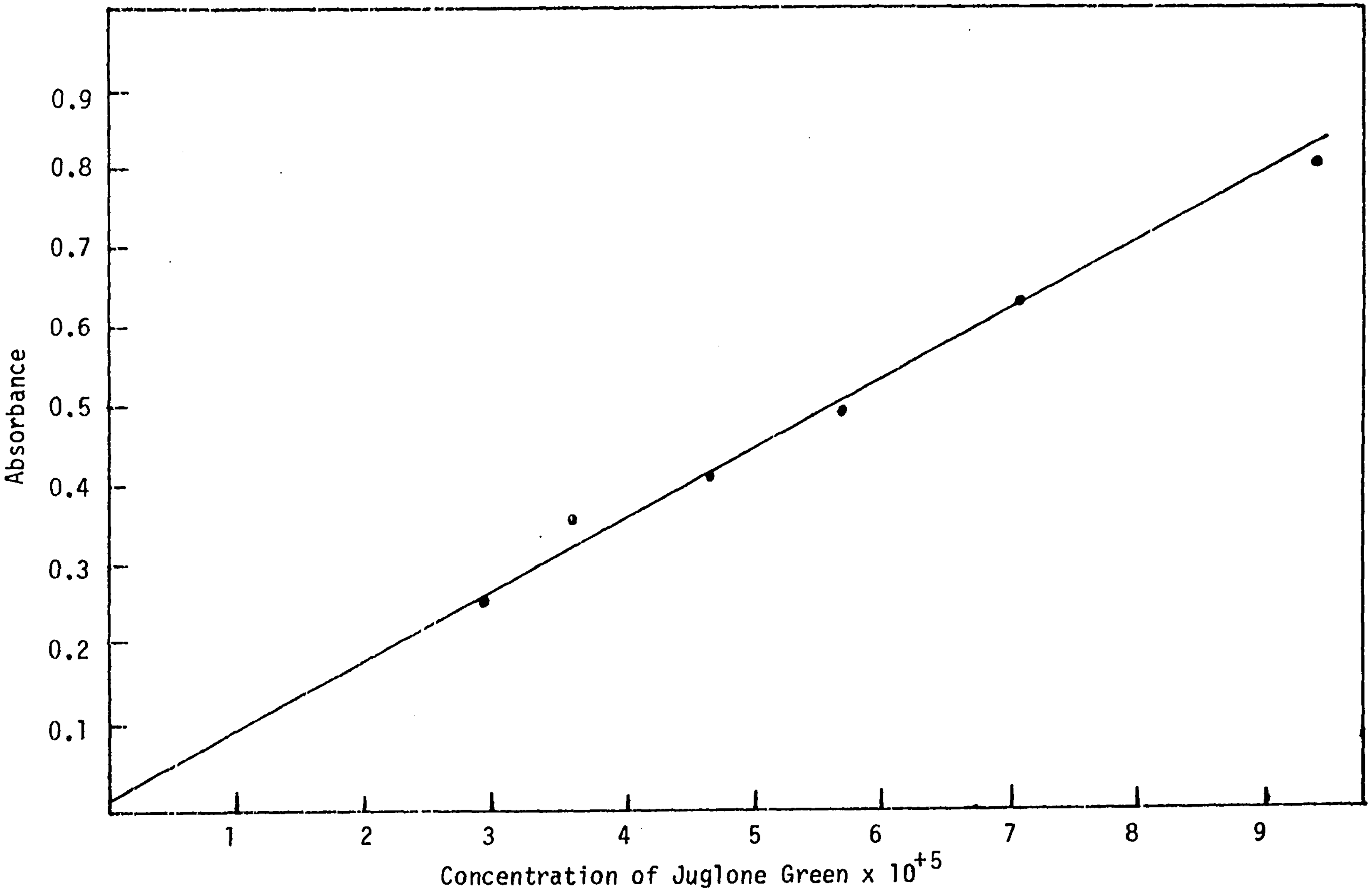


Figure 10. Beer's law plot for juglone green at 522 nm $\varepsilon = 8.70 \times 10^{+3}$ l-mol^{-1}-cm^{-1}.

graph, the system conforms well to Beer's law; the molar absorptivity coefficient, ε, is 8700 $l\ mol^{-1}\ cm^{-1}$.

3. Determination of the dissociation constant of juglone green as an acid

When the pH of an alkaline solution of juglone green is lowered a color change from purple to yellow-brown occurs. From the titration data obtained in Chapter V it was found that juglone green can be titrated as the sodium salt of a weak acid. Using spectrophotometry, an attempt was made to determine the acid dissociation constant. This determination requires that the system conform to Beer's law; the preceeding experiment verified that absorbance was proportional to concentration.

A series of sodium bicarbonate-sodium hydroxide buffers were prepared over the pH range 7 to 11. In this region juglone green passes through a color change from purple on the alkaline side to yellow at the acidic side. Using the previously prepared stock solution of juglone green, 2.00 ml of juglone green was added to 50.00 ml of each buffer. The pH of each solution was recorded and the visible absorption spectrum obtained.

The plot of absorbance at 522 nm versus pH obtained from this data, the novel way of handling such a system, did not give the expected S-shaped curve. The absorbance was found not to change significantly as a function of pH. In view of this result, further experiments were performed to determine the stability of juglone green in alkaline and acidic solutions.

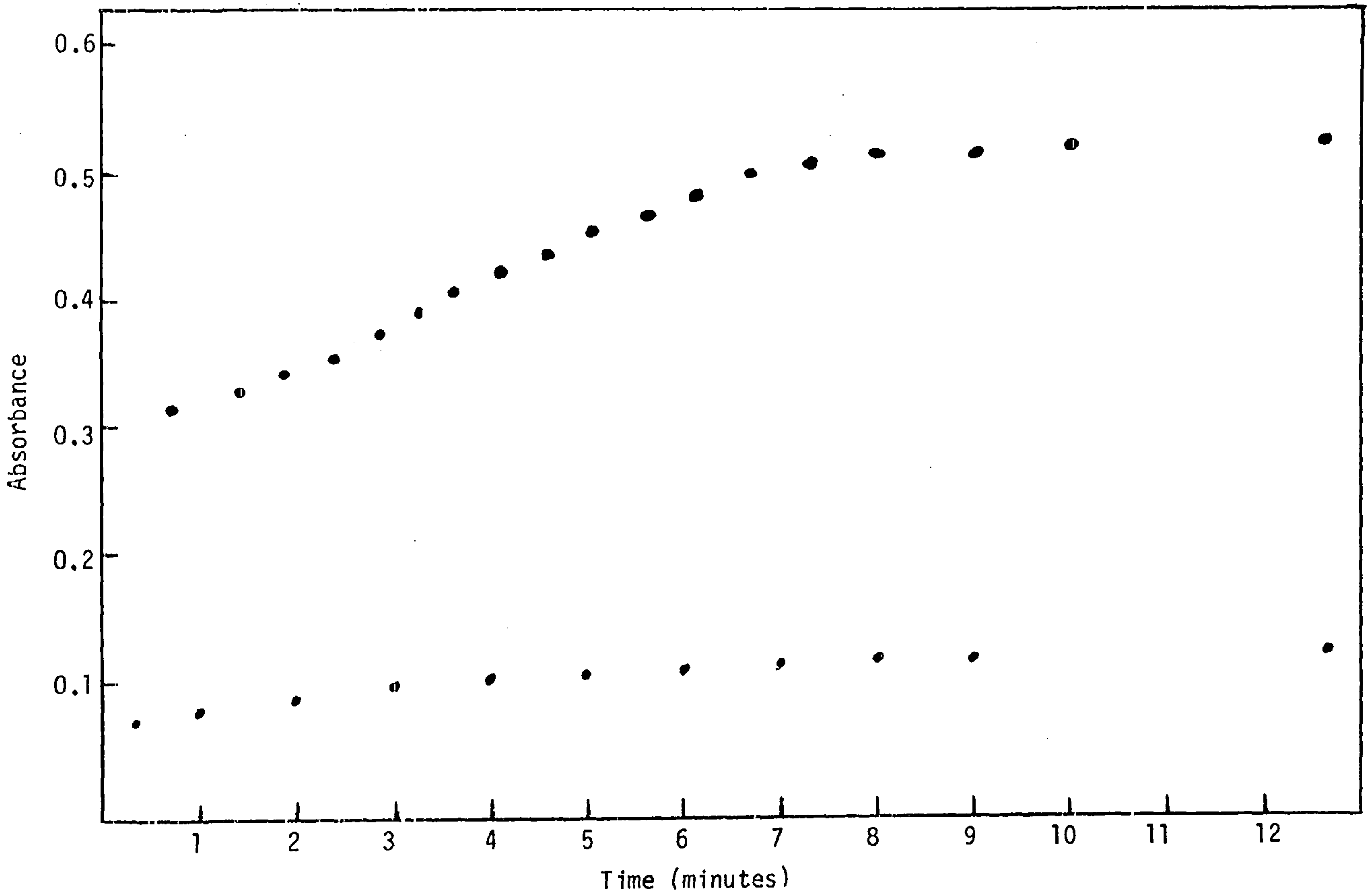
4. Stability of juglone green in alkali

The stability of juglone green and juglone in alkali was determined by dissolving each compound in 1 M sodium hydroxide and recording the absorption spectra as a function of time. The wavelength maximum for juglone was found to shift from 520 nm to 475 nm with a shoulder at 450 nm, and finally the maximum shifted to 440 nm. This shift took place within an hour after dissolution in alkali. Juglone changes visually from purple to a dark wine-red color. After several hours in the alkaline solution, the color is dark brown. This instability of juglone in alkali has been attributed to oxidative decomposition (43).

