PREPARATION AND STUDY OF SOME NITROGEN ANALOGS OF QUINONES AS POTENTIAL TUMOR INHIBITORS

By

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OF QUINONES AS POTENTIAL TUMOR INHIBITORS

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TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	HISTORICAL	4
III.	EXPERIMENTAL	17
	Preparation of Compounds	17
	2,6-Dibromo-4-nitrophenol and 4,6-Dibromo-2-nitrophenol	17
	2,6-Dichloro-4-nitrophenol and 4,6-Dichloro-2-nitrophenol	18
	2,6-Dihalo-4-aminophenol Chlorostannate and 4,6-Dihalo-2-aminophenol Chlorostannate	20
	N,2,6-Trihalo-1,4-benzoquinone 4-Imine and N,4,6-Trihalo-1,2-benzoquinone 2-Imine	21
	N-Halo-1,4-benzoquinone 4-Imines	23
	Indoaniline Derivatives With Substituents on the Quinone Ring	24
	Quinone 4-Oximes	25
	3-Halo-1,4-benzoquinone 4-Oximes	27
	2,4-Dinitro-1-naphthol	29
	Ammonium Salt of 2,4-Dinitro-1-naphthol	31
	2,4-Diamino-1-naphthol Dihydrochloride	31
	2-Amino-1,4-naphthoquinone 4-Imine Hydrochloride	32
	N,N'-Diacetyl-2-amino-1,4-naphthoquinone 4-Imine	33
	Attempts to Synthesize Other Compounds	34
	Proof of Structures of Compounds	35
	Studies of Compounds	50
	Stability Studies	50
	Toxicity Studies	69
	Inhibition of Ascitic Sarcoma 180	69
	Oxidation-Reduction Potentials	79
IV.	RESULTS AND DISCUSSION	81
BIBLIOGRAPHY		84

LIST OF TABLES

Table		Page
I.	Dibromonitrophenols	18
II.	Dichloronitrophenols	19
III.	N,2,6-Trihalo-1,4-benzoquinone 4-Imines	22
IV.	N,4,6-Trihalo-1,2-benzoquinone 2-Imines	23
V.	N-Halo-1,4-benzoquinone 4-Imines	24
VI.	Indoaniline Derivatives	26
VII.	Quinone 4-Oximes	28
VIII.	3-Halo-1,4-benzoquinone 4-Oximes	30
IX.	Elemental Analyses of New Compounds	37
X.	Infrared Spectral Assignments for Quinone 4-Oximes	38
XI.	Infrared Spectral Assignments for 1,4-Benzoquinone 4-Imines	39
XII.	Infrared Spectral Assignments for 1,2-Benzoquinone 2-Imines	39
XIII.	Infrared Spectral Assignments for Indoanilines	40
XIV.	Hydrolysis of 1,4-Benzoquinone 4-Oxime	56
XV.	Hydrolysis of 2-Chloro-1,4-benzoquinone 4-Oxime	57
XVI.	Hydrolysis of 2-Methoxy-1,4-benzoquinone 4-Oxime	58
XVII.	Hydrolysis of N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine	59
XVIII.	Hydrolysis of N,2,6-Tribromo-1,4-benzoquinone 4-Imine	60
XIX.	Hydrolysis of N,2,6-Trichloro-1,4-benzoquinone 4-Imine	61
XX.	Hydrolysis of N-Chloro-2,6-dibromo-1,4-benzoquinone 4-Imine	62

LIST OF TABLES (Continued)

Table		Page
XXI.	Hydrolysis of N,4,6-Trichloro-1,2-benzoquinone 2-Imine . .	63
XXII.	Hydrolysis of N,4,6-Tribromo-1,2-benzoquinone 2-Imine. .	64
XXIII.	Hydrolysis of N-Chloro-1,4-benzoquinone 4-Imine.	65
XXIV.	Hydrolysis of N,N-Dimethylindoaniline.	66
XXV.	Hydrolysis of 2-Chloro-N,N-dimethylindoaniline	67
XXVI.	Hydrolysis of 2-Methyl-N,N-dimethylindoaniline	68
XXVII.	Hydrolysis Rates of the Compounds--Summary	70
XXVIII.	Toxicities of Compounds.	71
XXIX.	Inhibition of Ascitic Sarcoma 180 by Quinone 4-Oximes. .	73
XXX.	Inhibition of Ascitic Sarcoma 180 by N-Halo-1,4-benzoquinone 4-Imines	74
XXXI.	Inhibition of Ascitic Sarcoma 180 by N,2,6-Trihalo-1,4-benzoquinone 4-Imines.	75
XXXII.	Inhibition of Ascitic Sarcoma 180 by N,N-Dimethylindoaniline Derivatives.	76
XXXIII.	Inhibition of Ascitic Sarcoma 180 by Indophenol Derivatives.	77
XXXIV.	Inhibition of Ascitic Sarcoma 180--Summary	78
XXXV.	Oxidation-Reduction Potentials of the Compounds.	80

LIST OF FIGURES

Figure		Page
1.	Proton Magnetic Resonance Spectrum of 2-Chloro-1,4-benzoquinone 4-Oxime in Dioxane -- 100 MHz.	43
2.	Infrared Spectrum of 2-Chloro-1,4-benzoquinone 4-Oxime -- Nujol Mull	44
3.	Proton Magnetic Resonance Spectrum of N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine in Acetone-d_6 -- 100 MHz . .	45
4.	Infrared Spectrum of N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine -- Nujol Mull.	46
5.	Proton Magnetic Resonance Spectrum of N-Bromo-4,6-dibromo-1,2-benzoquinone 2-Imine in Acetone-d_6 -- 100 MHz.	48
6.	Infrared Spectrum of N-Bromo-4,6-dibromo-1,2-benzoquinone 2-Imine -- Nujol Mull.	49
7.	Proton Magnetic Resonance Spectrum of N-Chloro-1,4-benzoquinone 4-Imine in Acetone-d_6 -- 100 MHz	51
8.	Infrared Spectrum of N-Chloro-1,4-benzoquinone 4-Imine -- Nujol Mull	52
9.	Proton Magnetic Resonance Spectrum of 2-Chloro-N,N-dimethylindoaniline in Acetone-d_6 -- 100 MHz	53
10.	Infrared Spectrum of 2-Chloro-N,N-dimethylindoaniline -- Nujol Mull	54

CHAPTER I

INTRODUCTION

Quinones and their derivatives occur widely in nature and many have important biological properties such as the ability to inhibit the growth of tumors and to destroy bacteria[1-2]. The structure-antitumor relationships among 1500 quinone derivatives have recently been described[3]. The biological properties of the quinones may be related to their protein-binding characteristics[4-7] and/or their oxidation-reduction potentials.

Mitomycin C, a quinone derivative that controls tumors of both animals and man, has been shown to be a strong inhibitor of the synthesis of nucleic acids[8]. Schwartz et al.[9] demonstrated that the reduction of the benzoquinone ring of the mitomycins to a dihydrobenzoquinone was an essential step for biological activity. Iyer and Szybalsky[10] showed that a NADPH (reduced form of nicotinamide-adenine dinucleotide phosphate)-dependent quinone reductase system was involved in the reductive activation step. Recently, Kinoshita and coworkers[11,12] reported a positive correlation between both antineoplastic and antimicrobial activity of a series of mitomycin derivatives and their reduction potentials. These investigators[11,12] also provided evidence that neither the carbamyl group nor the aziridine ring of the mitomycins was essential for biological activity. The essential portion of the mitomycins was considered to be the benzoquinone ring. Furthermore, since Cater and Philips[13] found a significantly lower oxidation-reduction potential for tumor tis-

sue than for most normal tissues, it is conceivable that a differential will exist between normal tissue and some cancers with compounds requiring bioreduction. Thus it was visualized by Lin et al.[14] that the NADPH-dependent enzyme system that reduces the mitomycins *in vivo*, which apparently is relatively nonspecific and therefore possesses the ability to reduce a variety of mitomycin-like derivatives[10-12], will also act upon the benzoquinones and naphthoquinones. Furthermore, it seems reasonable to assume that in the neoplastic cells of solid tumors distal to blood vessels, which traditionally are extremely resistant to chemotherapy, the oxygen tension is decreased, thereby creating conditions conducive to an increase in the ratio of reduced and oxidized pyridine nucleotide coenzyme. Such cells should theoretically be particularly sensitive to quinones which require bioreduction prior to exertion of their growth-inhibitory capacities. As a result, compounds of this type may be particularly useful against certain solid tumors.

In consideration of these concepts, a series of quinone imines which have structural resemblance to the quinones, with different substituent groups so as to have different transport properties and different capabilities of reacting with the active site, were synthesized. The structures of these compounds were proven by elemental analysis, infrared spectrometry, and nuclear magnetic resonance spectroscopy. Since the stabilities of these compounds in aqueous solution are important to their biological properties, the rates of hydrolysis were measured. Because the oxidation-reduction potentials of these compounds may play an important role in their biological functions, these were measured and some correlations with antitumor activities were attempted. Acute toxicities of these compounds in Swiss mice were determined and their activities in

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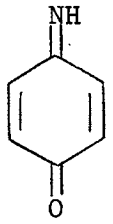
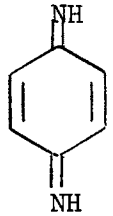
CHAPTER II

HISTORICAL

Early in the twentieth century, more than eighty years after 1,4-benzoquinone had been prepared, Willstätter began extensive and productive studies of 1,4-benzoquinone mono- and diimines (I and II) and related compounds.[15,16]

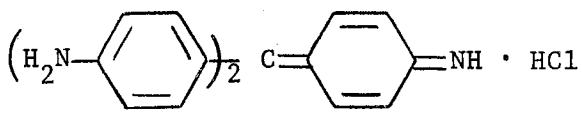


I



II

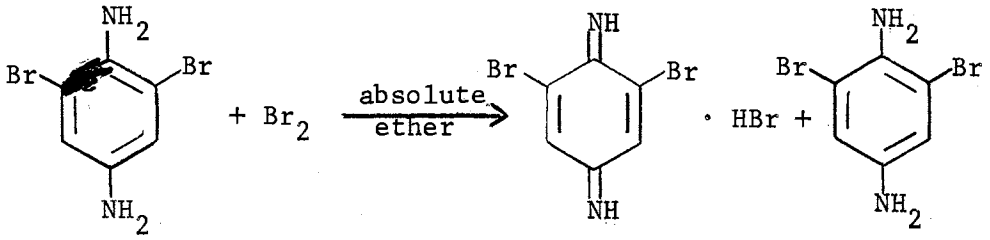
1,4-Benzoquinone monoimine (I) and diimine (II) are closely related to p-aminophenol and p-phenylenediamine, respectively. They may be regarded as derived from 1,4-benzoquinone by replacing one or both of the oxygen atoms by imino groups. At the time, circa 1880, when Nietzki's classical formula for the triphenylmethane dyes (III) was accepted it



III

was supposed that I and II would show powerful absorption in the visible spectrum and attempts were made to prepare them in order to test this prediction. Both compounds (I and II), however are quite unstable, and it was only by working with special precautions that Willstätter eventually isolated them. The mono-imine proved to be sulfur-yellow and the di-imine to be colorless.

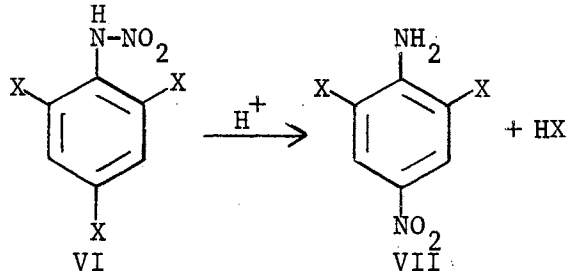
At approximately the same time as Willstätter's work on the quinone imines, an interesting approach to the synthesis of these compounds was taken by Jackson at Harvard.[17] He reasoned that heavy substitution of the ring might make the quinonoid form more stable. An attempted bromination of 2,6-dibromo-p-phenylenediamine (IV) produced a dark green precipitate having the characteristics of an equimolar mixture (V) of the hydrobromides of the quinone diimine and the starting diamine.



IV V

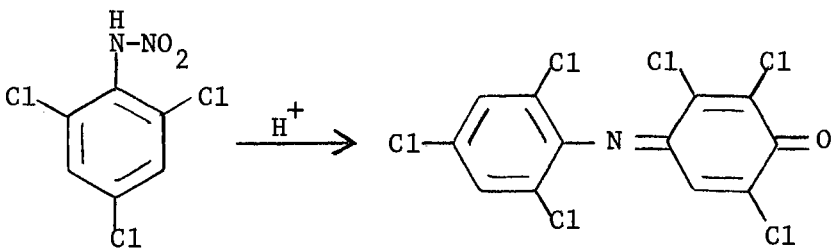
Similar results were obtained with p-phenylenediamine.

The rearrangement of N-nitroanilines (VI) to the ring-substituted isomers (VII), often with the displacement of halogen, is accompanied by



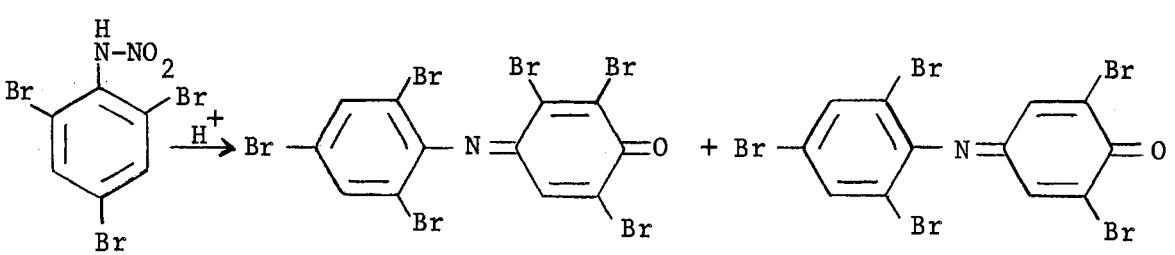
VI VII

side reactions giving magenta-colored products. A study of this reaction has been reported.[18] When a 4-chloro atom which resists displacement is present, the anil (IX) was obtained.



VIII IX

An attempted synthesis[19] of the corresponding tribromo compound (X) produced a mixture of (XI) and (XII).



X XI XII

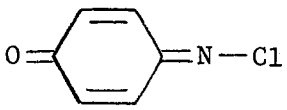
The suggestion was made that these quinonoid structures may be related to intermediates in the rearrangement of nitroanilines.

Long before the structures of the intermediates were determined, dye chemists had oxidized p-phenylenediamines with potassium dichromate, ferricyanide, and permanganate.[20,21] In fact, a wide variety of oxidizing agents can be used for the preparation of quinone diimines. Willstäter's use of silver ion is interesting in view of its subsequent use by photographic chemists.

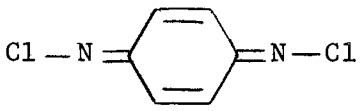
Present-day color photography is based on the discovery by Fischer[22,23] that exposed silver halide enhances the rate of the oxidative condensation of p-phenylenediamines with suitable molecules to form dyes. A wide variety of substituted p-phenylenediamines and p-aminophenols show these characteristics, but for practical photographic reasons the N,N-dialkyl substituted compounds have been most extensively investigated.[24]

During the past twenty years a number of studies have appeared which represent significant contributions to our understanding of the chemistry of quinone imines and related compounds.

1,4-Benzoquinone monoimine and diimine can be prepared by two methods. (1) When p-aminophenol and p-phenylenediamine are oxidized with bleaching powder, 1,4-benzoquinone chloroimine (XIII) and the dichlorodiimine (XIV) are formed.