Juglone green proved to be more stable than juglone in alkali. The absorption maximum also shifted to shorter wavelengths but the shift was only 30 nm and took place over a two day interval.

After dissolving the compound in alkali, and before recording the first spectrum, a period of one to two minutes elapsed. To find out what was happening in the initial minutes, a few milligrams of each compound was dissolved in alkali and immediately placed in the Bausch & Lomb Spectronic 20. The absorption was measured as a function of time and the results are shown for juglone and juglone green in Figure 11.

From the data in Figure 11, one can see the increased stability of juglone green in alkali as compared to juglone. The curve for juglone green was shifted down 0.2 absorbance units to facilitate comparison.



Top curve: Juglone
Bottom curve: Juglone Green

Figure 11. Absorbance as a function of time for juglone and juglone green in alkali.

5. Stability of juglone green in acid

Because solutions of juglone green in alkali proved stable, the phenomena observed in the attempt to determine the dissociation constant became more puzzling. Stability in acid solution became of interest.

A small amount of the previously prepared stock solution of juglone green in alkali was acidified with 1 M hydrochloric acid. The yellow solution of juglone green turned brown rapidly. Initially this yellow-brown color was thought to be normal but closer inspection revealed that the initial color was a bright yellow and very rapidly darkened. This instability in acid was found to be characteristic of aroxyl radicals. According to Buchachenko (44), phenoxyl radicals when protonated form unstable species and decompose rapidly.

Juglone is stable indefinitely in acid solutions.

6. Conclusions

From the information obtained using ultraviolet spectrophotometry, juglone green was found to be more stable in alkali than juglone. Juglone green in alkaline solution is not oxidized by air as is juglone. This instability at low pH provides further evidence for a radical, which is unstable when protonated and decomposes rapidly.

VII. ADDITIONAL OBSERVATIONS ON JUGLONE GREEN

Many organic chemists who had been doubtful of the existence of semiquinone radicals have been converted to their acceptance only after observing the results of such magnetic measurements — L. Michaelis.

A. Changes in the NMR Spectrum of Juglone During Disproportionation

The nuclear magnetic resonance (NMR) spectrum of juglone dissolved in D_6-DMSO was obtained at intervals during the disproportionation, which takes place rather slowly in DMSO. The NMR of juglone dissolved in D_6-DMSO is shown at two intervals in Figure 12.

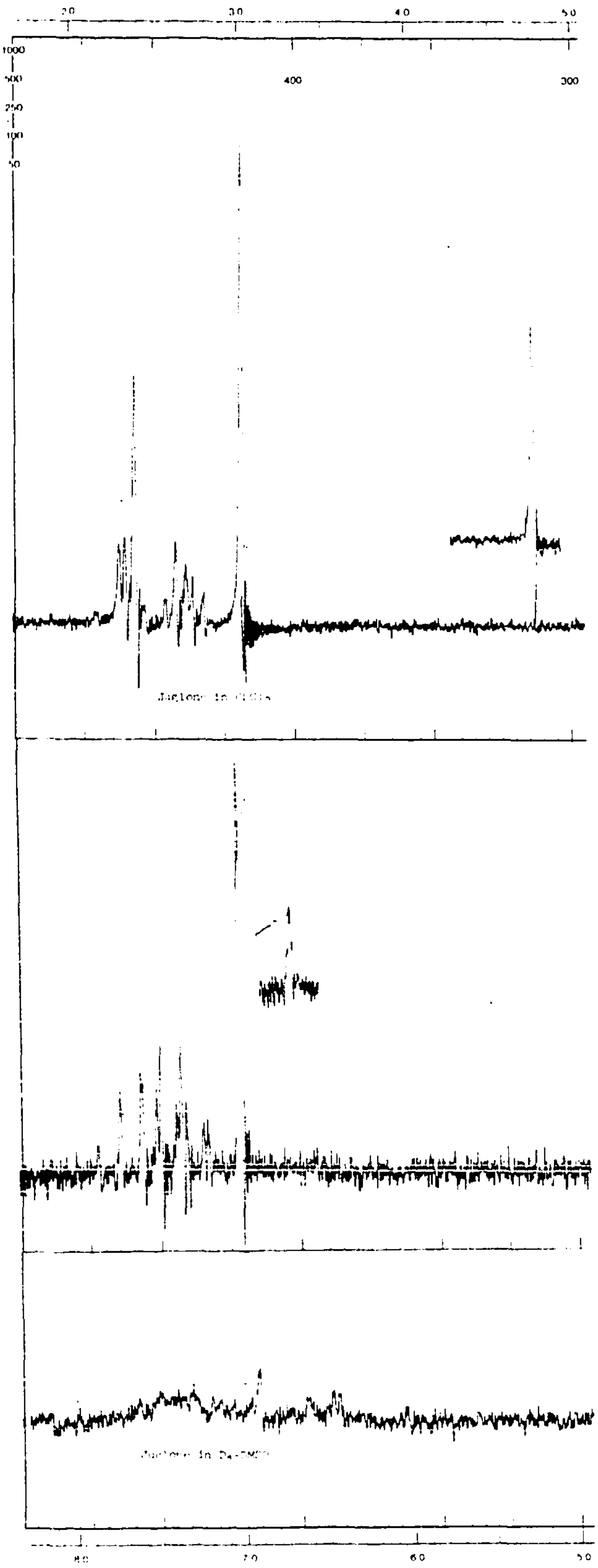
The spectrum of juglone in deuterated chloroform is given for reference, Figure 12, top spectrum; juglone does not disproportionate in chloroform. Assignments of the individual peaks have been previously reported (Michels (4), p. 20). The peaks at $\delta = 6.9$ corresponds to the two quinonoid protons. These quinonoid protons remained unaffected as the juglone disproportionated. The protons at δ 7.2 to 7.8, corresponding to the three protons in the phenolic ring, changed as disproportionation progressed, Figure 12, middle spectrum. The peaks broadened and were ultimately wiped out (Figure 12, bottom spectrum), owing to the effect of the unpaired electron in juglone green. The change in the molecule was evidently occurring in the phenol ring.

Figure 12. NMR spectrum of juglone during disproportionation.

Top: Freshly prepared solution in deuterated chloroform (stable solution).

Middle: Solution in deuterated dimethylsulfoxide after 15 minutes.

Bottom: Solution in deuterated dimethylsulfoxide after one hour.


2.0
3.0
4.0
5.0
1000
500
250
100
50
400
300
Juglone in CDCl3
Juglone in D6-DMSO
8.0
7.0
6.0
5.0

B. Magnetic Susceptibility

1. Background

The electron spin resnonance spectrum of juglone green in the solid state indicated the presence of a free electron. Unfortunately, the electron spin resonance method yields no information as to the concentration of free electrons in the sample (number of unpaired electrons).

Because juglone green is a radical and stable in the solid state, a measurement of the magnetic susceptibility of the material may possibly yield information from which the number of unpaired electrons can be calculated.

Free radicals are paramagnetic because the unpaired electron is a charged spinning body which gives rise to a magnetic field. This field will interact with any applied field. Relating measurements of the paramagnetic susceptibility to the concentration of free electrons in a sample is not straightforward because the measured susceptibility is composed of two quantities, the contribution of the paramagnetic moment and the contribution of the diamagnetic moment.

Diamagnetism is a property of all molecules resulting from the movement of electrons in filled orbitals. A diamagnetic substance has no magnetic moment when exposed to the field. The induced magnetic moment for any molecular species is always negative and relatively weak. A paramagnetic substance has a permanent magnetic dipole which is always positive and comparatively strong.

For free radicals and molecules in which the diamagnetic susceptibility has not been measured, the diamagnetic contribution can be calculated using an empirical method devised by Pascal (45). As a first approximation, the diamagnetic susceptibility of a molecule can be calculated by the addition of the diamagnetic susceptibility of the component atoms. The specific contribution of each atomic species has been evaluated empirically by Pascal. Slight "structural corrections" to the additivity rule must be applied to account for effects of aromatic rings and other structural features of the molecule.

2. Gouy balance

The magnetic susceptibility of juglone green was determined using a Gouy balance. The Gouy apparatus was constructed from the following components:

The balance was a standard double-pan analytical balance having a sensitivity of 0.1 mg.

The electromagnet was an Alpha Scientific Lab Model AL7500 used in conjunction with the AL7500 power supply. The magnet had pole caps of 4" diameter which were separated by 3/4 of an inch. All measurements were made at the same field strength of 7000 gauss.

The sample tube was constructed from pyrex tubing and had a thin glass septum dividing it into two compartments. The upper compartment was filled with solid; the lower was filled with air.

The Gouy apparatus was calibrated using $HgCo(CNS)_4$ and $(Ni(en)_3)S_2O_3$ both of which are stable, pure, not hygroscopic and pack easily. The susceptibility of each compound is well-known.

3. Calculations

The magnetic susceptibility per mole of substance X_m, is obtained by multiplying the specific susceptibility, X, by the molecular weight M of the compound:

$$X_m = (X)(M) \qquad (17)$$

Since the measured molar susceptibility of a compound is the sum of the paramagnetic and diamagnetic contributions, the susceptibility of only the unpaired electron $X_{m,p}$ may be obtained from the additive relationship:

$$X_m = X_{m,p} + X_{m,d} \qquad (18)$$

where $X_{m,p}$ is the paramagnetic molar susceptibility and $X_{m,d}$ is the diamagnetic molar susceptibility. The paramagnetic susceptibility depends on the temperature of the substance according to the Curie law:

$$X_{m,p} = C/T \qquad (19)$$

where C is the Curie constant and T is the absolute temperature. The diamagnetic susceptibility is not temperature dependent.