XIII

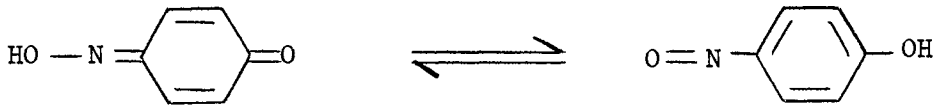


XIV

These are respectively yellow and colorless crystalline compounds, volatile in steam and hydrolyzed by dilute acids to 1,4-benzoquinone and ammonia. If these compounds are treated with hydrogen chloride in dry ether, chlorine is liberated as with other N-chloro compounds and the imines are formed. (2) The better method is to oxidize p-phenylenediamine or p-aminophenol, by dissolving it in dry ether and shaking the solution with silver oxide or lead peroxide in the presence of anhydrous

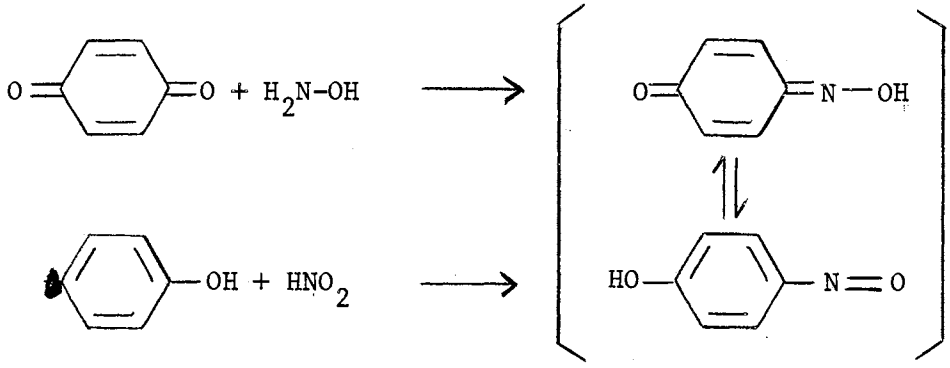
sodium sulfate to remove the water produced. Isolation of the products is difficult and their preparation only succeeds if it is carried out in the dark and the materials are carefully dried. 1,4-Benzoquinone diimine forms colorless explosive crystals. It polymerizes readily, especially in the presence of light or acids, and in aqueous solution it darkens and decomposes; its molecular weight in boiling acetone corresponds to the simple formula. 1,4-Benzoquinone diimines with alkyl groups attached to the ring polymerize less readily and are more stable, but all are readily hydrolyzed to ammonia and a quinone, even by boiling water. 1,4-Benzoquinone imine resembles the diimine but is even more unstable: it decomposes at room temperature in daylight in a few seconds.

Important work has been reported on quinone monoximes (XV) especially in regard to their tautomerism with nitrosophenols[25] (XVI).



XV XVI

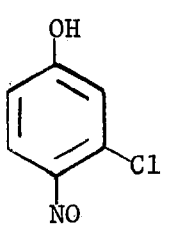
The two common methods of preparing these compounds both produce the same product mixture.



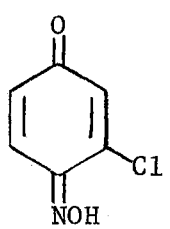
This system has been the subject of a great amount of study since the early part of the twentieth century. Extensive early studies of Hodgson[26,27] are well summarized in the literature[28]. They also provide data which lead to some interesting recent research.

In 1923 Hodgson and Moore reported studies which gave chemical evidence that they had succeeded in isolating 3-chloro-1,4-benzoquinone 4-oxime and 3-chloro-4-nitrosophenol as individual compounds. A number of reinvestigations have been made of this problem and the intriguing possibilities presented by it[29,30]. These latter studies have generally confirmed Hodgson's conclusions concerning the tautomerism of most quinone monoximes with p-nitrosophenols, but strongly questioned the isolation of tautomers in the 3-chloro case. In the early 1950's a series of papers appeared which seem to explain this difficulty[31].

When the nitrosation of 3-chlorophenol is carried out in aqueous media of low acidity the product melts at approximately 140°. When the same reaction is performed in sulfuric acid a product melting at 174° is obtained. These materials are believed to have benzenoid (XVII) and quinonoid (XVIII) structures respectively.



XVII



XVIII

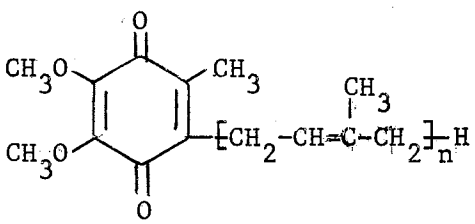
The low-melting product is unstable to light and can be transformed into the higher melting one by treatment with acid or alkali. All the evi-

dence indicates that the high-melting material is a homogeneous substance which in solution is an equilibrium mixture of benzenoid and quinonoid tautomers.

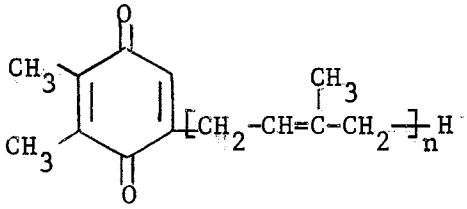
Early evidence implying that in solution 1,4-benzoquinone oxime exists as a tautomeric mixture came from a study of its methylation products[32,33]. 1,4-Benzoquinone oxime (XV) would be expected to give an O-methyl derivative, $Me\text{-}O\text{-}N{=}C_6H_4{=}O$, a known compound formed by the action of O-methylhydroxylamine on 1,4-benzoquinone, and this is indeed formed by the action of either diazomethane or methyl iodide and alkali on 1,4-benzoquinone oxime.

Quinones have been shown to function catalytically in animals, plants, and micro-organisms. These compounds are found in the subcellular organelles which contain the multi-enzyme respiratory complex responsible for the storage of energy in phosphate bonds through oxidative metabolism. Although these structures were known to contain large amounts of lipoidal materials, it is only recently that the nature and the function of these lipids are becoming apparent. The quinones appear to be spatially oriented in the respiratory complex and function as "electron transformers" by shuttling electrons between other respiratory coenzymes. In addition, these lipid-soluble cofactors may play a direct role in oxidative phosphorylation.

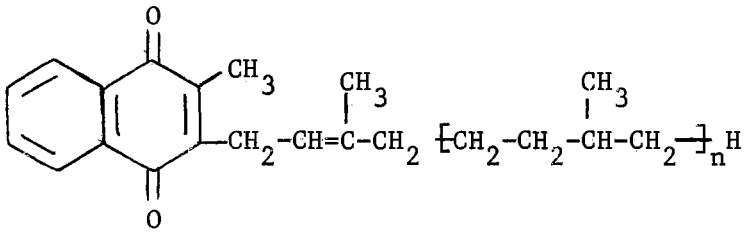
Some of the naturally occurring quinones are:



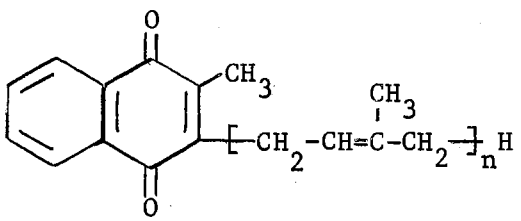
Coenzyme Q - Ubiquinone Series



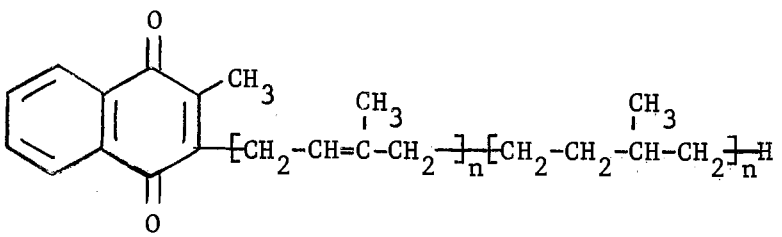
Plastoquinone Series



Vitamin K_1 Series



Vitamin K_2 Series



Vitamin K_n Series

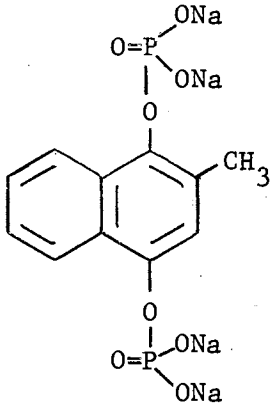
These have been shown to be active in biological systems. Although these quinones are structurally different, their biological activity may be related to certain common chemical properties. The quinonoid structure, which undergoes oxidation and reduction with ease, provides the basis for their catalytic role in electron transport. Furthermore, the biologically active quinones have oxidation-reduction potentials which are commensurate with their position in the electron transport chain.

Phosphorylated quinols have been proposed as participants in oxidative phosphorylation. Such structures are chemically and thermodynami-

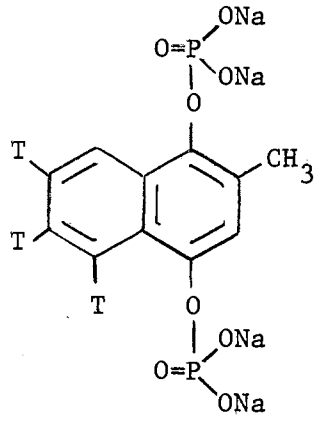
cally capable of yielding an intermediate with phosphate-bond energy. It is also of interest to note that phosphorylated quinols, on hydrolysis, can give rise to structures having the characteristic resonance of other energy-rich phosphorylated compounds.

A series of quinones and related compounds have been studied as radiation sensitizers in treatment of cancer.

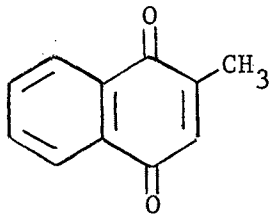
In 1946, J. S. Mitchell and D. H. Marrian conducted extensive studies on a number of quinone-type of compounds as radiation sensitizers. The first compounds to be studied were:



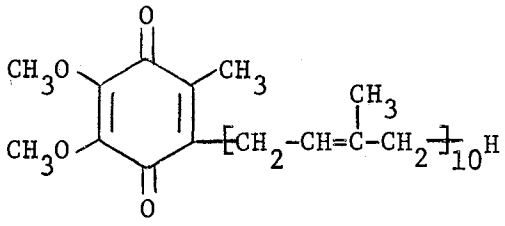
2-Methyl-1,4-naphthoquinol-bis(disodium phosphate). (Synkavit)



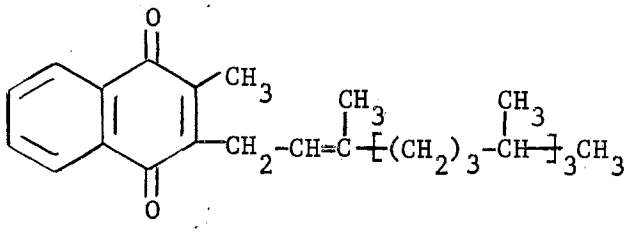
2-Methyl-5,6,7-tritritio-1,4-naphthoquinol-bis(disodium phosphate) (TRA 119)



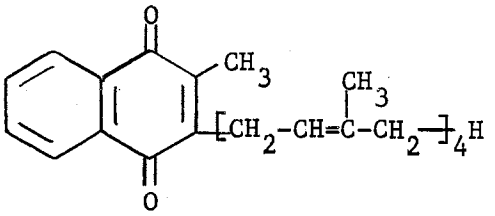
2-Methyl-1,4-naphthoquinone (menadione)



Coenzyme Q-10
Ubiquinone-10 (Q-10)



Vitamin K_1 (α-Phylloquinone)



Tetraprenyl Menaquinone (MK-4)

From a biochemical point of view, the selective uptake of synkavit and TRA-119 into certain malignant cells is of interest, particularly in relation to the recent findings of low concentrations of ubiquinone in some tumors, and of the greater increase in the activity of certain oxidative enzymes produced by the addition of ubiquinone (Q-10) or menadione in tumors than in homologous normal tissue[34].

A fundamental idea which has become widely recognized in recent years[35] is that an important factor in limiting the radiocurability of malignant tumors is the proportion of relatively radioresistant anoxic cells. One of the problems in the study of synkavit is to determine whether the enzymic oxidation of cell constituents such as the reduced coenzymes $NADH_2$ (DPNH) and $NADPH_2$ (TPNH) by menadione in anoxic as well as in oxygenated cells is an important factor in the radiosensitization

of tumors in radiotherapy. The chemical studies of oxidative phosphorylation using quinol mono- and diphosphates, including synkavit,[36] are very revelant to this problem. Dephosphorylation of the monophosphates must accompany and not precede the oxidation. The measured changes in redox potentials[13] are consistent with the suggestion that synkavit is taken up into the tumor cells in phosphorylated form, and then rapidly hydrolyzed and oxidized to give menadione.

In trying to elucidate the mechanisms of radiosensitization, it is important to consider the evidence concerning the role of some of the quinones, e.g. synkavit and menadione, in relevant biochemical processes, especially in relation to biochemical differences between malignant and normal cells. In recent years, the discovery of ubiquinone has been associated with a great interest in the biochemistry of quinones, including menadione.

An attempt has been made by J. S. Mitchell and D. H. Marrian[37] to summarize recent biochemical evidence in the form of a possible scheme for the respiratory chain of oxidative phosphorylation, showing the effects of addition of menadione and the role of ubiquinone, together with the phosphorylation steps and the site of action of certain chemical inhibitors which appear relevant to the present problem.[38-40] It must be emphasized that such a scheme is tentative and that further knowledge is likely to modify it.

Slater[38] concluded that menadiol enters the respiratory chain in the region of cytochrome b. It appears that menadione can couple the oxidation of extra-mitochondrial $NADH_2$ (DPNH) and $NADPH_2$ (TPNH) to the mitochondrial respiratory chain. The reaction involving DT diaphorase (di- and tri-phosphopyridine nucleotide diaphorase) is specific for cer-

tain quinones; the only highly active quinone is menadione among a series of related compounds and it is particularly interesting to note that vitamin K (phylloquinone and menaquinones) and some benzoquinones including coenzyme Q-10 are inactive. There is evidence for a number of menadione-dependent diaphorases in the mitochondrial and supernatant fractions of various cells.

Wenner et al.[39] showed by means of ^{14}C-labelled glucose that menadione greatly increases oxidation at carbon-1 in ascites tumor cells but had no effect on the oxidation at carbon-6, which suggests that it stimulated the pentose phosphate shunt pathway. The rate-limiting reaction in $NADPH_2$ oxidation appears to be between flavoprotein and cytochrome C[40]. Moreover, Callner and Ernster[41] demonstrated that addition of menadione to ascites tumor cells in the presence of glucose prevented the inhibition of respiration by amytal and abolished the Crabtree effect. Under some conditions, in the presence of menadione, $NADPH_2$ has been oxidized more readily than $NADH_2$. It is of interest, in view of the similar NAD content of brain and tumors that synkavit and menadione increases the oxygen uptake in brain in the presence of glucose. This increase has been shown to be due to increased oxidation of the carbon-1 of glucose and provides additional evidence for a stimulation for the pentose phosphate pathway of glucose metabolism by synkavit and menadione.

Risse and Tiedemann[42] have shown that phenanthraquinone is involved in a cyclic process of oxidation and reduction in Ehrlich ascites tumor cells under aerobic conditions, $NADH_2$ being the hydrogen donor for the reduction. During the outo-oxidation of the corresponding quinol by atmospheric oxygen, radicals and hydrogen peroxide were formed. These

radicals and hydrogen peroxide were regarded as responsible for the inhibition of glycolysis.

It appears that not all of the quinones that concentrate in tumors act as clinical radiosensitizers; this may be because of the unsuitable value of the oxidation-reduction potential of the parent quinones. It is reasonable to assume that the "active form" of synkavit within the cell is menadione. The observed selective concentration in tumor cells under some circumstances is probably related to low levels of ubiquinone concentration in some tumor cells and also in certain normal cells. Menadione may replace the ubiquinone in certain biochemical processes in some tumor cells.

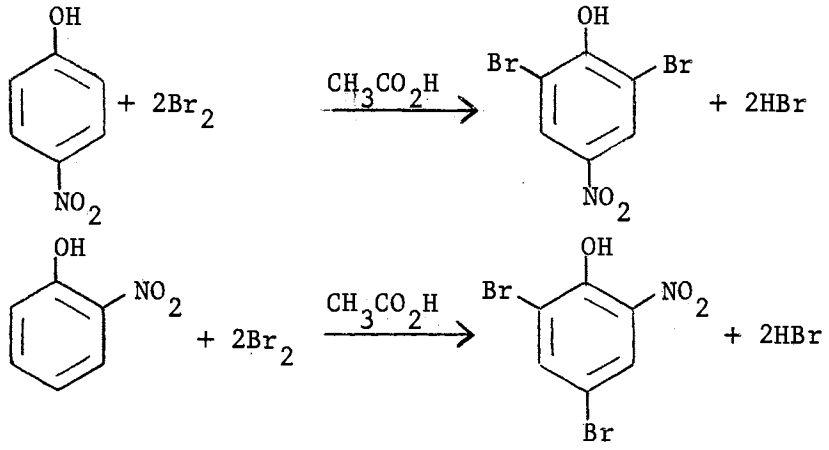
For some experimental tumors in mice it has been noted that menadione increases the activity of the lactic dehydrogenase system, inhibits aerobic glycolysis, increases the pentose phosphate shunt pathway, reduces the concentration of ATP, and inhibits part of the incorporation of purine nucloeotides into RNA. It is suggested that a fundamental effect of menadione is to lead to oxidation of $NADH_2$ (DPNH) and especially of $NADPH_2$ (TPNH) in tumor cells.

CHAPTER III

EXPERIMENTAL

Preparation of Compounds

2,6-Dibromo-4-nitrophenol and 4,6-Dibromo-2-nitrophenol[43]



In a 250-ml round-bottom flask fitted with a dropping funnel and a tube leading to a gas trap to carry off the hydrogen bromide, 27.8 g (0.2 mole) of p-nitrophenol (or o-nitrophenol) was dissolved in 83 ml of glacial acetic acid. To this solution at room temperature was added dropwise with stirring during the course of one hour a solution of 75.0 g (0.47 mole) of bromine in 70 ml of glacial acetic acid. After the addition of the bromine the reaction mixture was stirred for 15 minutes and then warmed on the steam bath (internal temperature about 85°) for 20

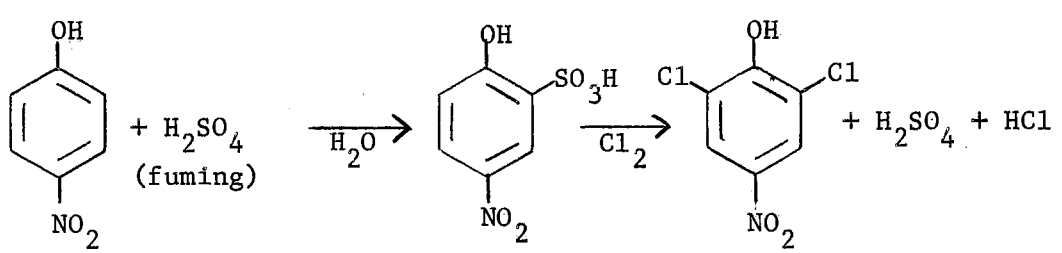
minutes in order to remove as much of the excess bromine as possible. The last trace of bromine was removed by passing a stream of air into the reaction mixture, which then had a yellow or brown color. The mixture was treated with 110 ml of cold water, stirred until cool, and allowed to stand in ice, or in an ice chest overnight. The pale yellow crystalline product was collected on a Buechner funnel and washed first with 50 ml of 50 percent aqueous acetic acid and then thoroughly with water. It was dried in a vacuum desiccator over sodium hydroxide. Some data on the dibromonitrophenols are given in Table I.

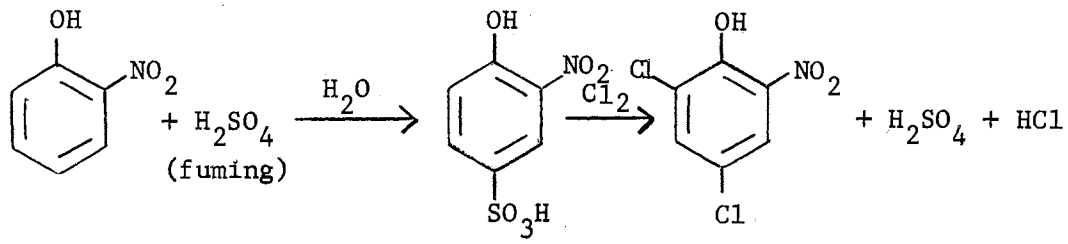
TABLE I

DIBROMONITROPHENOLS

Compound	Appearance of Crystals	Yield, %	Melting Point Found, °C	Melting Point Reported, °C	Reference
2,6-Dibromo-4-nitrophenol	nearly colorless	96-98	140-141	138-140	43
4,6-Dibromo-2-nitrophenol	yellow	87-88	114-115	115-117	44

2,6-Dichloro-4-nitrophenol and 4,6-Dichloro-2-nitrophenol[45]





The sulfonic acid was prepared by the gradual addition of 3 ml of fuming sulfuric acid (in the case of p-nitrophenol) or 5 ml of fuming sulfuric acid (in the case of o-nitrophenol) to 4 g (0.029 mole) of nitrophenol (either ortho or para). In the case of o-nitrophenol this addition was done with cooling to avoid charring of the mixture. After the addition was completed the mixture was allowed to stand for an hour and then diluted with water and treated with chlorine until a copious precipitate of yellow substance formed. The solid was collected, washed, and crystallized a few times. Some data on the dichloronitrophenols are given in Table II.

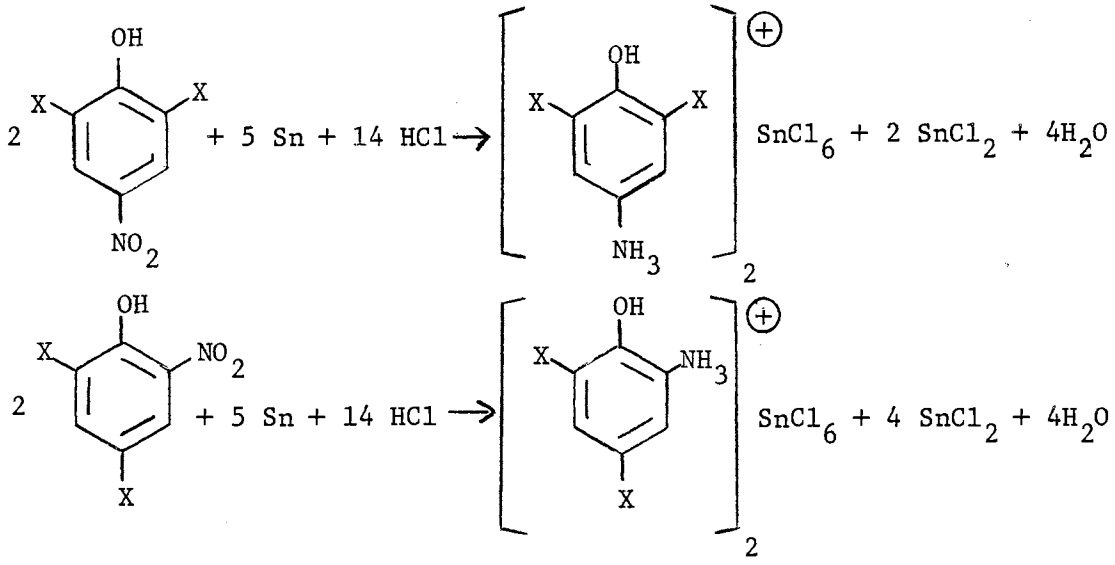
TABLE II

DICHLORONITROPHENOLS

Compound	Appearance of Crystals	Yield, %	Melting Point		Reference
			Found, °C	Reported, °C	
2,6-Dichloro-4-nitrophenol	light-brown	82.7	123-124	124-125	45
4,6-Dichloro-2-nitrophenol	yellow	98	122-123	122-123	45

2,6-Dihalo-4-aminophenol Chlorostannate and

4,6-Dihalo-2-aminophenol Chlorostannate[43]



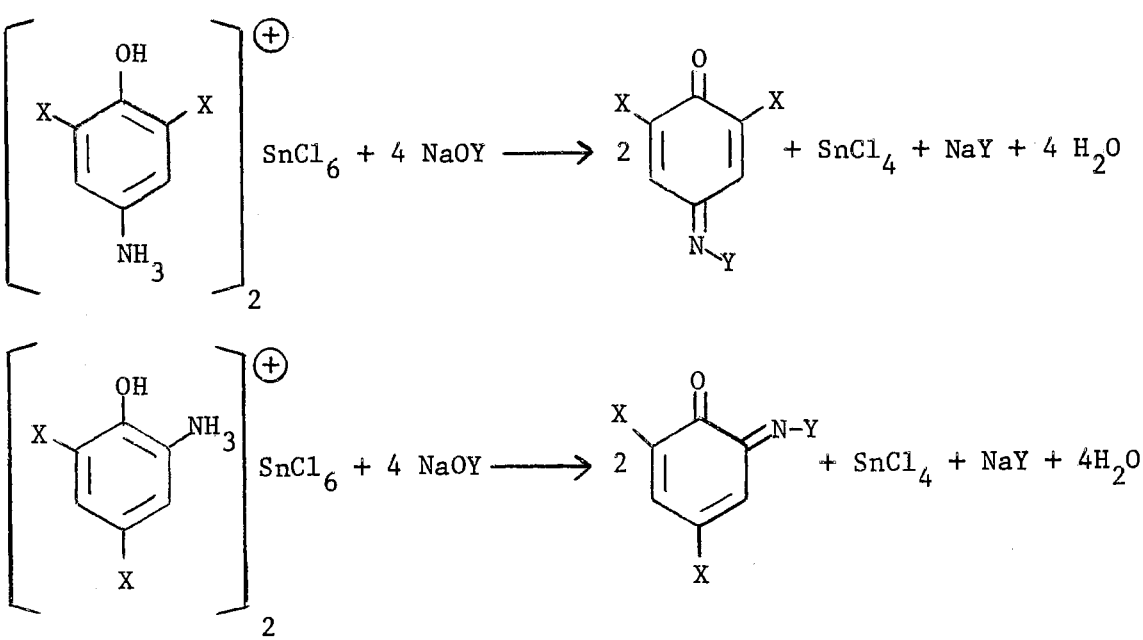
where X = Cl, Br

In a 250-ml round-bottom flask was placed 0.05 mole of the appropriate dihaloaminophenol with 30 ml (0.36 mole) of concentrated hydrochloric acid (sp. gr. 1.19), 30 ml of water, and 18.5 g of mossy tin. Capryl alcohol (0.3 ml) was added to control foaming and the mixture was heated in an open flask with stirring on the steam bath until the reaction started. The reaction may be vigorous at the outset and it is well to heat cautiously during the initial stages. Hydrochloric acid and water were added from time to time and foaming was controlled by the addition of a part of the water. A total of 52 ml (0.62 mole) of concentrated hydrochloric acid and 90 ml of water were added during the course of the reaction. When the first vigorous reaction was over the mixture was heated strongly until the dihalonitrophenol had all dis-

solved and the hot solution (at about 85°) was filtered through a layer of decolorizing carbon ("Norit A") on a hot Buechner funnel. The filtrate (which usually was colorless) was cooled to 0° with stirring for a half hour or allowed to stand in a cool place overnight. The product, which separated in the form of colorless or slightly yellow needles, was collected on a Buechner funnel and washed with cold dilute hydrochloric acid (one volume of concentrated acid to one volume of water). The material was usually colorless and was used directly for the preparation of the trihalobenzoquinone imine. After the solid was dried in a vacuum desiccator over sodium hydroxide, the yield of tin salt was almost quantitative.

N,2,6-Trihalo-1,4-benzoquinone 4-Imine and

N,4,6-Trihalo-1,2-benzoquinone 2-Imine[43]



where X = Cl, Br

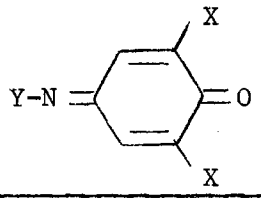
Y = Cl, Br

In a 250-ml beaker was placed a solution of 11.5 g (0.29 mole) of sodium hydroxide in 17.5 ml of water and 100 g of cracked ice; 0.152 mole of halogen (Cl_2 or Br_2) was added to the mixture. About 80 per cent of the ice was melted by the operation.

In a 500-ml beaker 0.0127 mole of tin salt was dissolved in 120 ml of water and 1.2 ml of concentrated hydrochloric acid. Solution was effected by warming the mixture to 40-50°, after which the solution was cooled to 15-17° and 60 g of cracked ice was added. The sodium hypohalite solution was then added all at once with vigorous stirring under the hood. A yellow to orange precipitate of the trihalobenzoquinone imine separated immediately. As soon as the sodium hypohalite solution was added with stirring, 12 ml of concentrated hydrochloric acid was added in order to keep the tin salt in solution. The solution was stirred for 20 minutes in order to increase the particle size of the imine. The fine yellow to orange precipitate was filtered from the solution and washed with 150 ml of 5 per cent hydrochloric acid to remove tin salts. The product was dried in a vacuum desiccator over sodium hydroxide. Some data on the trihalobenzoquinone imines are given in Tables III and IV.

TABLE III

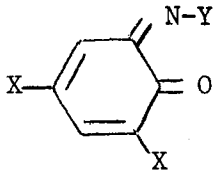
N,2,6-TRIHALO-1,4-BENZOQUINONE 4-IMINES:



Substituents		Appearance of		Melting Point		
X	Y	Crystals	Yield, %	Found, °C	Reported, °C	Reference
Br	Br	yellow-brown	74-76	98-100	---	--
Br	Cl	yellow-orange	84-87	80-81	80-82	43
Cl	Cl	yellow	85-86	65-66	66.5-67	53
Cl	Br	yellow	82-84	95-96	---	--

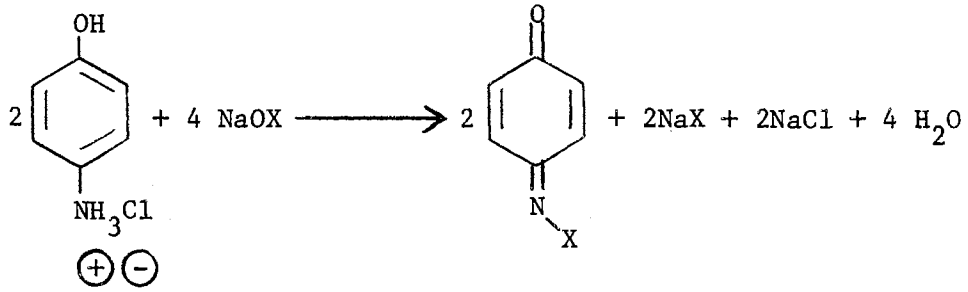
TABLE IV

N,4,6-TRIHALO-1,2-BENZOQUINONE 2-IMINES:



Substituents X	Y	Appearance of Crystals	Yield, %	Melting Point Found, °C	Reported, °C	Reference
Br	Br	orange-brown	58-59	74-75	---	--
Cl	Cl	brown	57-59	46-47	---	--

N-Halo-1,4-benzoquinone 4-Imines



In a 250-ml beaker was placed a solution of 11.5 g (0.29 mole) of sodium hydroxide in 17.5 ml of water, and 100 g of cracked ice; 0.152 mole of halogen (Cl_2 or Br_2) was added to the mixture. About 80 percent of the ice was melted by the operation.

In a 500-ml beaker 3.7 g (0.0245 mole) of p-aminophenol hydrochloride was dissolved in 120 ml of water and 1.2 ml of concentrated hydrochloric acid. Solution was effected by warming the mixture to 40-50°, after which it was cooled to 15-17° and 60 g of ice was added. The sodium hypohalite solution was then added all at once with vigorous stirring under the hood. A yellow to orange precipitate of N-halobenzoquinone imine separated immediately. The mixture was stirred for 20 minutes in order to increase the particle size. The fine yellow to

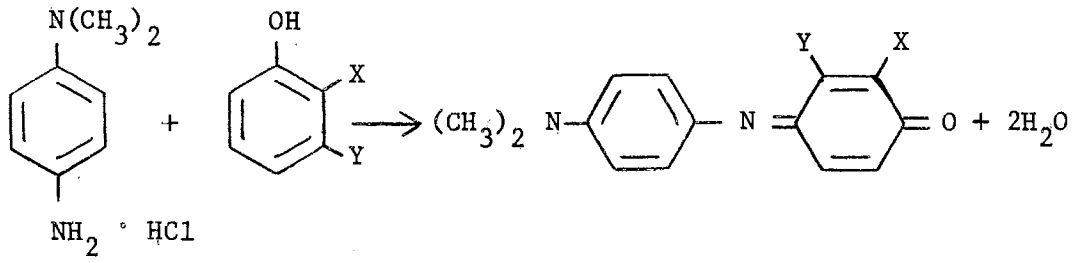
orange precipitate was filtered out and washed with 150 ml of 5 percent hydrochloric acid. The product was dried over a glass tray in a vacuum desiccator over sodium hydroxide. Some data on the N-halo-1,4-benzoquinone imines are given in Table V.

TABLE V

N-HALO-1,4-BENZOQUINONE 4-IMINES

Halogen in Compound	Appearance of Crystals	Yield, %	Melting Point Found, °C	Melting Point Reported, °C	Reference
Chloro	yellow	71-73	83-84	84-85	46
Bromo	dark yellow	63-65	67-68 (dec)	67 (dec)	56

Indoaniline Derivatives With Substituents on the Quinone Ring[47,48]



where X = H, Cl, CH_3, OCH_3, $N(CH_3)_2$, $NHCOCH_3$; Y = H;

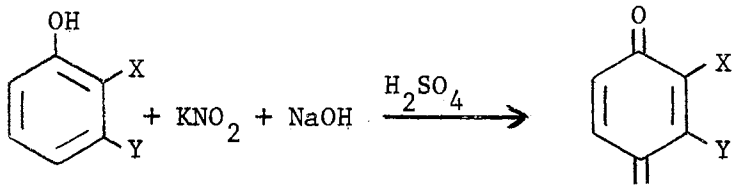
or X + Y = benzo

A solution of 37.4 g (0.22 mole) of silver nitrate in 200 ml of water was added slowly to a well-stirred solution containing 14.6 g (0.25 mole) of sodium chloride and 0.3 g of gelatin in 200 ml of water. The gelatin had been dissolved separately in 10-20 ml of water by soaking at room temperature for several hours, then warming to 50° to dis-

solve. To the suspension of finely divided silver chloride was added, successively, a solution of 20 g of sodium carbonate monohydrate in 100 ml of water, and a solution of the appropriate phenol (0.025 mole) in 100 ml of 95 percent ethanol. Finally a solution of 4.75 g (0.0275 mole) of N,N-dimethyl-p-phenylenediamine hydrochloride in 200 ml of water was added slowly with vigorous stirring. Color developed immediately, and stirring was continued for thirty minutes. Ethyl acetate (or ether) (200 ml) was then added to the mixture, the whole was stirred well and filtered to remove silver and residual silver chloride, and the residue was washed with a small portion of the solvent to remove adhering dye. The ethyl acetate layer containing the colored product was separated from the filtrate, washed with water until the wash water was nearly colorless (six to seven times), dried over anhydrous potassium carbonate, and finally evaporated to leave the colored solid. The crude compounds were obtained in 90-98% yields, all of them in a fairly high state of purity so that one or two crystallizations from a suitable solvent yielded products with the reported melting points.