Curie's law is usually not strictly obeyed but is replaced by the Curie-Weiss law:

$$X_{m,p} = C/(T + \Theta) \qquad (20)$$

where Θ is the Weiss constant. This constant arises from the mutual interaction of the molecular dipoles. Θ is always very small for free radicals (46) so the calculations are based on the simple Curie law.

The magnetic susceptibility per gram, X, is experimentally determined by making the following measurements:

A. Weight of empty tube, field off

B. Weight of empty tube, field on

C. Weight of tube filled to line with water, field off

D. Weight of tube filled to line with solid, field off

E. Weight of tube filled to line with solid, field on

Knowing the magnetic susceptibility per gram, X of the standard, the calibration constant may be calculated using the equation:

$$(X)(m) - (0.029 \times 10^{-6})V = \beta(\Delta - \delta) \qquad (21)$$

where V equals the volume of the tube (C-A)/d, d is the density of water at ambient temperature, δ equals the susceptibility of the tube B-A, Δ equals the susceptibility of the solid E-D, and m equals the mass of the solid D-A. β is the calibration constant.

Having obtained the value for X, $X_{m,p}$ can be obtained using equations 17 and 18, where $X_{m,d}$ is obtained from Pascal constants. If the $X_{m,p}$ obeys Curie's law, the effective magnetic moment may be calculated from the expression:

$$\mu_{eff} = \left\{\frac{3kX_{m,p}T}{N_{B}^{2}}\right\}^{\frac{1}{2}} = 2.83(X_{m,p}T)^{\frac{1}{2}} \qquad (22)$$

where k = Boltzmann's constant, B = Bohr magneton, N = Avogadro's number and T = absolute temperature.

The paramagnetic contribution to the susceptibility of a free radical is due to the spin of the unpaired electron. By assuming that the orbital motion of the electrons has no paramagnetic effect in the molecule (46) the number of unpaired electrons, n, may be calculated from the equation:

$$\mu_{eff} = (n(n + 2))^{\frac{1}{2}} \tag{23}$$

4. Results

The results of the experiment indicated that the number of unpaired electrons was 1.3 with an uncertainty of 0.1. The diamagnetic contribution was calculated based on Pascal constants. The magnetic susceptibility of quinhydrone, quinone and hydroquinone have been measured in an effort to determine the structure of quinhydrone and evaluate the correctness of Pascal constants. In two independent laboratories the agreement was found to be very good.

Table 5. Correlation of measured susceptibilities with Pascal constants.

Substance	$-\chi_{m,d} \times 10^6$ measured (47)	$-\chi_{m,d} \times 10^6$ measured (48)	$-\chi_{m,d} \times 10^6$ calculated (45)
Quinone	35.96	38.20	40.80
Hydroquinone	66.11	64.73	66.82
Quinhydrone	102.05	105.00	107.62

Analyzing the sources of error in this experiment, one source of error lies in the assumptions which were made in deriving the equations, that is, the empirical method used to obtain the diamagnetic contribution, the assumption that the Weiss constant is negligible and the assumption that the orbital motion of the electrons has no paramagnetic effect in the molecule.

In addition to error inherent in the approximations, the experimental error due to the actual measurement is also sizeable. The right hand side of equation 21 involves two terms, Δ and δ, each of which is a small difference between two large numbers. The difference is then taken between these two numbers, each of which have masses approaching the detection limit of the balance. Also, the purity, stability, hygroscopicity, and packing properties of juglone green contribute to the accuracy of the final value. These properties of the compound would all lead to low results. That is to say, any impurity, moisture, air channels in the packed tube or decomposition of the juglone green would lower the measured paramagnetic moment.

The value determined for n, 1.3, is disturbing. Although closer to 1 than 2, the number is sufficiently ambiguous in consideration of the error analysis as to keep the question, how many unpaired electrons are present in juglone green?, unsettled.

C. Evolution of Hydrogen

If two unpaired electrons are present in juglone green, and the molecular weight is 520, the formation of a biradical may be accompanied by the production of hydrogen:

$$3\ C_{10}H_6O_3 = C_{30}H_{16}O_9{\cdot\cdot} + H_2$$

The following experiment was designed to determine if hydrogen is evolved in the disproportionation of juglone to juglone green.

Approximately 10 mg of pure juglone was placed in a tube with a break seal on one end. The tube was evacuated to a pressure of 0.001 mm Hg on a mass spectrometer and sealed. The conversion of juglone to juglone green was then effected by submerging the sealed tube in a hot oil bath. At 154^{0} a yellow vapor formed and then turned green, this was followed by the deposition of solid juglone green on the walls of the tube.

The tube containing juglone green was then fused to a vacuum stopcock containing a steel ball (Figure 13). The dead space was evacuated on the mass spectrometer. The break seal was then broken by the steel ball and any gases which may have formed in the tube were introduced into the mass spectrometer. No hydrogen was detected.

A peak at m/e 1 was observed; this peak was presumably caused by the fragmentation of water resulting from a small leakage of air into the system.

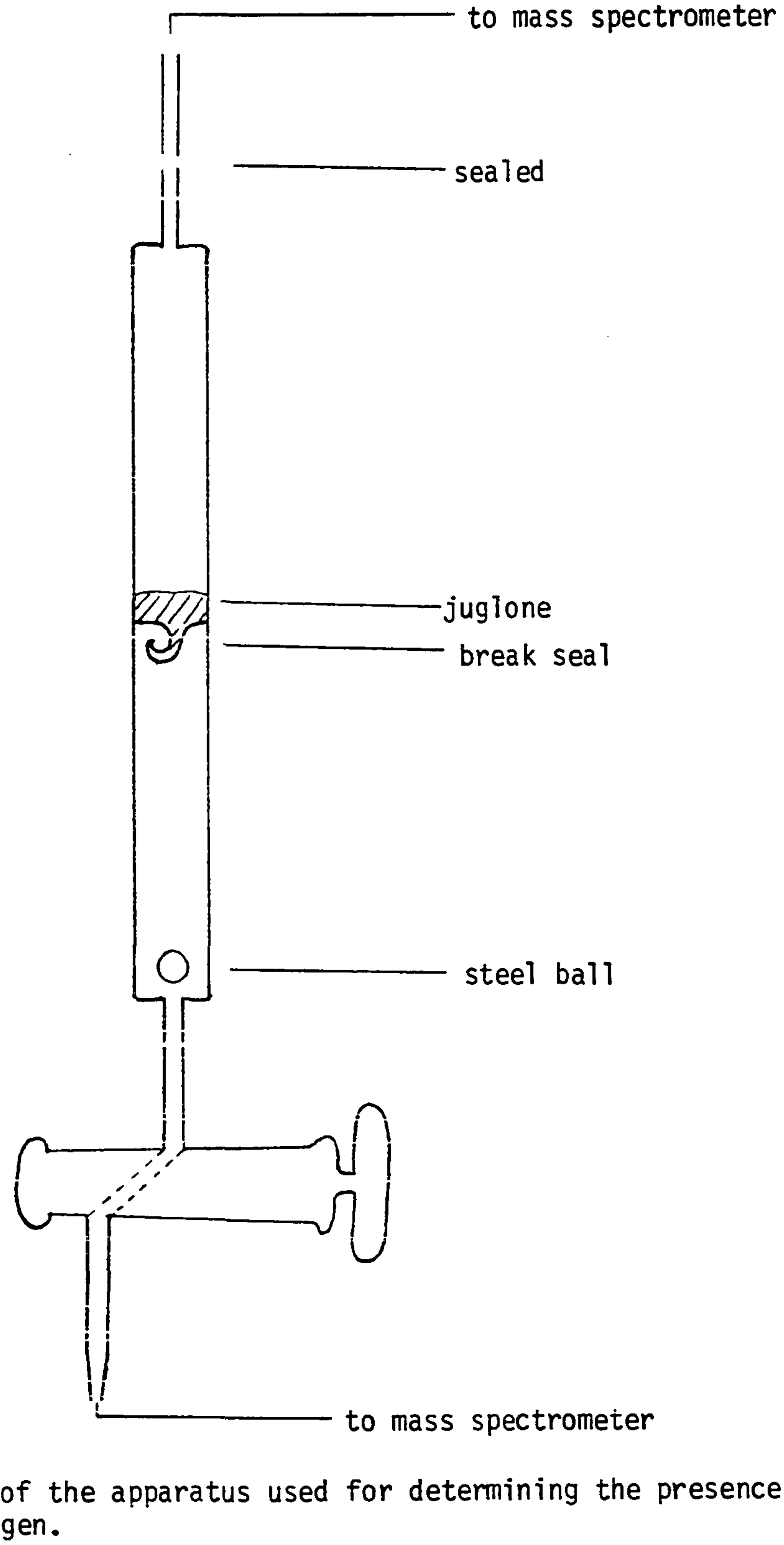


Figure 13. Diagram of the apparatus used for determining the presence of hydrogen.

VIII. OTHER NAPHTHOQUINONE TRIMERS

Each adds a little to our knowledge of nature, and from all the facts assembled there arises the grandeur — Aristotle.