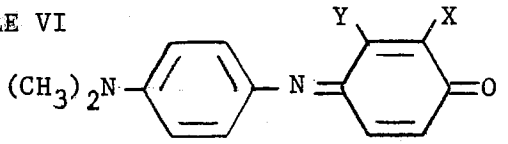
Attempts to prepare such compounds from catechol and resorcinol were unsuccessful. Dye formation was observed to occur, but the dyes were too unstable to permit isolation. Some data on indoaniline derivatives are given in Table VI.

Quinone 4-Oximes[49]



where X = H, OCH_3, Cl; Y = H or X + Y = benzo

TABLE VI

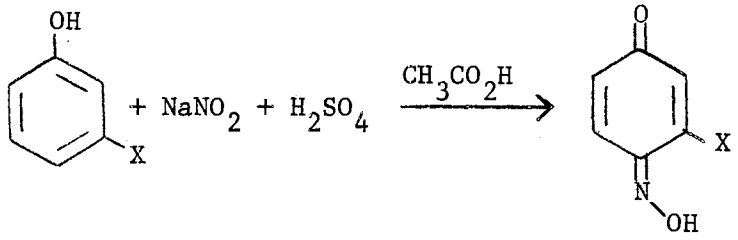
INDOANILINE DERIVATIVES: 

Substituents X	Substituents Y	Appearance of Crystals	Yield, %	Melting Point Found, °C	Melting Point Reported, °C	Reference
H	H	dark purple	98	161-162	161-163	47
CH_3	H	dark purple (green reflection)	96	124-125	123-124	47
OCH_3	H	dark purple	92	167-168	167-168	47
Cl	H	dark green	98	124-125 (dec)	125 (dec)	47
$NHCOCH_3$	H	dark purple (gold reflection)	94	185-186	184-185	47
X + Y = benzo		dark purple	93	143-144	*	48

*Compound is known, but no value for the melting point was found.

A solution of 6 g (0.064 mole) of phenol, 2.7 g (0.068 mole) of sodium hydroxide and 6.68 (0.078 mole) of potassium nitrite in 150 ml of water was cooled to -3°. To this solution was added with stirring 15 g (0.142 mole) of concentrated sulfuric acid in 40 ml of water at a rate sufficiently low to maintain the temperature of the reaction mixture at -3° to 0° (to avoid charring). The addition required about 30-45 minutes, after which the reaction mixture was stirred for an additional hour with continued cooling. The yellow to brownish crystalline precipitate was filtered out. The product was washed on the funnel with approximately 20 ml of ice-water and then air-dried. The yields of the quinone 4-oximes varied from 72 to 84 percent of the theoretical amount, all of them in fairly high state of purity, so that one or two crystallizations from a suitable solvent yielded products with the reported melting points. Some data on quinone 4-oximes are given in Table VII.

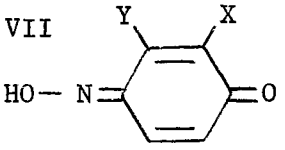
3-Halo-1,4-benzoquinone 4-Oximes[50]



where X = Cl, Br, I.

A solution of 0.016 mole of m-halophenol in 20 ml of glacial acetic acid was added below 20° to a solution of 2.0 g (0.03 mole) of sodium nitrite in 10 ml of concentrated sufuric acid which had previously been heated to 70°. The mixture was kept at 0° for approximately 10 minutes and then poured on ice; the 3-halo-1,4-benzoquinone 4-oxime was precipi-

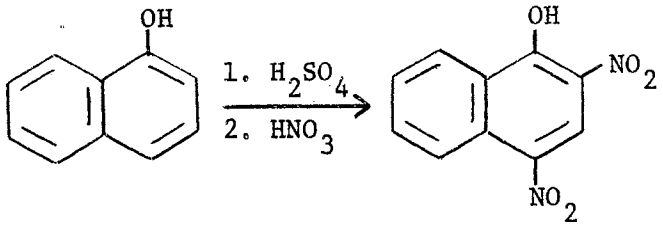
TABLE VII

QUINONE 4-OXIMES: 

Substituents X	Substituents Y	Appearance of Crystals	Yield, %	Melting Point Found, °C	Melting Point Reported, °C	Reference
H	H	brown	72	137-138 (dec)	137 (dec)	49
OCH_3	H	brown	74	174-175 (dec)	174.8-175.5 (dec)	54
Cl	H	grayish-brown	79	142-144 (dec)	142	55
X + Y = benzo		yellowish-brown	84	192-194 (dec)	190-192 (dec)	57

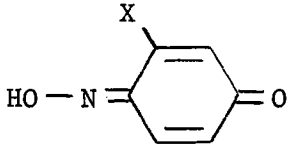
tated as a slightly yellow-brown solid. The products were obtained in yields of 79 to 85 percent of the theoretical amount and were of fairly high purity so that one crystallization from aqueous alcohol yielded products giving the reported melting points. Some data on 3-halo-1,4-benzoquinone 4-oximes are given in Table VIII.

2,4-Dinitro-1-naphthol[52]



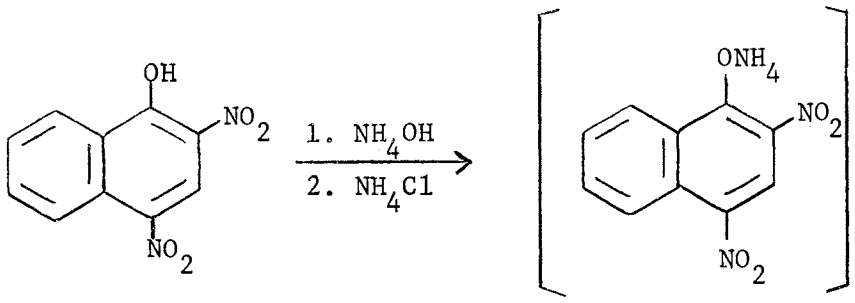
To 5 g (0.0347 mole) of pure α-naphthol was added very slowly 10 ml of concentrated sulfuric acid and the mixture was heated with swirling on the steam-bath for 5 minutes. The solid dissolved and an initial red color was discharged. The mixture was cooled in an ice bath and 25 ml of water added along with rapid cooling to 15°. Six milliliters of concentrated nitric acid was then added very slowly (dropwise) to the chilled aqueous solution while the temperature was kept around 15-20°. When the addition was completed and the exothermic reaction had subsided (1-2 min.), the mixture was warmed gently to 50° (on a water bath) for approximately one minute while the nitration product separated as a stiff yellow paste. The mixture was heated (on a steam bath) for another minute or so and water was added (about 75 ml) with vigorous stirring until the mixture was a smooth paste. The product was collected on a Buechner funnel and washed well with water. The yield of the product was 85-90 percent of the theoretical amount, which after one crystallization from ethanol gave a pure product melting at 138° (reported[52]: 138°).

TABLE VIII

3-HALO-1,4-BENZOQUINONE 4-OXIMES: 

Substituent X	Appearance of Crystals	Yield, %	Melting Point Found, °C	Melting Point Reported, °C	Reference
Cl	yellowish-brown	79	174-175	172-178	51
Br	yellowish-brown	82	188-190	189-190	51
I	yellowish-orange	84	194-196 (dec)	185-195 (dec)	51

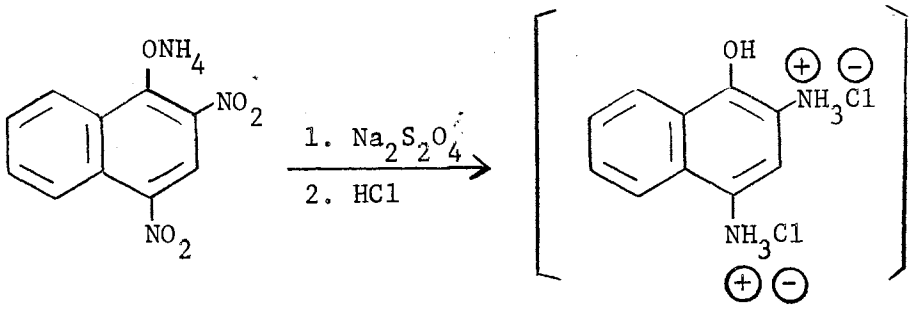
Ammonium Salt of 2,4-Dinitro-1-naphthol[52]



To a solution of 7.02 g (0.03 mole) of 2,4-dinitro-1-naphthol in 100 ml of water, 150 ml of hot water and 5 ml of concentrated ammonia solution (sp. gr. 0.90) were added and the mixture was heated to the boiling point with stirring until the solid was completely dissolved. The hot solution was filtered and 10 g of ammonium chloride was added to the filtrate to salt out the ammonium salt of the phenol, and the mixture was cooled in an ice bath. The product, which separated in the form of bright orange crystals, was collected on a Buechner funnel and washed with water containing 1-2 per cent ammonium chloride. The yield of product was about 85-90 per cent of the theoretical amount.

Dissolving the salt in hot water and acidifying it with hydrochloric acid gave the free 2,4-dinitro-1-naphthol which was crystallized from ethanol (Norite) melting at 138° (reported[52]: 138°).

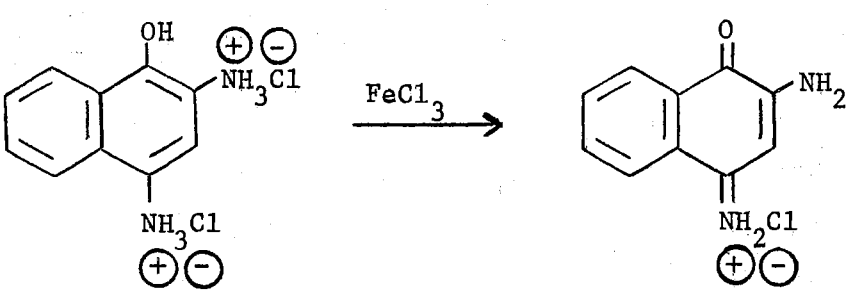
2,4-Diamino-1-naphthol Dihydrochloride[52]



In a 600-ml beaker was placed 7.5 g (0.03 mole) of the ammonium salt, 200 ml of water, and 40.0 g (0.23 mole) of sodium hydrosulfite. The mixture was stirred well until the original orange color disappeared and a crystalline tan precipitate formed. The solid was collected and washed with hydrosulfite solution (1-2 g of sodium hydrosulfite in 100 ml of water), avoiding even brief sucking of air through the cake after the reducing agent had been drained away. The solid was then transferred immediately into a beaker containing 6 ml of concentrated hydrochloric acid and 25 ml of water. The mixture was then stirred well in order to convert all the diamine to the dihydrochloride.

The acid solution, containing suspended sulfur and filter paper, was clarified by suction filtration through a moist bed of Norite (made by shaking 2 g of the decolorizing carbon with 25 ml of water in a stoppered flask to produce a slurry). The pink or colorless filtrate (2,4-diamino-1-naphthol dihydrochloride) was used immediately for the preparation of 2-amino-1,4-naphthoquinone 4-imine hydrochloride.

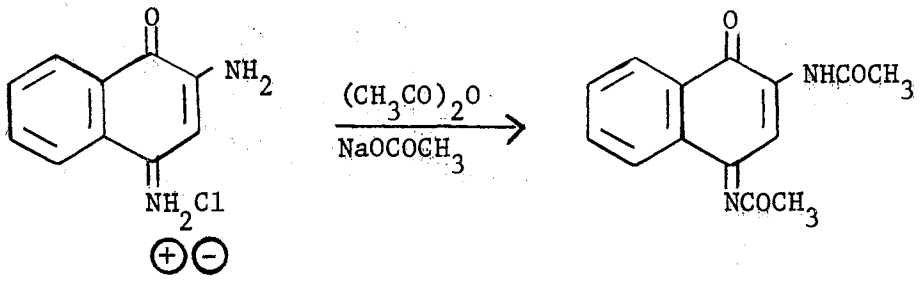
2-Amino-1,4-naphthoquinone 4-Imine Hydrochloride[52]



To 30 ml of a solution containing 7.41 g (0.03 mole) of 2,4-diamino-1-naphthol dihydrochloride was added 50 ml of 1.3 M ferric chloride

solution (made by dissolving 22.5 g of $FeCl_3 \cdot 6H_2O$ in 25 ml of water and 25 ml of concentrated hydrochloric acid). The solution was cooled in an ice bath, the mixture filtered on a Buechner funnel, and the solid washed with dilute hydrochloric acid (50 per cent solution). The red crystals were dried and crystallized from dilute hydrochloric acid (20 per cent solution). The yield of the product was about 78 percent of the theoretical amount; it melted at 288-289° (dec.).

N,N'-Diacetyl-2-Amino-1,4-naphthoquinone 4-Imine[52]

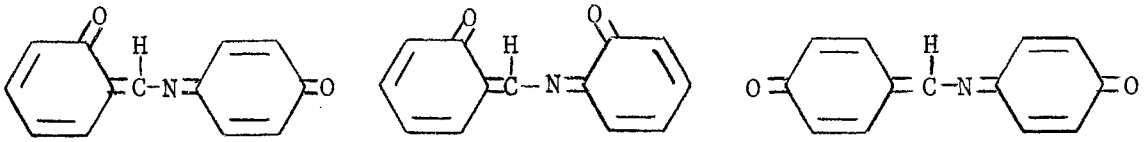


A mixture of 0.5 g (0.0024 mole) of 2-amino-1,4-naphthoquinone 4-imine hydrochloride, 0.5 g (0.006 mole) of sodium acetate (anhydrous), and 3 ml of acetic anhydride was stirred in a beaker and warmed gently on a hot plate (40-50°). With thorough stirring the red salt was soon changed into yellow crystals of the diacetyl derivative. As soon as the particles of red solid had disappeared the mixture was poured into a small amount of water (5-10 ml), and stirring was continued until the excess acetic anhydride had either dissolved or become hydrolyzed. The product was collected on a funnel and washed with water. The yield of the product was 72 percent of the theoretical amount, which after one crystallization from ethanol gave a pure product melting at 191-192°

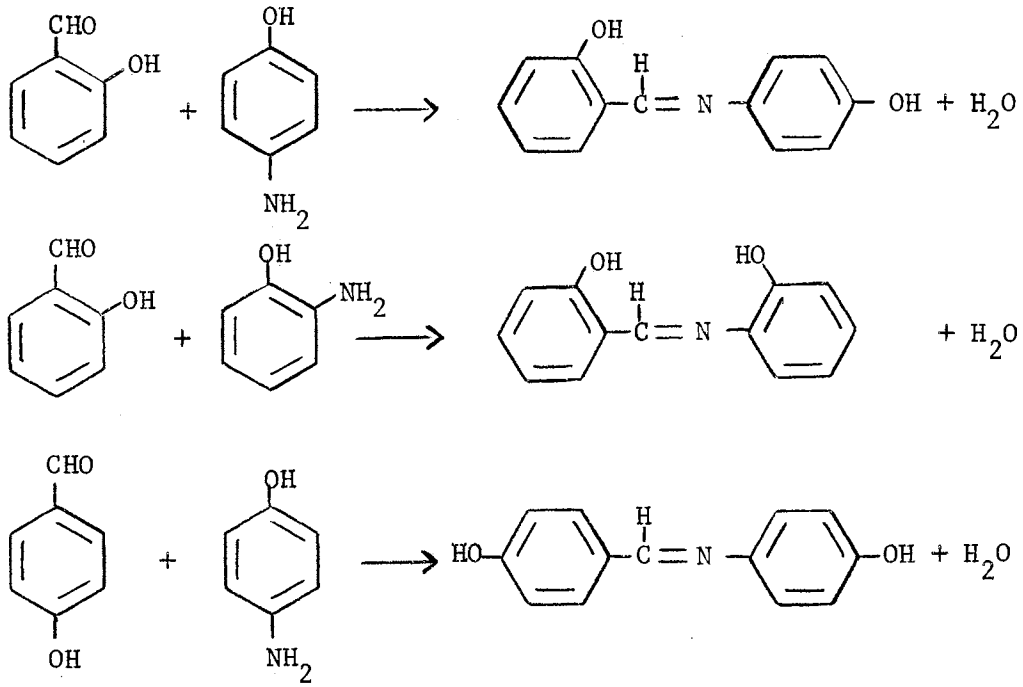
(reported[52]: 189-191°).