A. Introduction

The failure to obtain an NMR spectrum of juglone green, because of the presence of an unpaired electron in the molecule, made NMR data unavailable for determining the structure of juglone green. The slow deterioration of the NMR spectrum of juglone dissolved in D_6-DMSO did indicate that the protons in positions 2- and 3- of juglone were unaffected during the disproportionation. Attempts to cleave the juglone green molecule and isolate the components proved fruitless. Based on the chemistry of similar phenolic compounds, the oxidative coupling of juglone, which apparently occurs during the disproportionation, probably occurs at the 6- position. Some support for this comes from work described in this chapter on 5-hydroxy-1,4-naphthoquinones bearing substituents in various positions in the phenolic ring. Only those derivatives with the 6-position open formed compounds analogous to juglone green.

With the exception of 2,3,6-tribromojuglone, the derivatives were synthesized using the condensation of maleic anhydride with the appropriate phenol. All products were purified by vacuum sublimation and were characterized by mass spectrometry, infrared absorption spectrometry, and nuclear magnetic resonance spectrometry.

B. Experimental Work

The molecular weight and chemical formula of the compounds was obtained on a high resolution AEI Mass Spectrometer 902. The infrared absorption spectra were recorded in potassium bromide using the Beckman IR-33. The proton magnetic resonance spectra were recorded in deuterated chloroform using the Varian A-60 nuclear magnetic resonance spectrometer.

1. 2,3,6-Tribromojuglone

2,3,6-Tribromojuglone was synthesized by the direct bromination of juglone. To 0.1 g of pure juglone was added 3 ml of hot glacial acetic acid. An amount of 0.5 g of bromine was added to the mixture. The crystals which formed were filtered and purified by vacuum sublimation. The melting point was observed at 165^o, reported 170^o (49).

The infrared absorption spectrum is shown in Figure 14. No assignments of the individual bands have been made. The spectrum indicates that the compound contains a hydrogen bonded phenolic group and two carbonyl groups.

The nuclear magnetic resonance spectrum gave the expected pattern. The phenolic proton was observed at $\delta = 11.8$, at the same position found for the phenolic proton in juglone. This is rather surprising, one would expect the bromine groups to shift the phenolic proton absorption further downfield. Chemical shift values for all of the protons are given in Table 6.

The mass spectrum of 2,3,6-tribromojuglone showed a parent peak at m/e 408. The ion had a molecular composition of $C_{10}H_3Br_3O_3$. The

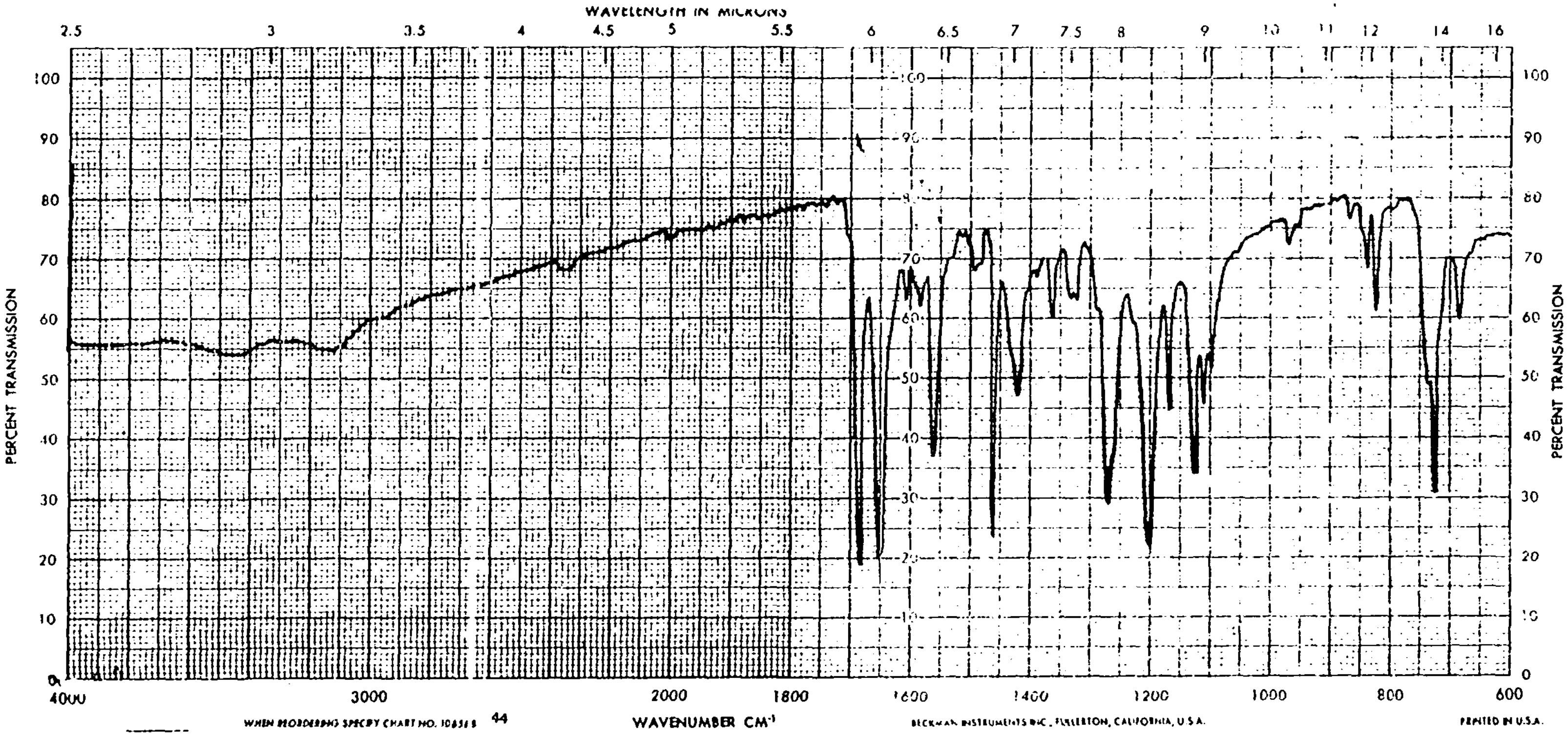


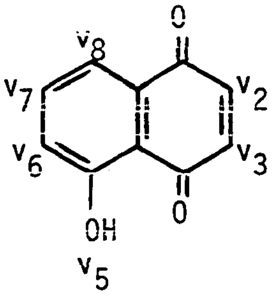
Figure 14. Infrared absorption spectrum of 2,3,6-tribromojuglone in potassium bromide.

Table 6. Summary of chemical shifts[a] of various naphthoquinones in $CDCl_3$.

Compound	v_2	v_3	v_5	v_6	v_7	v_8
Juglone	7.0	7.0	11.8	7.2	7.6	7.58
8-Chlorojuglone	7.05	7.05	12.78	7.41	7.7	----
2,3,6-Tribromojuglone	----	----	11.8	----	7.9	7.5
7-Me-8-Cl-juglone	6.8	6.8	12.73	7.24	2.32	----
6-Me-8-Cl-juglone-	7.0	7.0	13.1	2.35	7.6	----

[a] Delta values are referenced to TMS.

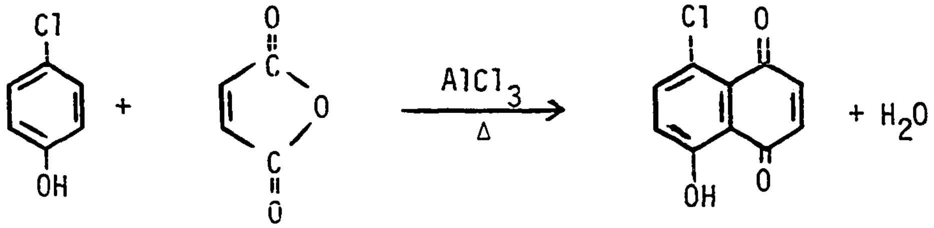
Numbering of Protons on Hydroxynaphthoquinones



molecule did not form a green trimeric compound on steam distillation or warming in dimethylsulfoxide. This failure to disproportionate indicates that the 2-, 3- or 6- position must be unsubstituted for disproportionation to occur.

2. 8-Chlorojuglone

8-Chlorojuglone was synthesized by the condensation of maleic anhydride and 4-chlorophenol:



a melt of 80 g of anhydrous aluminum chloride and 16 g of sodium chloride was heated to 150^0. To this solution a pulverized mixture containing 8 g of maleic anhydride and 4 g of 4-chlorophenol was added. The solution was cooled with stirring to 120^0 and the reaction was quenched with a mixture of hydrochloric acid and ice. After standing several hours, the precipitate was filtered, dried and placed in a Soxhlet extractor with light petroleum ether, Skelly A. The crude material obtained from the distillation of Skelly A was further purified by vacuum sublimation. The melting point obtained was 118^0, reported 120^0 (50).

The nuclear magnetic resonance spectrum of 8-chlorojuglone gave the expected pattern. Chemical shifts for the protons are reported in Table 6.

The infrared absorption spectrum is shown in Figure 15. The spectrum of the corresponding trimer is also presented. The absence of an OH stretching absorption in the spectrum of 8-chlorojuglone is probably the result of the strong intramolecular hydrogen bond between the phenolic proton and the adjacent carbonyl group (7). This hydrogen bond should be stronger in 8-chlorojuglone than juglone inasmuch as the chlorine group is electron-withdrawing.

During the recovery of 8-chlorojuglone, a dark green compound formed on the upper walls of the boiling flask of the Soxhlet extractor. The green material also formed during sublimation and on warming the 8-chlorojuglone in dimethylsulfoxide. The green compound was found to be highly insoluble, exhibit no melting point below 300°, and showed an absence of peaks in the nuclear magnetic resonance spectrum.

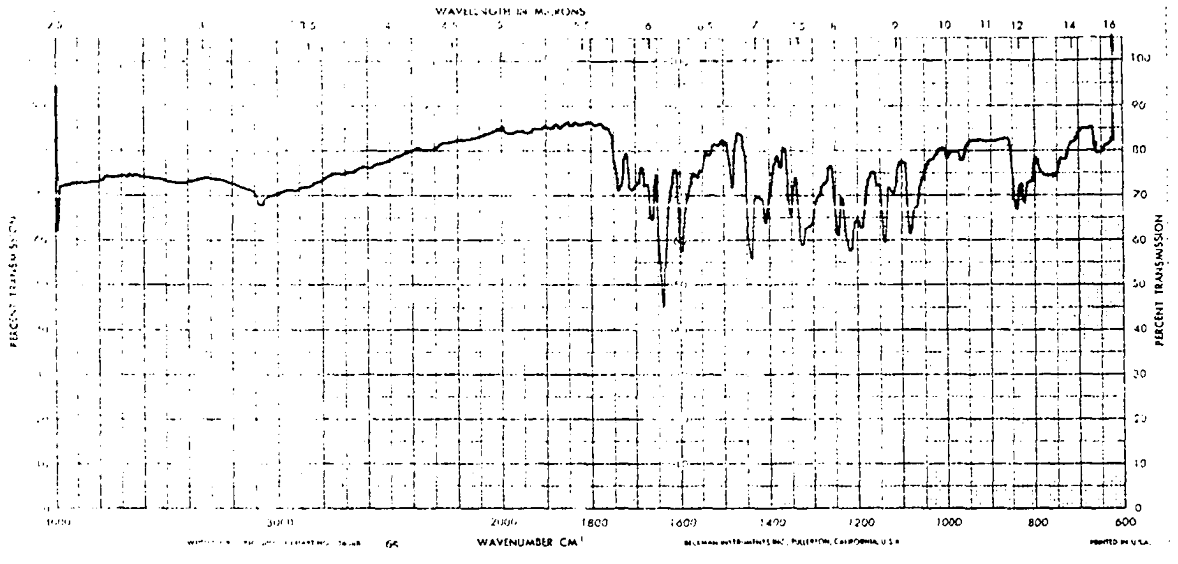
3. 7-Methyl-8-chlorojuglone

The synthesis used for 7-methyl-8-chlorojuglone was identical to that given above for 8-chlorojuglone. 3-Methyl-4-chlorophenol (4.2 g) was used in place of the 4-chlorophenol. The melting point was observed at 170°, reported 163° (51).

The nuclear magnetic resonance spectrum gave the predicted pattern, chemical shifts are shown in Table 6. The infrared absorption spectrum is presented in Figure 16 along with the corresponding trimer. Again, no hydroxyl stretch is observed due to the strong intramolecular hydrogen bonding to the carbonyl.

During the recovery process of 7-methyl-8-chlorojuglone from the filtrate, no green compound formed. However, when the derivative was

Figure 15. Infrared absorption spectrum of 8-chlorojuglone (top) and 8-chlorojuglone green (bottom) in potassium bromide.


WAVELENGTH IN MICRONS
6
7
9
10
11
12
14
16
100
90
80
70
60
50
40
30
10
0
PERCENT TRANSMISSION
3000
2000
1800
1600
1400
1200
1000
800
600
WAVENUMBER CM⁻¹
BECKMAN INSTRUMENTS INC., FULLERTON, CALIFORNIA, U.S.A.
PRINTED IN U.S.A.

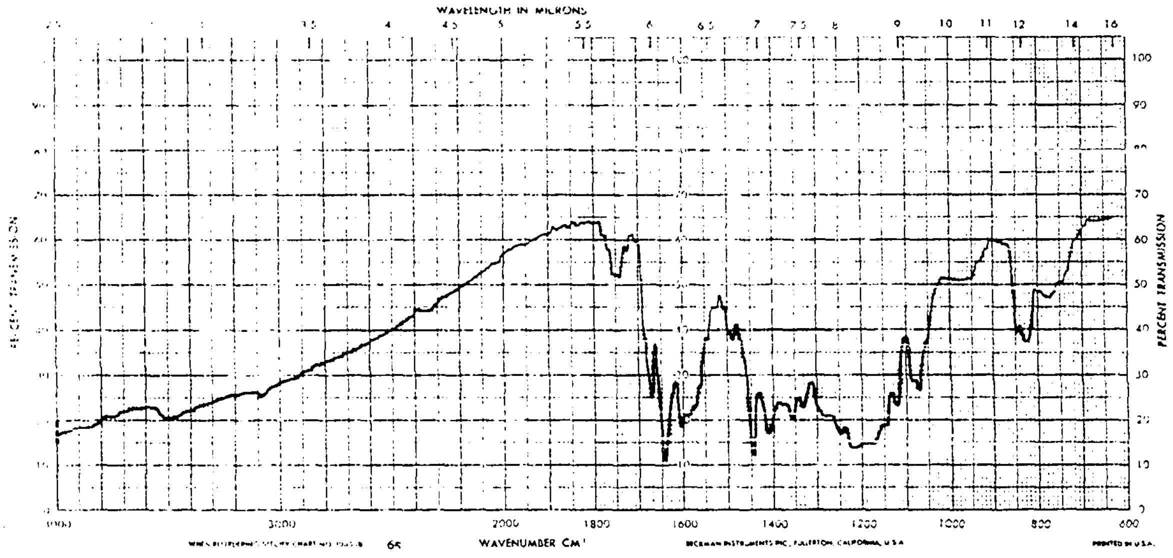

WAVELENGTH IN MICRONS
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
9
10
11
12
14
16
100
90
70
60
50
40
30
20
10
0
PERCENT TRANSMISSION
3000
2000
1800
1600
1400
1200
1000
800
600
WAVENUMBER CM⁻¹
BECKMAN INSTRUMENTS INC., FULLERTON, CALIFORNIA, U.S.A.
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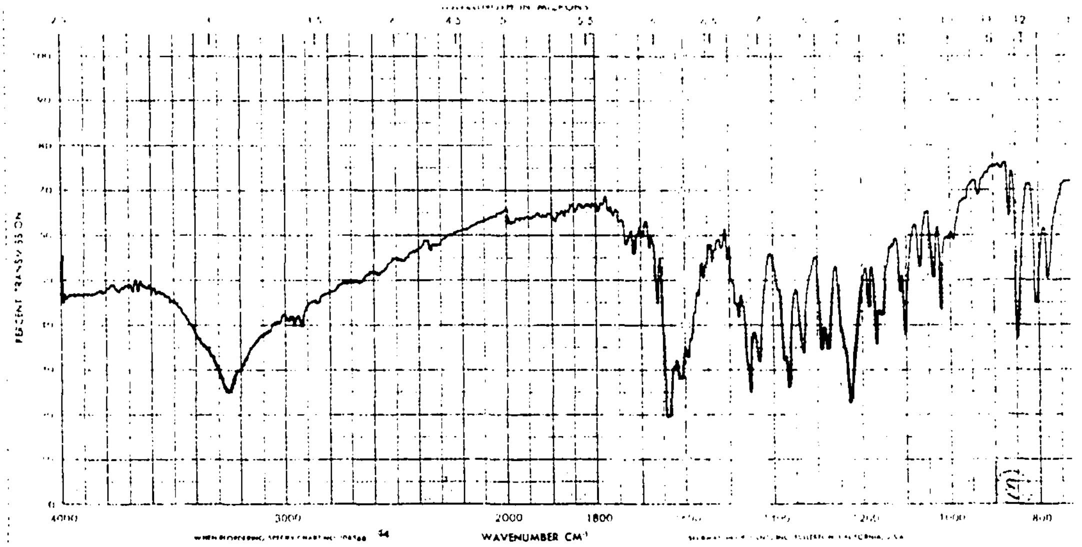
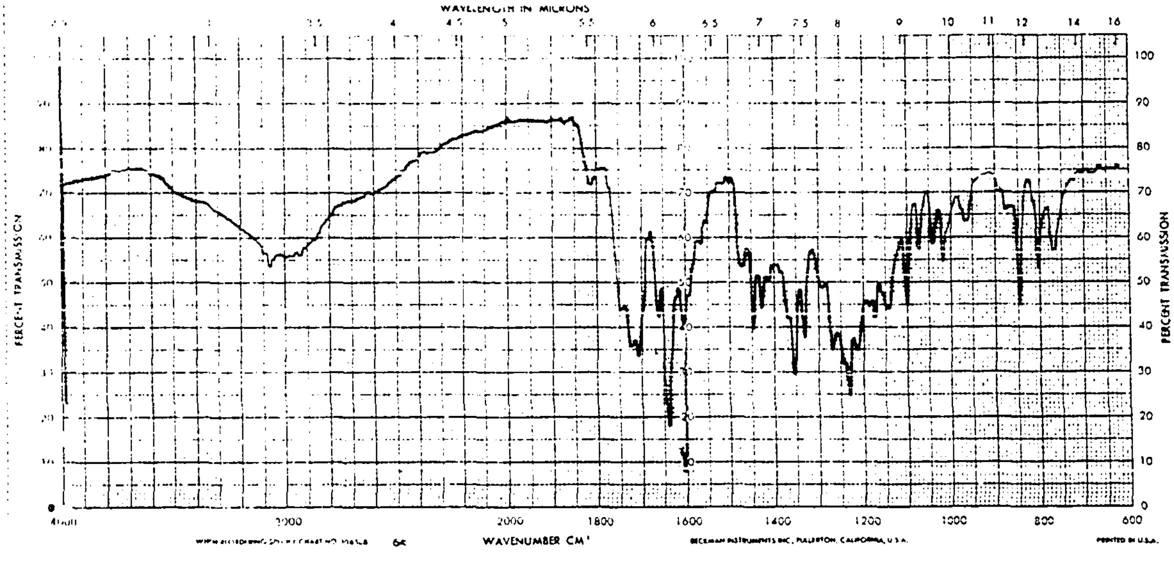
Figure 16. Infrared absorption spectrum of 7-methyl-8-chlorojuglone (top) and 7-methyl-8-chlorojuglone green (bottom) in potassium bromide.