Attempts to Synthesize Other Compounds

Several attempts were made to prepare the following compounds, none of which are described in the literature:



For this purpose the following Schiff bases were prepared by the general method described in the literature[58]:



Attempts were made to oxidize these compounds to the corresponding quinone imines. Different oxidizing agents were used but no pure product was obtained in any of the three cases. Because of rapid hydrolysis of these compounds in aqueous solution[59], the choices of the oxidizing agent and the solvent are limited. The reagents described in the following paragraphs which have been used on similar systems[60-63] were tried,

but unfortunately they did not give the desired products. From the results it was concluded that either the C=N bond breaks or cyclization takes place in the case of ortho hydroxy compounds. Some unreacted starting material also remains, which adds to the difficulty of separation and purification.

Commercial silver oxide and freshly prepared silver oxide in dioxane, ether, and acetone were used at reflux temperatures under nitrogen atmosphere over periods of 10 hours to 3 days. This reagent was used by Willstätter[60] for oxidation of catechol to 1,2-benzoquinone.

Tetrachloro-1,4-benzoquinone (Chloranil) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol were used at reflux temperature under nitrogen atmosphere over periods of 12 hours to 4 days. These reagents were used by Becker[61] for oxidation of some phenols.

Chromium trioxide in graphite (Seloxcette) in toluene was used at reflux temperature over periods of 14 hours to 4 days. This reagent was used by Lalancette[62] for oxidation of various alcohols to the corresponding aldehydes and ketones.

Lead tetracetate in pyridine and in benzene was used at reflux temperatures over periods of 8 hours to 2 days. This reagent was used by Partch[63] and others for the conversion of alcohols to the corresponding aldehydes and ketones.

Proof of Structures of Compounds

The structures of all compounds prepared were verified by elemental analysis, infrared spectroscopy, nuclear magnetic resonance studies, and/or by comparison with known compounds. The compounds that were prepared for the first time were studied most completely in order to corro-

borate the expected structures.

The purified new compounds were sent to M.H.W. Laboratories, Garden City, Michigan 48135 for analysis for carbon, hydrogen and nitrogen. The results of these analyses correspond to the values calculated for the expected compounds; they are shown in Table IX.

Infrared spectra of the compounds were determined on mineral oil mulls on a Beckman IR-5A Spectrophotometer with sodium chloride optics. Major absorption peaks are tabulated in Tables X, XI, XII, and XIII and complete spectra of some of the compounds are shown in Figures 2, 4, 6, 8, and 10.

The infrared (IR) spectra of these compounds have certain absorption peaks in common, two of which in particular were used as evidence of structure. One is a medium to strong absorption in the region between 1618 and 1668 cm^{-1} which is due to the C=O stretching mode and the other is a medium to strong absorption in the region between 1520 and 1580 cm^{-1}, which is absent in the spectra of the corresponding quinones. Thus it is associated to some extent with the C=N group and their smaller wavenumber in comparison with those for this group in some other compounds is explained[64] in terms of a strong conjugation with the quinonoid double bonds, particularly the C=O group, and consequent reduction of the double-bond character of the C=N bond.

The bonds which are characteristic of aromatic structures at 1660-2000 cm^{-1} and 667-1200 cm^{-1} could not be identified in quinone oximes and quinone imines. This fact coupled with the presence of the carbonyl absorption and the olefinic absorption together with the close overall similarity to the spectra of 1,4-benzoquinone makes it almost certain that the structure of these compounds in the solid state is quinonoid.

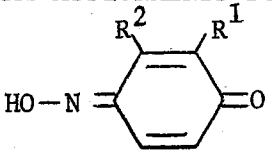
TABLE IX

ELEMENTAL ANALYSES OF NEW COMPOUNDS

Compound	Composition					
	Carbon, %		Hydrogen, %		Nitrogen, %	
	Calcd.	Found	Calcd.	Found	Calcd.	Found
N,2,6-Tribromo-benzoquinone 4-Imine	20.9	20.74	0.58	0.46	4.07	4.17
N-Bromo-2,6-di-chloro-1,4-benzo-quinone 4-Imine	28.20	28.13	0.79	1.00	5.40	5.48
N,4,6-Tribromo-1,2-benzoquinone 2-Imine	24.05	23.84	0.68	0.93	4.67	4.44
N,4,6-Trichloro-1,2-benzoquinone 2-Imine	34.22	33.98	0.99	1.23	6.65	6.41

TABLE X

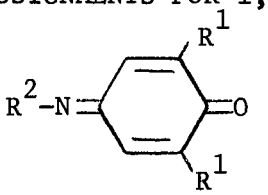
INFRARED SPECTRAL ASSIGNMENTS FOR QUINONE 4-OXIMES



Substituents		O-H Stretching,	C=O Stretching,	C=N and/or C=C Stretching,	O-H Bending,	C=C H Out of Plane Bending,	N-O Stretching,
R^1	R^2	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}
H	H	3210	1645	1563	1366	870	1000
Cl	H	3190	1640	1560	1430	830, 855 880, 895	1000
OCH_3	H	3220	1645	1579	1445	865	1005
H	Cl	3225	1642	1565	1440	885	990, 1018
H	Br	3250	1644	1564	1435	880	980
H	I	3260	1650	1625	1445	895	1010
$R^1 + R^2$ = benzo		3265	1655	1562	1440	910	1075

TABLE XI

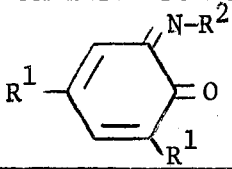
INFRARED SPECTRAL ASSIGNMENTS FOR 1,4-BENZOQUINONE 4-IMINES



Substituents		C=O Stretching,	C=N and/or C=C Stretching,	C=C-H Out of Plane Bending,	N-R^2 Stretching,
R^1	R^2	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}
H	Cl	1635	1580	865	705
H	Br	1640	1580	870	670
Cl	Cl	1647	1550	905	704
Cl	Br	1648	1554	908	695
Br	Br	1645	1560	907	692
Br	Cl	1648	1565	905	695

TABLE XII

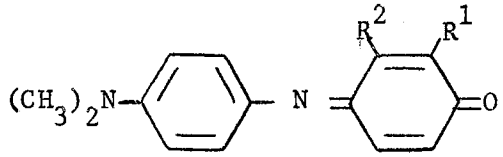
INFRARED SPECTRAL ASSIGNMENTS FOR 1,2-BENZOQUINONE 2-IMINES



Substituents		C=O Stretching,	C=N and/or C=C Stretching,	C=C-H Out of Plane Bending,	N-R^2 Stretching,
R^1	R^2	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}
Cl	Cl	1640	1550	906	710
Br	Br	1645	1545	908	725

TABLE XIII

INFRARED SPECTRAL ASSIGNMENTS FOR INDOANILINES



Substituents R^1	Substituents R^2	C=O Stretching, cm^{-1}	C=N and/or C=C Stretching, cm^{-1}	C=C H Out of Plane Bending, cm^{-1}	C-N Stretching, cm^{-1}
H	H	1620	1580	815	1424
Cl	H	1610	1570	815	1425
CH_3	H	1615	1575	810	1426
OCH_3	H	1615	1580	814	1425
$NHCOCH_3$	H	1615	1575	816	1427
$R^1 + R^2$ = benzo		1610	1570	817	1424

Jaffe[65] has calculated by the molecular-orbital method that the oxime form should be the more stable than the p-nitrosophenol form.

The infrared spectra of indoaniline derivatives are rather complex in that the bands which are characteristic of aromatic structure and the bands which are characteristic of quinonoid structures are both present.

For the nuclear magnetic resonance studies, a solution of about 10 weight % was prepared for each compound in a suitable solvent (acetone-d_6 or dioxane). Tetramethylsilane (TMS) was used as the reference standard either internally or externally. The spectra were obtained on a Varian XL-100 Analytical Nuclear Magnetic Resonance Spectrometer. Spectra of some of the compounds are shown in Figures 1, 3, 5, 7, and 9. The assignment of the signals follows.

Assignment of signals in NMR spectra of the quinone monoximes as indicated by Norris and Sternhell[66] are based on the expectation of relative deshielding of protons at C-3 (or C-5) in the isomer with the hydroxyl syn to the proton concerned.

In 1,4-benzoquinone monoxime the C=N-O fragment is rigid and non-linear (compared to other =N- systems[67]); therefore, pairs of protons at C-2, C-6 and C-3, C-5 are not equivalent. Thus there is produced a rather complex pattern with four slightly different screening constants for the four protons and different coupling constants $J_{2,3}$, $J_{5,6}$, $J_{3,5}$, and $J_{2,6}$. As a point of comparison, the pairs of protons at C-2 and C-6 and at C-3 and C-5 in p-nitrosophenol are equivalent since the nitroso group is freely rotating and a simple pattern is produced.

If detectable amounts of 1,4-benzoquinone oxime and p-nitrosophenol are both present the spectral pattern would be a superposition of both spectra, or else an averaged spectrum, depending upon the rate of ex-

change between the two forms. In either case the spectrum is temperature-dependent.

The NMR spectrum of 2-chloro-1,4-benzoquinone 4-oxime in dioxane (Figure 1) shows a singlet at 3.5 δ and seven doublets around 6.4 to 8.0 δ. The singlet at 3.5 δ contains four protons and is due to the solvent (dioxane). The doublet at 7.95 δ represents the proton at C-3 when it is syn with the hydroxyl group and the doublet at 7.45 δ represents the proton on C-3 when it is anti to the hydroxyl group. The two doublets centered around 7.25 δ represent the proton on C-5 when it is syn with the hydroxyl group and the two doublets centered around 7.75 δ represent the proton on C-5 when it is anti to the hydroxyl group. This splitting pattern of the proton at C-5 is due not only to the syn and anti isomerism but also to the presence of the adjacent proton (proton on C-6). And, finally the doublet at 6.55 δ represents the proton at C-6 and the splitting is caused by the adjacent proton (proton at C-5).

Inspection of the IR spectrum of 2-chloro-1,4-benzoquinone 4-oxime, (Figure 2) as a representative of the other oximes, reveals the fact that the quinone oxime form of p-nitrosophenol is the predominant form.

Assignment of the signals in the NMR spectra of quinone imines, as indicated by Saito and Nukada[68], is based on the fact that the lone pair of electrons of the nitrogen atom causes a separation between syn and anti proton signals.

The NMR spectrum of N-bromo-2,6-dichloro-1,4-benzoquinone 4-imine in acetone-d_6 (Figure 3) shows a multiplet around 2.1 δ, a singlet at 2.65 δ, and two doublets around 7.5-8.0 δ. The multiplet around 2.1 δ and singlet at 2.65 δ both are due to the small amount of undeuterated

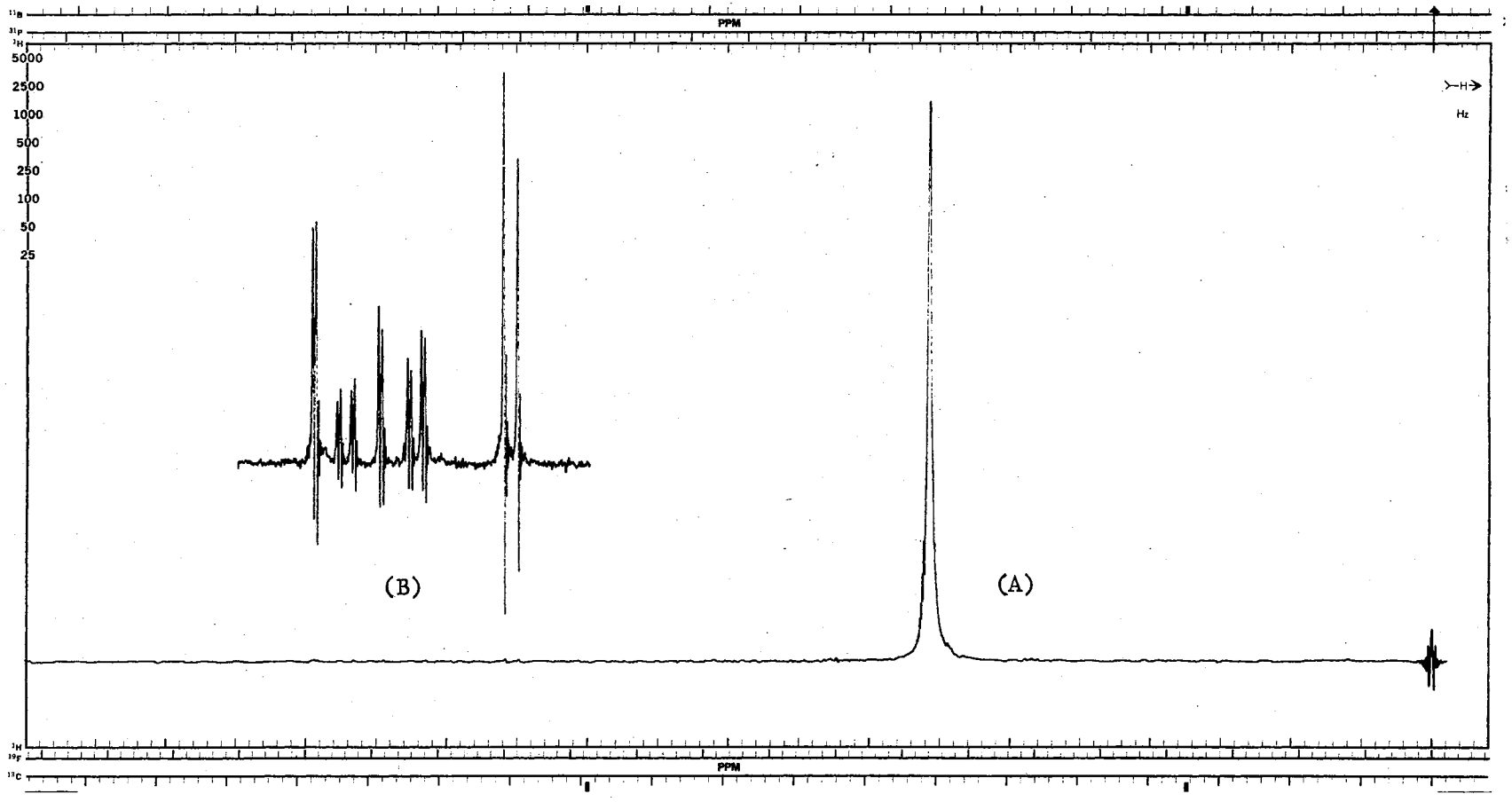


Figure 1. Proton Magnetic Resonance Spectrum of 2-Chloro-1,4-benzoquinone 4-Oxime in Dioxane -- 100 MHz. R.F. ... (A) 50, (B) 68 dB; S.W. ... 1000 Hz; S.A. ... (A) 0.32, (B) 6.3; F.B. ... 2 Hz; S.T. ... (A) 250, (B) 500 sec; S.O. ... 83701 Hz

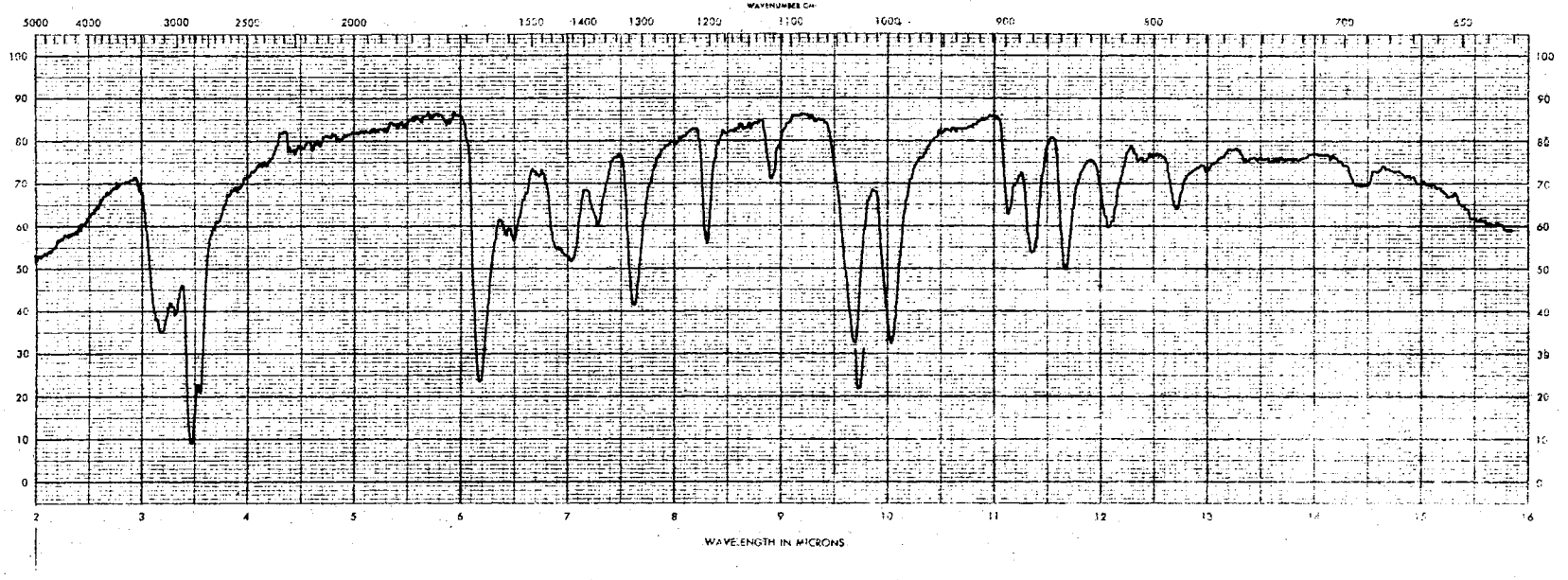


Figure 2. Infrared Spectrum of 2-Chloro-1,4-benzoquinone 4-Oxime -- Nujol Mull

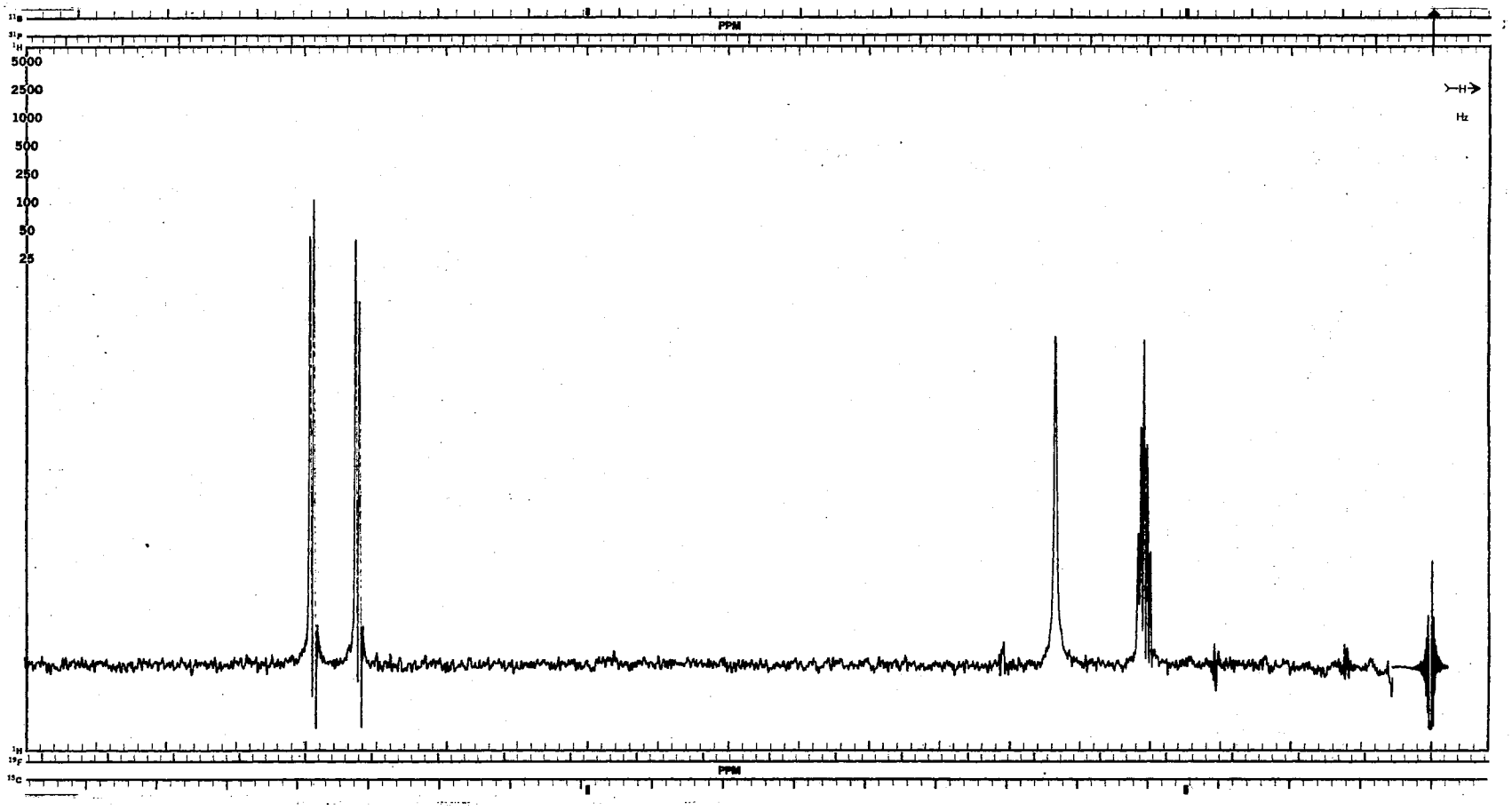


Figure 3. Proton Magnetic Resonance Spectrum of N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine in Acetone-d_6 -- 100 MHz. R.F. ... 72 dB; S.W. ... 1000 Hz; S.A. ... 16; F.B. ... 1 Hz; S.T. ... 500 sec; S.O. 83701 Hz

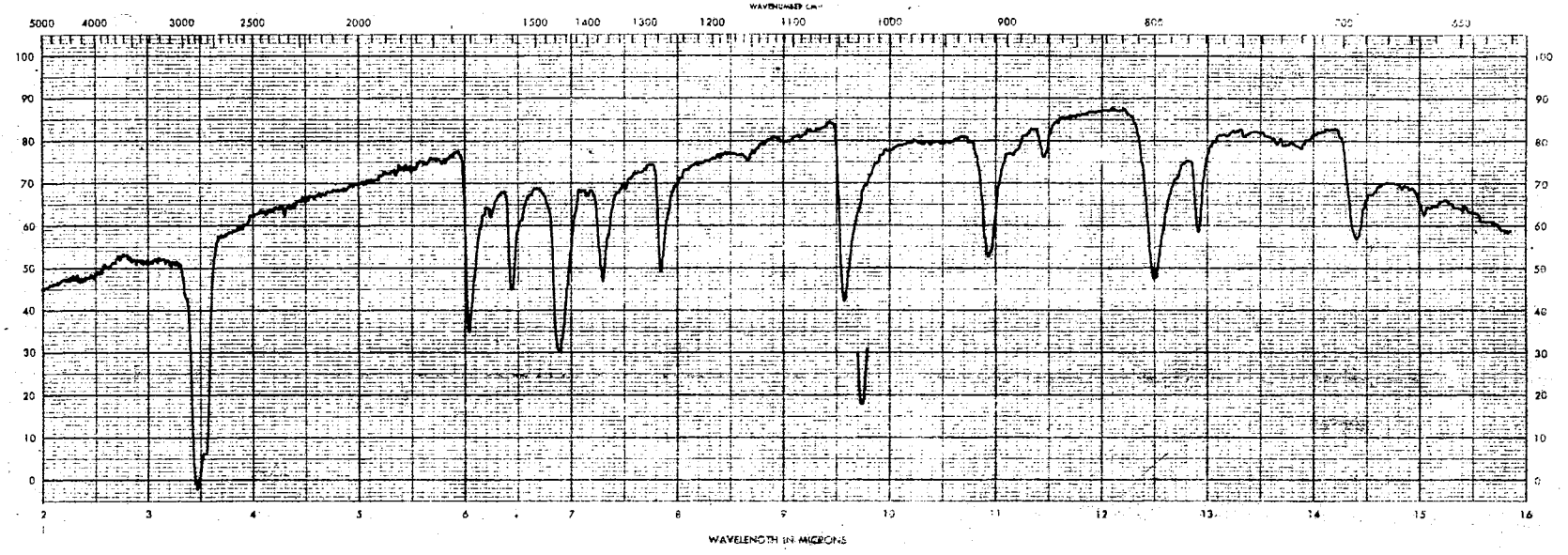


Figure 4. Infrared Spectrum of N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine -- Nujol Mull

solvent (acetone-d_6) used. The doublet at 7.62 δ represents the proton on C-5 when it is anti with the bromoimino group. The doublet at 7.95 δ represents the proton on C-3 when it is syn with the bromoimino group. The most reasonable interpretation[68] of the downfield shift is based on the formation of a hydrogen bond between the proton ortho to the imine group and the aprotic solvent. When a hydrogen bond is formed with the approach of the base (proton acceptor) to the ortho proton, the base on the syn side suffers from the steric hindrance due to the halogen atom bonded to the nitrogen atom, while there is no such effect on the anti side. The IR spectrum is in Figure 4.

The NMR spectrum of N,4,6-tribromo-1,2-benzoquinone 2-imine in acetone-d_6 which is shown in Figure 5 shows two doublets one centered around 6.5 δ and the other one around 7.2 δ. The doublet around 6.5 δ represents the proton on C-5 when it is anti with the bromoimino group, and the doublet around 7.2 δ the same proton when it is syn with the bromoimino group. Downfield shift is based on the same argument as for N-bromo-2,6-dichloro-1,4-benzoquinone 4-imine, except here the proximity of the C=O to the bromoimino group causes the further downfield shift. The IR spectrum is shown in Figure 6.

The NMR spectrum of N-chloro-1,4-benzoquinone 4-imine in acetone-d_6, which is shown in Figure 7, shows a multiplet around 2.1 δ, a singlet at 2.65 δ, and a multiplet around 6.5-8.0 δ. The multiplet around 2.1 δ and singlet 2.65 δ both are due to the solvent (acetone-d_6) used. The four doublets centered around 7.55 δ represent the C-3 and C-5 protons being coupled with C-2 and C-6 protons plus the effect of syn-anti isomerism. The quintet centered around 6.6 δ represents the protons on C-2 and C-6. One would have expected to see only two doublets for these

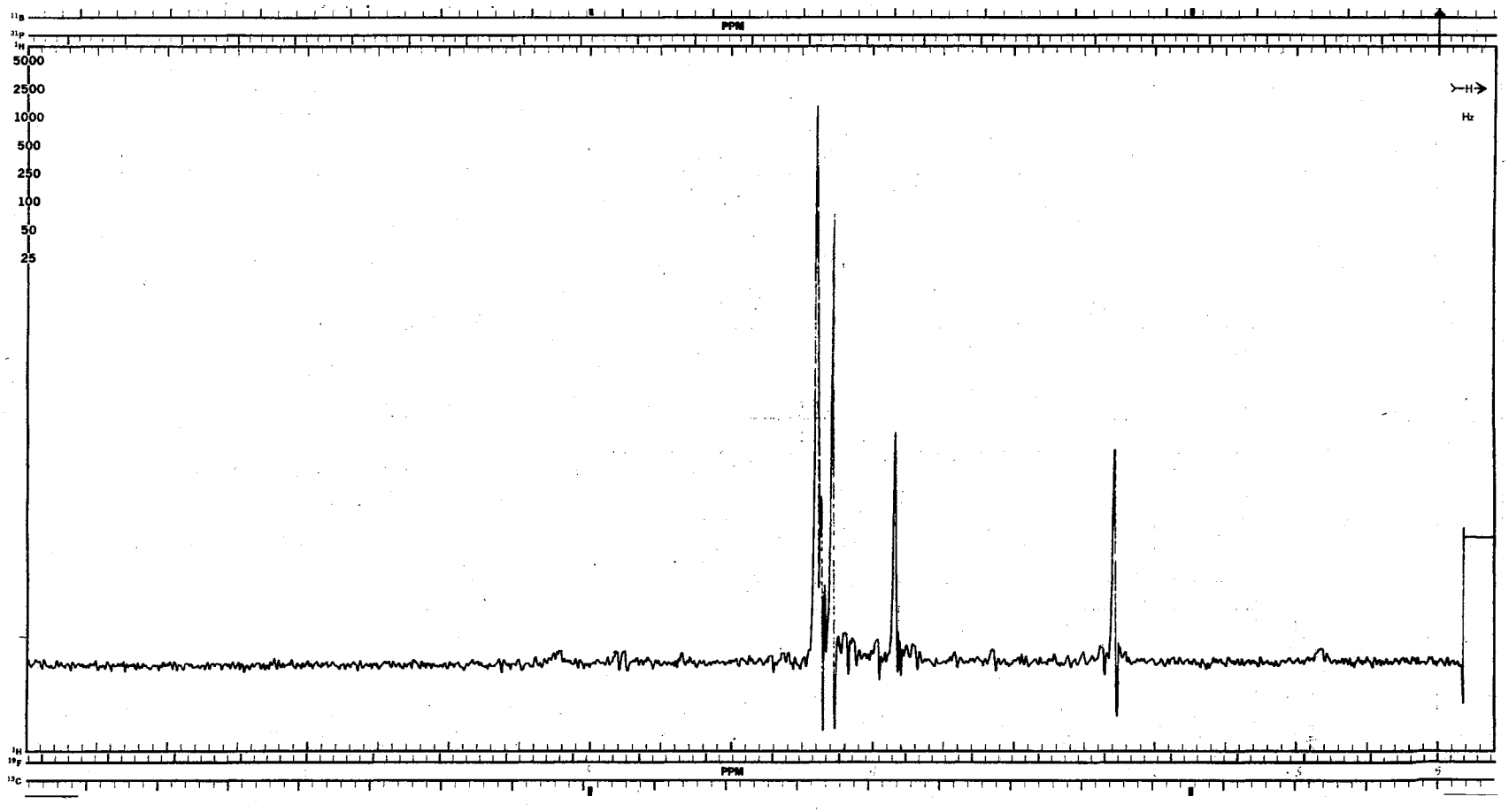


Figure 5. Proton Magnetic Resonance Spectrum of N-Bromo-4,6-dibromo-1,2-benzoquinone 2-Imine in Acetone-d_6 -- 100 MHz; R.F. ... 60 dB; S.W. ... 500 Hz; S.A. ... 2.5; F.B. ... 2 Hz; S.T. ... 250 sec; S.O. ... 84201

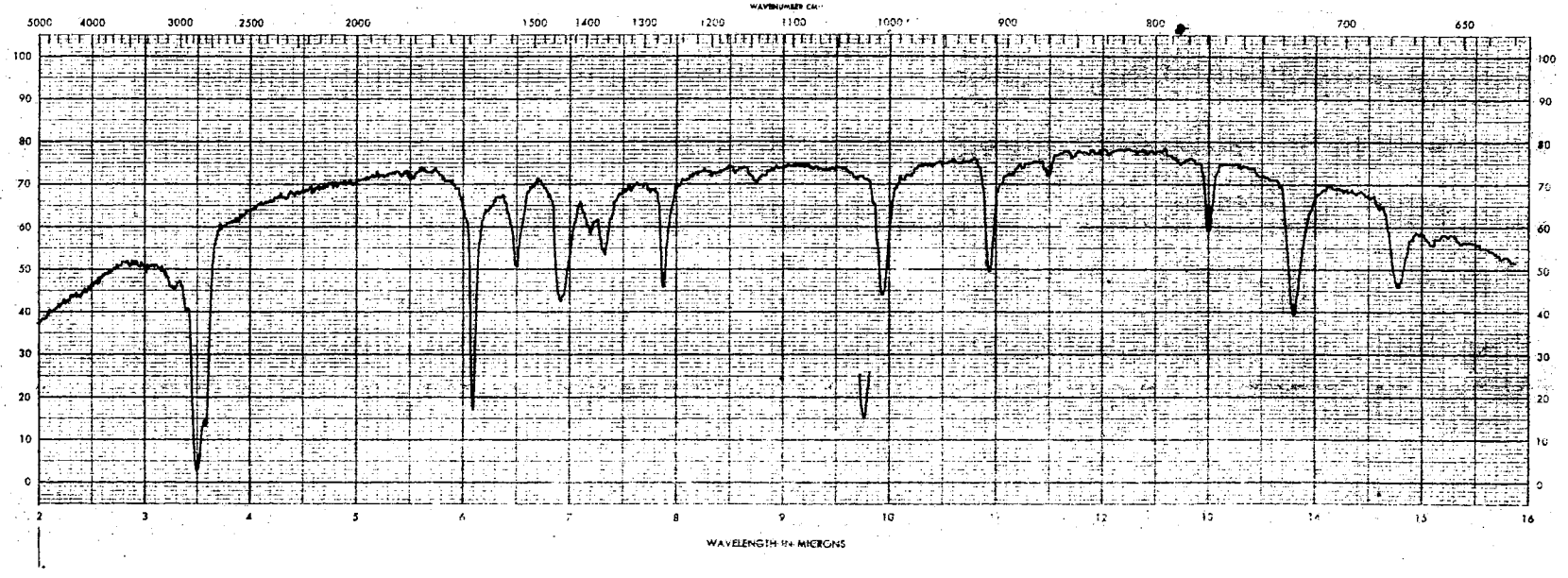


Figure 6. Infrared Spectrum of N-Bromo-4,6-dibromo-1,2-benzoquinone 2-Imine -- Nujol Mull

two protons, but since the couplings are identical, the result is a quintet. The IR spectrum is in Figure 8.