WAVELENGTH IN MICRONS
PERCENT TRANSMISSION
4000
3000
2000
1800
800
WAVENUMBER CM⁻¹


WAVELENGTH IN MICRONS
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
9
10
11
12
14
16
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
0
4000
3000
2000
1800
1600
1400
1200
1000
800
600
WAVENUMBER CM⁻¹
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further purified by sublimation, a very small amount of the dark green trimer formed. The green material possessed many of the properties of juglone green in terms of solubility and absence of peaks in the proton magnetic resonance spectrum.

4. 6-Methyl-8-chlorojuglone

6-Methyl-8-chlorojuglone was synthesized using the same procedure given for 8-chlorojuglone. 2-Methyl-4-chlorophenol (4.2 g) was used in place of the 4-chlorophenol. The melting point was observed at 158° with decomposition.

The infrared absorption spectrum is shown in Figure 17. The nuclear magnetic resonance spectrum gave the expected pattern of 4 singlets. The chemical shift values and assignments are given in Table 6.

As with 2,3,6-tribromojuglone, where the position ortho to the hydroxyl group is also substituted, no green compound formed. The 6-methyl-8-chlorojuglone was synthesized in addition to the tribromojuglone since in the tribromojuglone molecule the ortho positions on the quinonoid ring were also substituted. Absence of a green compound with 6-methyl-8-chlorojuglone confirms that substitution occurs on the phenolic ring ortho to the hydroxy group.

No previous reference to 6-methyl-8-chlorojuglone has been found during a careful examination of the literature.

The maleic anhydride condensation with 4-chloro-2-methylphenol resulted in a 23 per cent yield of the naphthoquinone. The dark red-orange crystals sublime at 65° in a vacuum. The carbon, hydrogen

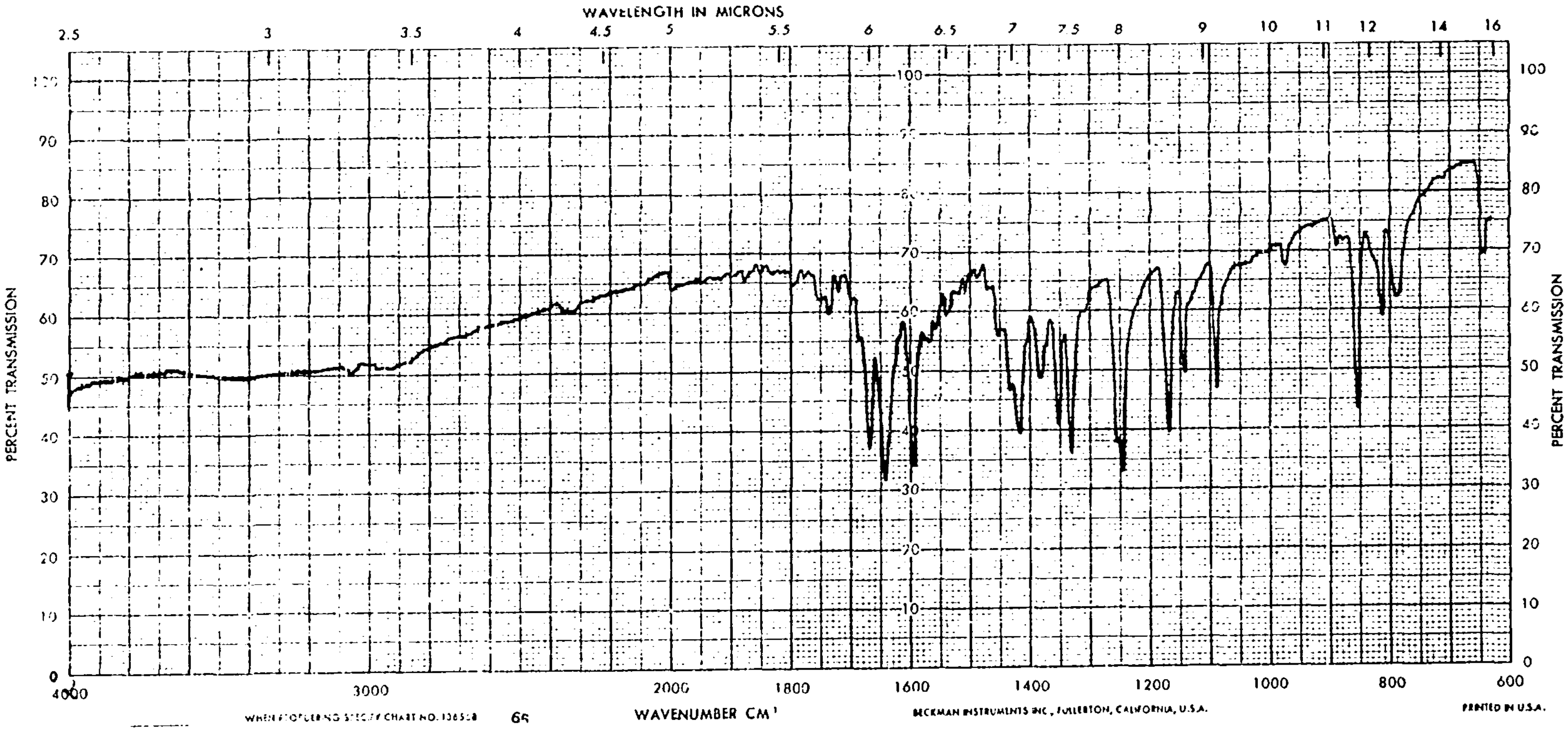


Figure 17. Infrared absorption spectrum of 6-methyl-8-chlorojuglone in potassium bromide.

and chlorine analysis obtained from Chemalytics Laboratories was: C, 58.63; H, 3.05; Cl, 15.37, the calculated values being: C, 59.46; H, 3.15; Cl, 15.77.

C. Conclusions

The results of these studies show that disproportionation of naphthoquinones to trimeric radicals held together by hydrogen bonding is not unique to juglone. Oxidative coupling in hydroxynaphthoquinones can occur where the position ortho to the phenolic group is unsubstituted.

IX. THE STRUCTURE OF JUGLONE GREEN

> *"From a drop of water," Holmes had written, "A logician could infer the possibility of an Atlantic or a Niagara without having seen or heard of one or of the other — A Conan Doyle.*

Based on the evidence presented in preceding chapters, I conceive juglone green to have been formed by the disproportionation of juglone and to have the composition and structure described in the following paragraphs. An abbreviated summary of the findings on which the conclusions are based is given in Table 7.

1. *Disproportionation Involved. Molecular Weight and Composition.* The disproportionation involves three molecules of juglone, two molecules of the juglone being oxidized and one reduced, the oxidized and reduced species then coupling to form a molecular compound. Overall, the reactions are:

$$3\ C_{10}H_6O_3 = \underset{\text{(Mol. Wt. 522.5)}}{C_{30}H_{18}O_9} \qquad (24)$$

or

$$3\ C_{10}H_6O_3 = \underset{\text{(Mol. Wt. 520.5)}}{C_{30}H_{16}O_9} + H_2 \qquad (25)$$

The molecular weight by the Rast method, 475 ± 50 (Chapter V, Part B, p. 40), and by mass spectrometry, 520 ± 2 (Chapter V, Part A, p. 37), indicate that juglone green is a trimer of juglone. The molecular weight calculated from titration data on the assumption that two replaceable hydrogen atoms are involved, 520 ± 5 (Chapter V, Part C, p. 43) is

Table 7. Summary of observations on juglone green.

Molecular Weight	520 ± 2
Composition	$C_{30}H_{16}O_9$, $C_{30}H_{17}O_9$, or $C_{30}H_{18}O_9$
Mass Spectrum	Weak parent peak m/e 520 Weak peak m/e 345 Intense peaks m/e 174, 175, 176
Neutralization Titration	Equivalent weight 260 ± 2
Infrared Spectrum	Hydrogen-bonded OH groups present; One or more carbonyl groups present; Aroxyl radical probably present
Solubility	Insoluble in water and most organic solvents
Nuclear Magnetic Resonance	No peaks
Carbon-13 NMR	No peaks
Electron Spin Resonance	Strong signal indicating a free electron
Arc Emission	No metals present
Electrochemical Response	0.040 V/pH

also not sufficiently accurate to decide between the two empirical formulas just proposed.

The determinations of the carbon and hydrogen content of juglone green (Chapter III, p. 21) agree best with $C_{30}H_{17}O_9$ but the errors in the determinations are probably sufficiently great to not preclude $C_{30}H_{18}O_9$ and $C_{30}H_{16}O_9$.