Analysis of the NMR and IR spectra of these three N-halobenzoquinone imines as representatives of the other quinone imines, proves the quinonoid structure of these compounds.

The NMR spectrum of 2-chloro-N,N-dimethylindoaniline in acetone-d_6, which is shown in Figure 9 shows a multiplet around 2.1 δ, a singlet at 2.70 δ, another singlet at 3.1 δ and another multiplet around 6.5-7.65 δ. The multiplet around 2.1 δ and singlet at 2.70 δ both are due to the solvent (acetone-d_6) used. The singlet at 3.1 δ which contains six protons represents the two methyl groups on the nitrogen attached to the aromatic ring. The assignment of the signals to the multiplet around 6.5-7.65 δ is difficult because of the close similarities of the protons on the quinonoid ring and the aromatic ring. However, the exact assignment of the signals can be accomplished by decoupling. From comparison of the NMR spectrum of 2-chloro-1,4-benzoquinone 4-oxime with that of 2-chloro-N,N-dimethylindoaniline, the doublet at 7.6 δ can probably be assigned to the proton on C-3 of the quinonoid part and the two doublets centered around 7.35 δ can be assigned to the C-5 proton of the quinonoid part and the doublet around 6.9 δ to the C-6 proton on the quinonoid part. The rest of the signals can be identified with accuracy only by decoupling. The IR spectrum is in Figure 10.

Studies of Compounds

Stability Studies

The relative stabilities of the compounds in aqueous solutions buffered at pH 7.0 at a temperature of 24° were determined spectrophoto-

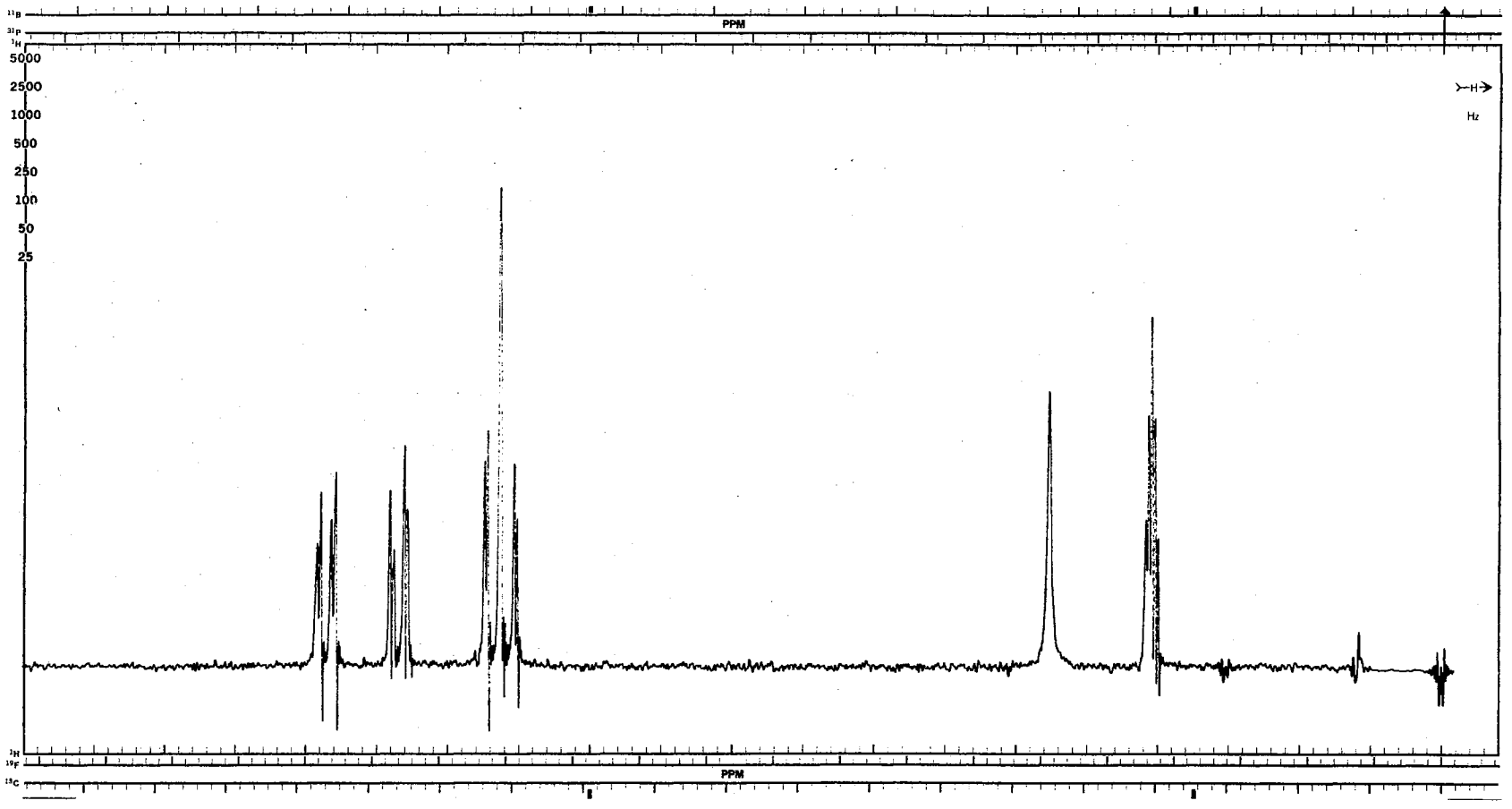


Figure 7. Proton Magnetic Resonance Spectrum of N-Chloro-1,4-benzoquinone 4-Imine in Acetone-d_6 -- 100 MHz. R.F. ... 69 dB; S.W. ... 1000 Hz; S.A. ... 6.3; F.B. ... 2 Hz; S.T. ... 250 sec; S.O. ... 83701 Hz

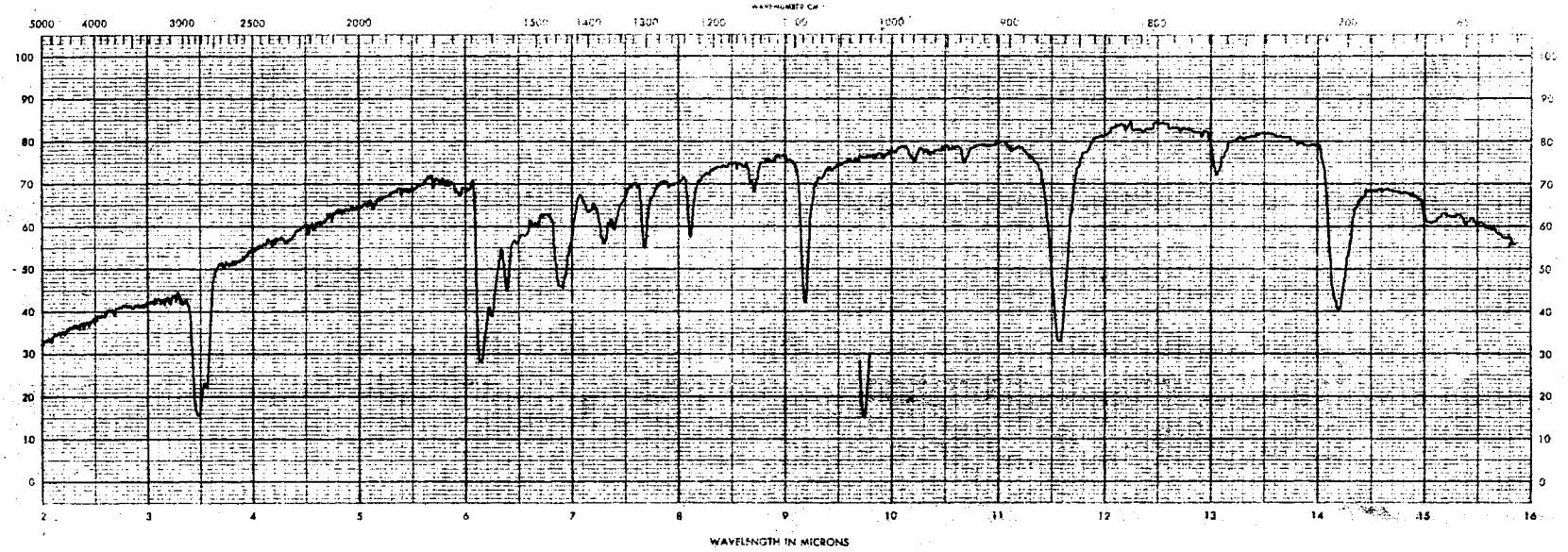


Figure 8. Infrared Spectrum of N-Chloro-1,4-benzoquinone 4-Imine -- Nujol Mull

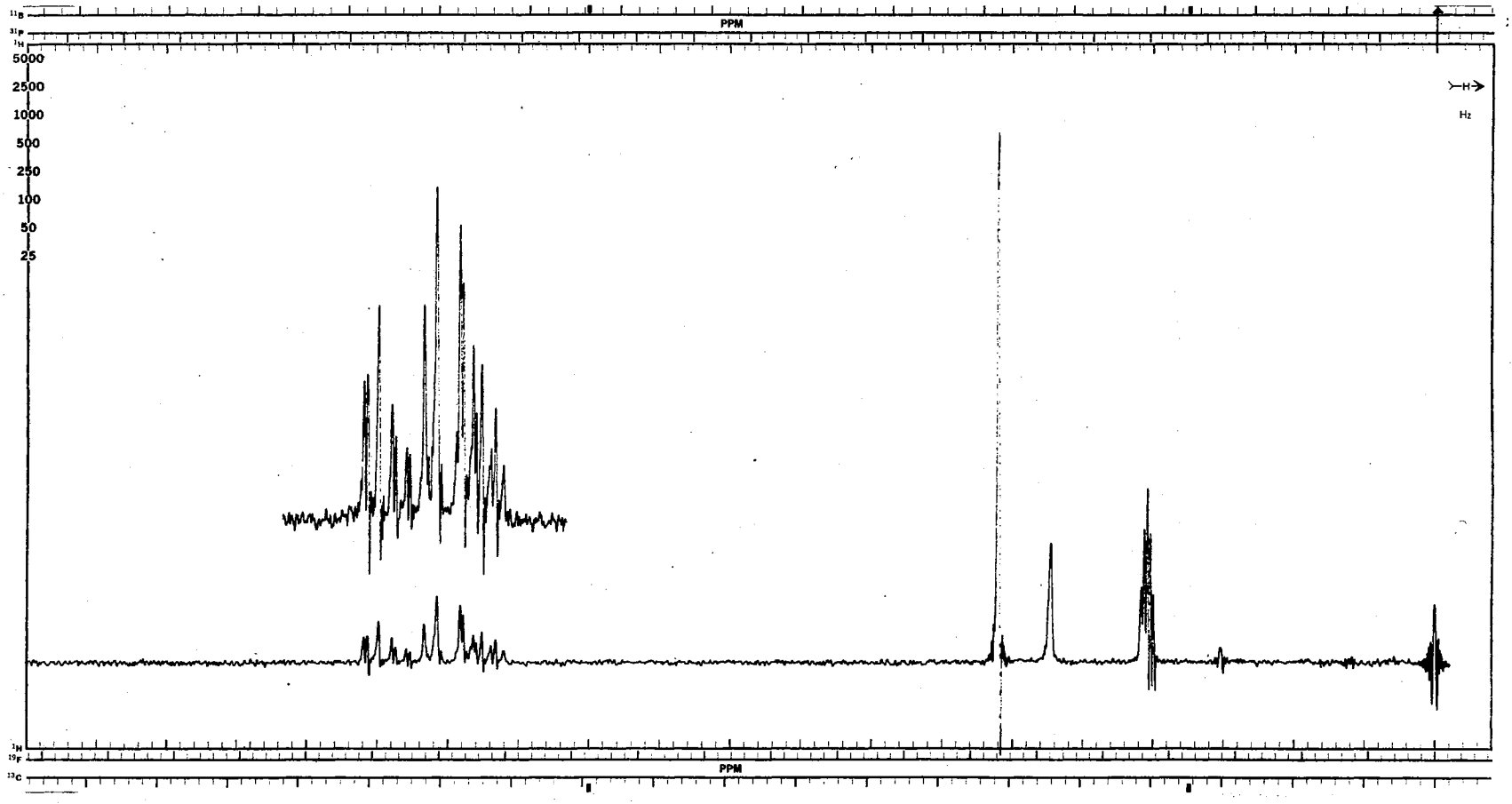


Figure 9. Proton Magnetic Resonance Spectrum of 2-Chloro-N,N-dimethylindoaniline in Acetone-d_6 -- 100 MHz. R.F. ... (A) 69, (B) 74 dB; S.W. ... 1000 Hz; S.A. ... (A) 5, (B) 16; F.B. ... 2 Hz; S.T. ... 250 sec; S.O. ... 83701 Hz

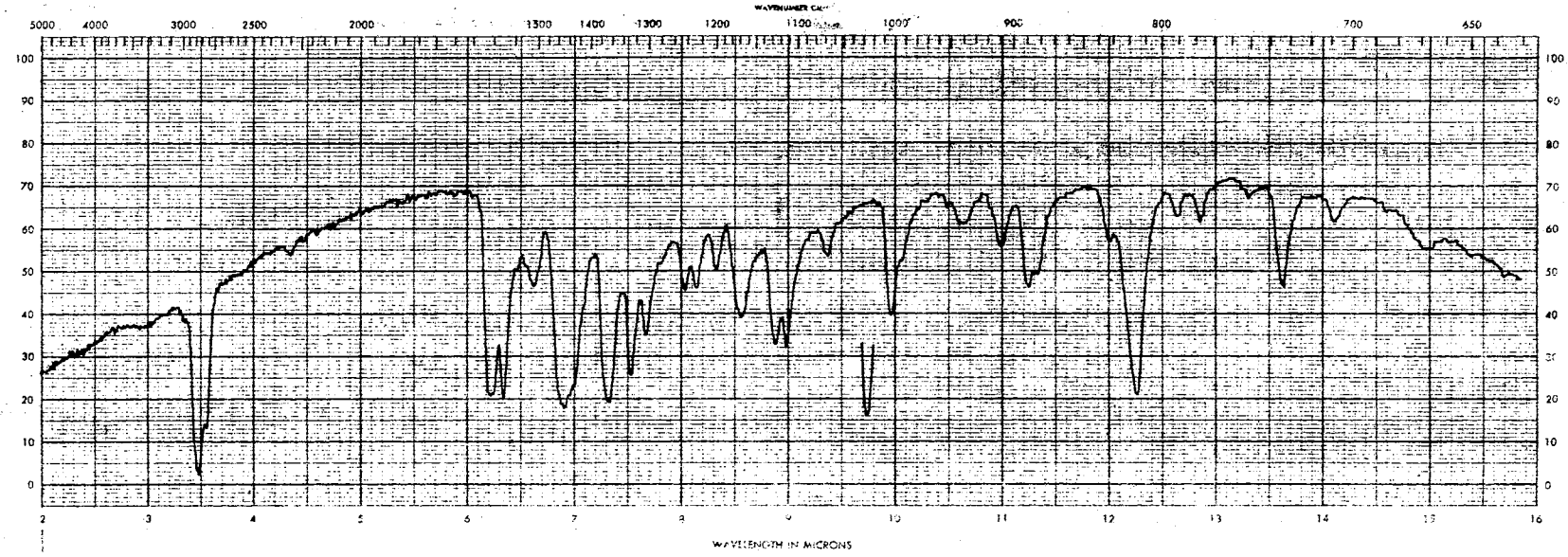


Figure 10. Infrared Spectrum of 2-Chloro-N,N-dimethylindoaniline -- Nujol Mull

metrically. The buffer solution contained 1.219 g (0.01 mole) of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) in one liter of solution. Enough hydrochloric acid was added to bring the pH to 7.0. The compounds were first dissolved in a small amount of absolute alcohol (because of solubility problems with some of the compounds in buffer solution) and the solutions then diluted to the desired concentration with buffer solution. Spectrophotometric readings were determined on a Cary 14 Recording Spectrophotometer; the cuvettes used were one centimeter in thickness. Data are given in Tables XIV through XXVI.