2. *Oxidative Coupling and Hydrogen Bonding Involved.* The two molecules of juglone which are oxidized during the disproportionation undergo oxidative coupling. In more detail then, equations (24) and (25) become:

$$3\ C_{10}H_6O_3 = (C_{20}H_{10}O_6)\{\not H\}(C_{10}H_8O_3) \qquad (26)$$

(Mol. Wts. 346.3 and 176.1)

$$3\ C_{10}H_6O_3 = (C_{20}H_9O_6\cdot)\{\not H\}(C_{10}H_7O_3\cdot) + H_2 \qquad (27)$$

(Mol. Wts. 345.3 and 175.2)

the superscript dot indicating an unpaired electron and the symbol $\{\not H\}$ representing a hydrogen bond. (Note that $\{\not H\}$ is not a representation of a chemical, all hydrogen atoms in the molecule being included in the empirical formulas enclosed in the parentheses.)

The mass spectrum of juglone green (Chapter V, Part A, p. 37) obtained with very high excitation techniques showed peaks of low intensity at 520 ± 2 and 345 ± 2, and intense peaks at 176, 175 and 174, there being no uncertainty in these latter masses. Few discernible peaks occurred between 520 and 345 and relatively few between 345 and 176. Juglone green apparently consists of two entities of molecular weights 345 ± 2, and 174 to 176 held together by very weak bonds. A similar pattern was found in the mass spectrum of quinhydrone, that is, a parent peak for the molecular compound and few peaks between it and the masses corresponding to quinone and hydroquinone. By analogy the bonding between the component entities of juglone green is also hydrogen bonding.

The infrared spectrum of juglone green (Chapter VI, Part A, p. 48) indicates that there is present in the molecule one or more hydrogen bonded hydroxyl groups, an aroxyl group and carbonyl groups, In the latter respect, juglone green resembles juglone in which there are present one simple carbonyl group and another carbonyl group hydrogen bonded to the neighboring hydroxyl group. The infrared spectrum thus supports the hypothesis that the component parts of juglone green are held together by hydrogen bonding.

3. *Juglone Green a Radical.* Juglone green is a radical, that is, a compound containing an unpaired electron.

The presence of an unpaired electron is inferred from the absence of any bands from the proton magnetic resonance spectrum (NMR) and from the carbon-13 nuclear magnetic resonance spectrum (Chapter VI, Parts B and C).

The NMR spectra were obtained on an aqueous, alkaline solution and on a dimethylsulfoxide solution. Juglone green presumably exists as the molecular compound in dimethylsulfoxide, the solution being green. In alkali, the color is purple and presumably the hydrogen bonding is lost by the neutralization of the phenolic groups; nevertheless no NMR signal was obtained and juglone green presumably exists in solution as the two, separated component parts, each bearing an unpaired electron (see 4., below).

The very strong signal in the electron spin resonance spectrum (e.s.r.) of juglone green is direct evidence for the presence of an unpaired electron. The signal appeared in the spectrum both of solid juglone green and of a solution of juglone green in dimethylsulfoxide.

The absorption band in the infrared spectrum of juglone green at 1590 cm^{-1} (Chapter VI, Part A, p. 48) indicates the presence of an aroxyl group, C-O· (unpaired electron).

4. *Juglone Green a Biradical.* Juglone green is probably a biradical that is, two unpaired electron are present in the molecule.

Unfortunately, the electron spin resonance method does not provide a measure of the number of unpaired electrons in a radical and the proof is not direct. The e.s.r. spectrum does indicate that there is no unpaired electron interaction so that if two unpaired electrons are present, the system is a biradical as opposed to a triplet state (Forrester, Hay and Thomson (41)).

Unfortunately, also the molecular weight of juglone green is not known with sufficient precision that the question can be settled by the stoichiometry of the disproportionation. That at least one unpaired electron is present rules out the stoichiometry expressed by equation (26) and favors equation (27), formation of the biradical; but a third stoichiometry, in which a monoradical is formed, also is possible:

$$3\ C_{10}H_6O_3 = (C_{20}H_9O_6\cdot)\{\cancel{H}\}(C_{10}H_8O_3) + \tfrac{1}{2}H_2 \qquad (28)$$

(Mol. Wt. 521.5)

The monoradical so formed has the merit that it agrees with the empirical formula best agreeing with the carbon and hydrogen content, that is $C_{30}H_{17}O_9$: it is not compatible with the less convincing arguments offered in the next two paragraphs.

As discussed separately below, results of the titration of juglone green with standard acid (Chapter V, Part C, p. 42) is best explained by the addition of hydrogen ions to two unpaired electrons.

As also discussed separately below the change in the reduction potential of solutions saturated with juglone green changes with pH in an unusual manner; again, the behavior observed can be explained best on the basis of two unpaired electrons.

5. *Reduction Potential.* As reported in Chapter IV, p. 32, the reduction potential of solutions saturated with juglone green changes 40 mV per pH unit, the potential being shifted negative by the removal of oxygen from the solution and positive by the addition of oxygen, the changes not being reproducible.

This experiment with juglone green was prompted by the striking similarity in physical appearance of juglone green to quinhydrone. The response of the reduction potential to pH was again similar and it is indeed tempting to interpret the response by analogy to the theory of the quinhydrone electrode (see Chapter IV, p. 28). The response of 40 mV/pH unit implies, through the Nernst electrode potential equation, that the oxidized and reduced species of the couple are interrelated by protons and electrons in the ratio of two to three. Unfortunately no stoichiometry can be written using the oxidized and reduced forms of the molecular (hydrogen bonded) compounds, empirical formulas for which are derived in equations (26), (27), and (28), and protons and electrons in the ratio of two to three, without writing, on the right-hand side of the equation, the reduced species in two, differently charged forms. Thus, for example, for the empirical formula for juglone green derived in equation (27), $(C_{20}H_9O_6\cdot)\{\cancel{H}\}(C_{10}H_7O_3\cdot)$, the couple would be:

$$C_{20}H_9O_6\cdot + 4H^+ + 6e^- = C_{10}H_7O_3\cdot + C_{10}H_6O_3\cdot\cdot \qquad (29)$$

This violates the assumption on which the theory was based.

The situation is complicated by several factors: The trimeric nature of juglone green, the presumably dimeric character of the oxidized species, the presence of an unpaired electron in the oxidized specie, or in the reduced specie, or in both. It may be that the couple actually involves molecular hydrogen reversibly; the response of the potential to oxygen suggests this. More experimental work is required.

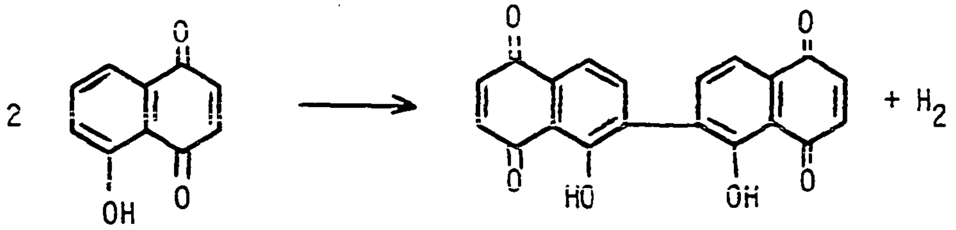
6. *Titration of Unpaired Electrons with Standard Acid.* The titration curve and data obtained by dissolving juglone green in standard alkali and back-titrating with standard acid (Chapter V, Part C, p. 42) are best explained as the titration of two unpaired electrons with acid. The average value for the dissociation constants of these unpaired electrons is $pK_A = 7.7$.

The acid dissociation constant of the phenolic group is normally between 10^{-10} and 10^{-14} (pK_A 10 to 14); the dissociation constant for juglone, for example in dioxane-water is 13.9 (Kido, Fernelius and Haas (52)). Titration of aroxyl radicals with acid has been reported

(Buchachenko (44)). It is reported also that the protonated forms are unstable, decomposing rapidly.

Juglone green is soluble in alkaline solution, a behavior expected of phenolic compounds. In the back titration with standard acid the standard alkali is neutralized and the phenolate ions converted to phenol groups, reactions which are complete when the first end-point is reached. A second point of inflection is present at two equivalents of acid added beyond the first point of inflection. No discernible point of inflection is present in the titration curve at one equivalent of acid added indicating the values of the two dissociation constants are closely the same; the ratio of the two dissociation constants is probably less than 100. The titration can only be explained as the addition of a hydrogen ion to each of two unpaired electrons.

7. *Oxidative Coupling During Disproportionation.* The internal oxidation which occurs during the disproportionation of juglone is oxidative coupling of two molecules of juglone in the 6-positions:

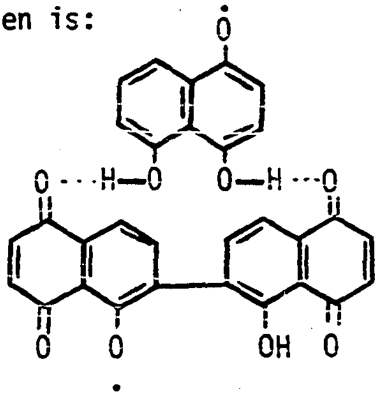


The peak found in the mass spectrum of juglone at 345 ± 2 corresponds in mass to two molecules of juglone. Presumably the coupling was oxidative, by the removal of two hydrogen atoms. Oxidative coupling is widespread in nature, enzymes commonly catalyzing the formation and coupling of phenoxy radicals (53). Such coupling commonly takes place ortho to phenolic groups, in the 6-position in juglone. Hooker (54), for example, found that lawsone (2-hydroxy-1,4-naphthoquinone), an isomer of juglone, on oxidation yielded 3,3'-bis(2-hydroxy-1,4-naphthoquinone).

The change in the NMR spectrum of juglone dissolved in D_6-DMSO with time, Chapter VII, Part A, p. 70, indicates that the protons at positions 2 and 3 (those in the quinonoid ring) remain unaffected during the disproportionation. Whereas, the splitting pattern of the three protons in the phenolic ring were altered as the conversion progressed.

Indirect evidence for the 6,6'-union was obtained from a study of certain substituted juglones (Chapter VIII). Compounds in which the 6-position was blocked by a substituent, as in 6-methyl-8-chlorojuglone and 2,3,6-tribromojuglone, did not form a molecular compound. However, when the 7- or 8-positions of juglone were blocked but the 6-position left unsubstituted as in 7-methyl-8-chlorojuglone and 8-chlorojuglone, the corresponding insoluble green compound was formed.

8. *Structure Proposed for Juglone Green.* Putting together the evidence presented in the foregoing eight chapters, I conclude that juglone green is:



$= C_{30}H_{16}O_9\cdot\cdot$

$= (C_{20}H_9O_6\cdot)\{H\}(C_{10}H_7O_3\cdot)$

X. SPECULATION ABOUT JUGLONE GREEN

As imagination bodies forth
The forms of things unknown, the poet's pen
Turns them to shape, and gives to airy nothing
A local habitation and a name —

A Midsummer Night's Dream

The relative ease with which juglone is converted to juglone green and the astonishing properties of this free radical material lead inevitably to speculation as to the possible presence of it in natural products and as to the role the sort of chemistry which produced it may play in synthetic chemistry. The speculations raised here are first framed as questions and then the background and a possible attack on the particular problem are discussed.

1. Is the green color of the skin of the ripening walnut juglone green rather than chlorophyll?

This may possibly be true inasmuch as juglone is present in the pericarp of the unripe walnut as a glucoside of the reduced form hydrojuglone. Hydrolysis and oxidation could well occur in the skin on exposure to air. Proof should be easy: peel off the skin of the pericarp, shred and obtain the e.s.r. spectrum of the solid.

2. Is juglone green allelopathic, or phrased another way is the property of juglone of poisoning plants exerted first by conversion to juglone green?

Free radicals are usually very reactive chemically because of the tendency to gain an additional electron and have a complete octet. This tendency for juglone green to react and pair up the free electrons, could result in the allelopathic property ascribed to juglone.

Juglone is reported to be toxic to tomatoes, potatoes and other important economic crops (55). The actual mechanism of transfer of juglone from it's natural source, the walnut tree is still not known. Conceivably, the transfer mechanism may involve conversion to juglone green. To test the allelopathicity of juglone green, plants could be treated with dilute aqueous solutions and see if the toxic effects are similar to those reported in juglone poisoning.

3. Is juglone green present in, and indeed, perhaps make up the bulk of the tar formed during the preparation of juglone by oxidation of 1,5-naphthalenediol?

In the oxidation of 1,5-naphthalenediol to juglone, a large amount of tar is formed. Because the tar is dark brown in color, it is difficult to visually detect any juglone green. To determine if in fact juglone green is present, the e.s.r. spectrum of the tar could be run, assuming no other stable radicals are present.

4. If the answer to 3 is yes, can juglone green be converted to juglone by 1) dissolving the tar in alkali, 2) reducing cleavage with hydrosulfite or zinc dust, and 3) acidification to pH less than 5.5 and steam distilling?

This raises the question of the nature of the substance or substances present when juglone green is dissolved in alkaline solution. No NMR spectrum can be obtained, the hydrogen bonding postulated for juglone green must be broken, and the conclusion must be that the separate oxidized and reduced components are present as free radicals. The titration data support this.

5. Juglone stuns fish. Does juglone green stun fish? Try them both on gold fish.

6. Does juglone green react with free radical reagents?

7. Does juglone green form derivatives with metal ions?

XI. SUMMARY

> *I learned this at least, by my experiment; that if one advances confidently in the direction of his dreams, and endeavors to live the life which he has imagined, he will meet with a success unexpected in common hours — Henry David Thoreau.*

A review has been made of the proposal initially presented in my Master of Science thesis to synthesize an oxidation-reduction couple bearing a chelating group.

The initial work on this project, the synthesis of juglone, has been plagued by low yields because of the formation of tarry by-products.

Repeatedly during the synthesis and purification of juglone green a dark green, very insoluble material has been obtained. This dark green material has been shown to have unusual properties and has been investigated in detail in the work described in this dissertation. The material has been given the common name *juglone green.*

Methods for the synthesis of juglone have been reviewed.

A new reagent for the oxidation of 1,5-naphthalenediol, involving pyridinium chlorochromate as the oxidizing agent, has been tested. The yields of juglone, 7 per cent, have not been greatly improved over earlier procedures but the method lends itself to preparations on a large scale.

The synthesis of juglone by the condensation of phenol and maleic anhydride has been found to fail.

The conditions under which juglone green is formed, during the extraction, steam distillation and sublimation of juglone, has been examined in detail.

A survey has been made of the literature and only three references to juglone green have been reported. The 1885 paper of Mylius has been found to be of most interest.

The formation of juglone green has been found to be a disproportionation of juglone; oxygen and water are not involved in the disproportionation.

The disproportionation of juglone during heating has been found to depend on pH, the conversion to juglone green being rapid and complete in alkaline solution and slow below pH 5.5.

Juglone green has been produced during the anodic oxidation of 1,5-naphthalenediol.

The carbon and hydrogen content of juglone green has been determined; the empirical formula calculated from these data is $C_{30}H_{18}O_9$, $C_{30}H_{17}O_9$, or $C_{30}H_{16}O_9$, the uncertainty in the analysis not permitting further differentiation.

Juglone green has been found to bear a striking resemblance to quinhydrone in that the reduction potential of a solution saturated with juglone green depends on the hydrogen ion concentration of the solution. The response, however, has been found to be 40 mV per pH unit in contrast to the 60 mV per pH unit obtained with quinhydrone.

The theory of the quinhydrone electrode has been reviewed. Application of the same theory to juglone green leads to the conclusion that the oxidized species and reduced species present in juglone green are interrelated by a reaction involving two hydrogen ions and three electrons.

Oxygen has been found to alter the potential of the juglone green couple but the behavior is not reproducible.

The molecular weight of juglone green has been determined by three independent methods: mass spectrometry, the Rast method, and titration; the molecular weight is close to 520 and juglone green is apparently a trimer of juglone.

The mass spectrum of juglone green has been interpreted as indicating that juglone green is a molecular compound, total mass 520 ± 2, consisting of molecules of masses 345 ± 2 and 176, 175 and 174.

The various forms of spectrophotometry have been used to investigate the properties of juglone green. By infrared spectroscopy, juglone green has been found to contain hydrogen-bonded phenolic groups, and two or more different carbonyl groups. An intense peak appearing at 1590 cm^{-1}, not present in the spectrum of juglone or quinhydrone, has been assigned to a $\nu(C\text{-}O\cdot)$ absorption, characteristic of aroxyl radicals.

The proton magnetic resonance spectrum and the carbon-13 nuclear magnetic resonance spectrum have been obtained and found to have no peaks. This has been interpreted as caused by the presence in the molecule of an unpaired electron.

A strong signal has been found in the electron spin resonance spectrum of juglone green. Proof has, thus, been found that a free electron is present in the molecule.

The arc-emission spectrum has been obtained; no metals are present in juglone green.

The ultraviolet spectrum of juglone green has been recorded. Juglone green has been found to conform to Beer's law. Alkaline solutions of juglone green have been found to be relatively stable, but acid solutions decompose rapidly.

The disproportionation of juglone to juglone green has been observed to occur in dimethylsulfoxide. The nuclear magnetic resonance spectrum of juglone in dimethylsulfoxide has been recorded at intervals. The spectra indicate that the disproportionation involves the protons in the phenolic ring.

The number of electrons contained in juglone green has been determined by a measurement of magnetic susceptibility. The result, 1.3 electrons per molecule, is inexplicable and an error in the experiment is suspected.

An experiment has been designed to determine if hydrogen gas is evolved during the disproportionation of juglone. The result, as determined by mass spectrometry has been found to be negative, that is, no molecular hydrogen was observed.

Additional naphthoquinone trimers have been synthesized. Oxidative coupling has been found to occur when, and only when the 6-position (that ortho to the phenolic group) is unsubstituted.

6-Methyl-8-chlorojuglone, previously unknown, has been synthesized.

A structure for juglone green has been proposed based on the various findings being reported. The molecule is a trimer consisting of 6,6'-bis(5-hydroxy-1,4-naphthoquinone) hydrogen bonded to a molecule of hydrojuglone. The molecule is a biradical, that is, contains two unpaired electrons.

Some speculations as to the chemical and biological roles of juglone green have been offered. Suggestions for future work have been presented.

XII. LITERATURE CITED

> *By reading the old journals we come in contact with the masters of experimentation and of reasoning who have gone before. . . . They laid the foundations of the science of to-day and they have much to teach us as to methods of thinking and working . . . .*
>
> *If we are to add to human knowledge we must know exactly how far that knowledge has gone, at least to the point where we are to do the adding: we must find the end of a rope before we can splice a piece onto it — E.E. Reid.*

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