For a first-order reaction,

$$2.303 \ (\log c_1 - \log c_2) = k(t_2 - t_1)$$

where c_1 is the concentration at t_1 and c_2 the concentration at t_2. Since concentration is directly proportional to absorbance or optical density, A, the equation becomes

$$2.303 \ (\log A_1 - \log A_2) = k(t_2 - t_1) \ .$$

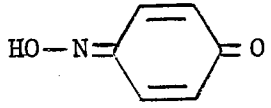
When the logarithms of the absorbance were plotted vs time, a straight line was obtained in each case. Since the slope of this line is $-k/2.303$, the rate constant can be calculated for the hydrolysis of each compound under these conditions. The half-time for the hydrolysis is the time required for the concentration of the compound to decrease to one-half of its starting value. Since this value is useful for our purpose, it was also calculated. Since

$$2.303 \ (\log 2 - \log 1) = kt_{\frac{1}{2}}$$

$$t_{\frac{1}{2}} = 0.693/k$$

TABLE XIV

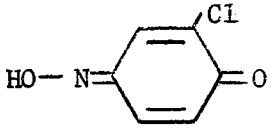
HYDROLYSIS OF 1,4-BENZOQUINONE 4-OXIME



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	305	1.570	0.040	1.530	0.1846
1215	305	1.490	0.045	1.445	0.1598
3775	305	1.650	0.185	1.465	0.1658
6100	305	1.430	0.020	1.410	0.1499
6720	305	1.550	0.140	1.410	0.1499

TABLE XV

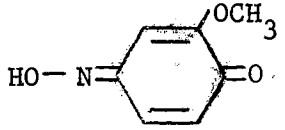
HYDROLYSIS OF 2-CHLORO-1,4-BENZOQUINONE 4-OXIME



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	264	1.760	0.130	1.630	0.2121
585	264	1.510	0.170	1.340	0.1271
1335	264	1.210	0.050	1.160	0.0644
2890	264	1.180	0.040	1.140	0.0569
4750	264	1.180	0.050	1.130	0.0530
5725	264	1.200	0.073	1.127	0.0499
6225	264	1.165	0.045	1.120	0.0492
6730	264	1.155	0.045	1.110	0.0453

TABLE XVI

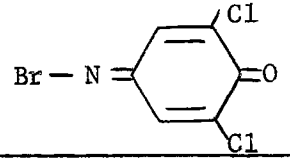
HYDROLYSIS OF 2-METHOXY-1,4-BENZOQUINONE 4-OXIME



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	322	1.510	0.130	1.380	0.1398
620	322	1.670	0.120	1.550	0.1903
1330	322	1.340	0.050	1.290	0.1105
2885	322	1.290	0.045	1.245	0.0951
4745	322	1.260	0.060	1.200	0.0791
5835	322	1.265	0.082	1.183	0.0729
6385	322	1.185	0.050	1.135	0.0550
7105	322	1.110	0.050	1.060	0.0253

TABLE XVII

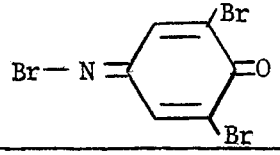
HYDROLYSIS OF N-BROMO-2,6-DICHLORO-1,4-BENZOQUINONE 4-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance A	Log A
		Solution	Solvent		
5	324	1.285	0.085	1.200	0.0791
15	324	1.240	0.085	1.155	0.0625
20	324	1.200	0.085	1.115	0.0472
60	324	1.050	0.085	0.965	-0.0155
330	324	0.680	0.045	0.640	-0.1938
665	324	0.235	0.102	0.133	-0.8761
1670	324	-----	-----	-----	-----

TABLE XVIII

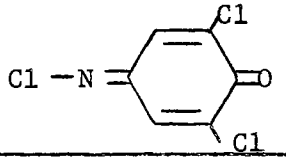
HYDROLYSIS OF N,2,6-TRIBROMO-1,4-BENZOQUINONE 4-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	328	1.440	0.130	1.310	0.1172
10	328	1.360	0.130	1.230	0.0899
15	328	1.300	0.130	1.170	0.0681
20	328	1.235	0.130	1.105	0.0433
27	328	1.160	0.130	1.030	0.0124
30	328	1.130	0.130	1.000	0.000
600	328	0.720	0.130	0.590	-0.2291
1290	328	-----	-----	-----	-----

TABLE XIX

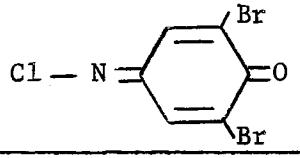
HYDROLYSIS OF N,2,6-TRICHLORO-1,4-BENZOQUINONE 4-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	314	1.380	0.130	1.250	0.0969
690	314	0.970	0.050	0.920	-0.0339
2295	314	0.675	0.045	0.630	-0.2007
4150	314	-----	-----	-----	----

TABLE XX

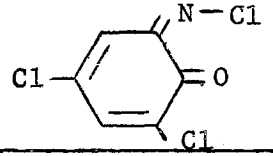
HYDROLYSIS OF N-CHLORO-2,6-DIBROMO-1,4-BENZOQUINONE 4-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	320	1.870	0.085	1.785	0.2516
15	320	1.845	0.085	1.760	0.2455
305	320	1.665	0.045	1.615	0.2081
640	320	1.495	0.100	1.395	0.1445
1625	320	0.865	0.060	0.800	-0.0969
3030	320	-----	-----	-----	------

TABLE XXI

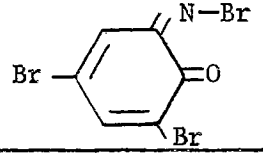
HYDROLYSIS OF N,4,6-TRICHLORO-1,2-BENZOQUINONE 2-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	278	1.520	0.080	1.440	0.1583
120	278	1.360	0.080	1.245	0.0951
560	278	1.035	0.048	0.987	-0.0057
1385	278	-----	-----	-----	------

TABLE XXII

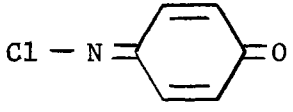
HYDROLYSIS OF N,4,6-TRIBROMO-1,2-BENZOQUINONE 2-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	220	0.590	0.075	0.515	-0.2882
25	220	0.470	0.075	0.405	-0.3925
360	220	0.445	0.050	0.395	-0.4034
700	220	0.455	0.085	0.370	-0.4318
1690	220	0.410	0.060	0.350	-0.4559
3100	220	-----	-----	-----	------

TABLE XXIII

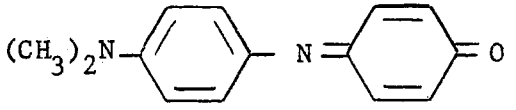
HYDROLYSIS OF N-CHLORO-1,4-BENZOQUINONE 4-IMINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	287	1.690	0.050	1.640	0.2148
4860	287	0.840	0.030	0.810	-0.0915
5675	287	0.664	0.145	0.520	-0.2840
7160	287	0.230	0.130	0.100	-1.000
7770	287	-----	-----	-----	-----

TABLE XXIV

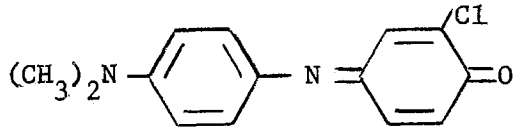
HYDROLYSIS OF N,N-DIMETHYLINDOANILINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	274	1.715	0.050	1.665	0.2214
1340	274	1.645	0.045	1.660	0.2041
4215	274	1.750	0.180	1.570	0.1959
6215	274	1.510	0.060	1.450	0.1731
7040	274	1.615	0.145	1.470	0.1673

TABLE XXV

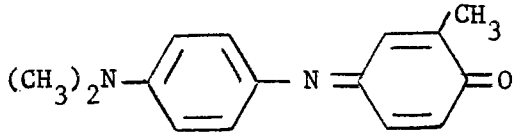
HYDROLYSIS OF 2-CHLORO-N,N-DIMETHYLINDOANILINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	285	0.650	0.130	0.520	-0.2840
600	285	0.600	0.150	0.450	-0.3468
1315	285	0.480	0.050	0.430	-0.3665
2910	285	0.470	0.045	0.425	-0.3716
4775	285	0.510	0.080	0.430	-0.3665
5705	285	0.510	0.090	0.420	-0.3768

TABLE XXVI

HYDROLYSIS OF 2-METHYL-N,N-DIMETHYLINDOANILINE



Elapsed Time, Min	λ_{max}, nm	Optical Density		Absorbance, A	Log A
		Solution	Solvent		
5	279	1.650	0.050	1.600	0.2041
1290	279	1.630	0.046	1.584	0.1997
4155	279	1.750	0.180	1.570	0.1959
6130	279	1.650	0.090	1.560	0.1931
6750	279	1.665	0.145	1.520	0.1818

The half-life for each compound under these conditions is listed in Table XXVII with the reaction rate constant.

Toxicity Studies

Determinations of the acute toxicities of the compounds were made by single-dose intraperitoneal injections into Swiss mice. The amount of a single dose was varied until one was found to kill approximately one-half of the animals within five days.

Six male or female Swiss mice weighing between 17 and 20 g were used in each treatment. The solutions were prepared fresh just before testing by weighing a given amount of sample and adding to it the amount of salt needed for an isotonic solution. This mixture was ground to a very fine powder and suspended in the proper volume of distilled water. Appropriate volumes of this suspension was then injected into the mice.

The LD_{50} of each compound is given in Table XXVIII expressed in terms of mg/kg of mouse as well as millimoles/kg of mouse.

Inhibition of Ascitic Sarcoma 180

The ascitic sarcoma 180 tumor cells used in this study were obtained originally from Frederic A. French of Mt. Zion Hospital, San Francisco, California, through living mouse carriers. Six female Swiss mice weighing between 17 and 20 g were used in each treatment. The solutions were prepared fresh each time just before testing by suspending a weighed amount of sample in saline solution.

Tumor cells to be used in inoculations were obtained by sacrificing one of the carrier mice with one week old tumor cells. The skin from the abdominal area of the mouse was removed and 2 ml of ascitic fluid

TABLE XXVII

HYDROLYSIS RATES OF THE COMPOUNDS--SUMMARY

Compound	-slope x 10^4	k x 10^4 hr^{-1}	$t_{\frac{1}{2}}$, hr
N,N-Dimethylindoaniline	4.3	9.9	700.
2-Chloro-N,N-dimethylindoaniline	6.3	14.5	478.
2-Methyl,N,N-dimethylindoaniline	1.5	3.5	2010.
1,4-Benzoquinone 4-Oxime	2.5	5.8	1210.
2-Chloro-1,4-benzoquinone 4-Oxime	9.6	22.	314.
2-Methoxy-1,4-benzoquinone 4-Oxime	10.1	23.3	298.
N-Chloro-1,4-benzoquinone 4-Imine	85.0	196.	35.3
N-2,6-Trichloro-1,4-benzoquinone 4-Imine	75.0	173.	40.0
N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine	810.0	1871.	3.7
N-2,6-Tribromo-1,4-benzoquinone 4-Imine	300.0	69.	10.4
N-Chloro-2,6-dibromo-1,4-benzoquinone 4-Imine	120.0	296.	23.3
N-4,6-Trichloro-1,2-benzoquinone 2-Imine	160.0	383.	18.1
N,4,6-Tribromo-1,2-benzoquinone 2-Imine	41.0	95.	72.8

TABLE XXVIII

TOXICITIES OF COMPOUNDS

Compound	LD_{50}, mg/kg	LD_{50}, mmole/kg
N,N-Dimethylindoaniline	80.	0.3535
2-Chloro-N,N-dimethylindoaniline	400.	1.5342
2-Methyl-N,N-dimethylindoaniline	27.	0.1124
2-Methoxy-N,N-dimethylindoaniline	25.	0.0975
2-Acetamido-N,N-dimethylindoaniline	70.	0.2471
1,4-Benzoquinone 4-Oxime	260.	2.1118
2-Chloro-1,4-benzoquinone 4-Oxime	240.	1.5232
2-Methoxy-1,4-benzoquinone 4-Oxime	120.	0.7835
3-Chloro-1,4-benzoquinone 4-Oxime	380.	2.4118
3-Bromo-1,4-benzoquinone 4-Oxime	400.	1.980
3-Iodo-1,4-benzoquinone 4-Oxime	400.	1.610
N-Chloro-1,4-benzoquinone 4-Imine	12.5	0.0883
N-Bromo-1,4-benzoquinone 4-Imine	40.	0.2150
N-2,6-Trichloro-1,4-benzoquinone 4-Imine	105.	0.4989
N-Chloro-2,6-dibromo-1,4-benzoquinone 4-Imine	63.	0.2104
N-2,6-Tribromo-1,4-benzoquinone 4-Imine	25.	0.0727
N-Bromo-2,6-dichloro-1,4-benzoquinone 4-Imine	20.	0.0981
Indophenol acetate	90.	0.3731
Indophenol sodium salt	50.	0.2260

was withdrawn with a 1-ml syringe with a 25-gauge needle. The fluid was diluted with saline to an approximate cell concentration of two million cells per 0.1 ml of solution. A 0.1-ml portion of this cell solution was injected into the intraperitoneal cavity of each mouse. Necessary precautions were made to prevent or minimize bacterial contamination. Administration of the drug was done once daily for three consecutive days, beginning 24 hours after tumor implantation. Mice used for controls were injected with isotonic saline solution each time. The mice were weighed on the first day of treatment and on the day after the last treatment and their average weight differences were calculated.

The mean survival time of treated animals was calculated by averaging the survival times of the six animals tested. The mean survival time of the control animals was calculated in the same manner. The effectiveness of the compounds in controlling this tumor system is expressed by the ratio of the average survival time of the treated animals to that of the control animals (T/C). For statistically significant effect, the value of T/C should be 125%. Values lower than 100% represent chemical toxicity. The data for each dose level are given in Tables XXIX through XXXIII. The antitumor data for all the compounds are summarized in Table XXXIV in several ways.

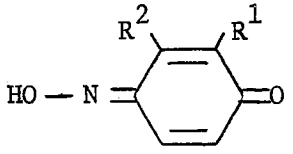
The optimum dose is that dose which administered according to this protocol gives the greatest increase in survival time with the least toxicity. This value is found by study of the dose-response curve. The optimum T/C is the value of T/C at the optimum dose.

The effective dose is the dose which will give a T/C value of 125%. This dose is found from the dose-response curve.

The maximum T/C is the largest value of T/C obtained with at least

TABLE XXIX

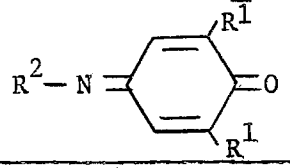
INHIBITION OF ASCITIC SARCOMA 180 BY QUINONE 4-OXIMES



Substituents R^1	R^2	Daily Dose, mg/kg	5-Day Survivors	Ave. Wt. Gain, % Treated	Ave. Wt. Gain, % Controls	Survival Times, Days Ave. ± Standard Error Treated	Survival Times, Days Ave. ± Standard Error Controls	T/C, %
H	H	100	6/6	+1.4	+3.5	12.8 ± 2.6	12.5 ± 6.2	103
OCH_3	H	52	6/6	+3.5	+3.5	13.3 ± 2.3	10.2 ± 3.1	131
		60	6/6	+0.4	+2.1	18.5 ± 10.5	10.7 ± 3.0	173
		70	5/5	+0.8	+3.5	13.0 ± 1.1	10.2 ± 3.1	128
		88	6/6	+2.7	+3.5	11.8 ± 2.5	10.2 ± 3.1	116
R^1, R^2 = benzo		50	6/6	+2.1	+3.5	13.3 ± 5.0	10.2 ± 3.1	131
		75	6/6	+2.5	+1.6	13.2 ± 1.5	14.2 ± 3.3	93
		125	5/6	+0.1	+1.6	10.3 ± 3.9	14.2 ± 3.3	73

TABLE XXX

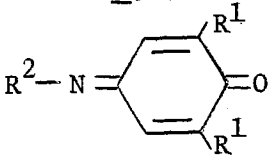
INHIBITION OF ASCITIC SARCOMA 180 BY N-HALO-1,4-BENZOQUINONE 4-IMINES



Substituents R^1	Substituents R^2	Daily Dose, mg/kg	5-Day Survivors	Ave. Wt. Gain, % Treated	Ave. Wt. Gain, % Controls	Survival Times, Days Ave. ± Standard Error Treated	Survival Times, Days Ave. ± Standard Error Controls	T/C, %
H	Cl	4.5	6/6	+0.1	+1.6	9.8 ± 3.3	16.2 ± 4.6	61
		5.0	5/5	-0.1	+0.7	21.7 ± 3.2	16.3 ± 4.7	133
		5.0	6/6	-1.5	+1.4	40.5 ± 21.5	16.5 ± 4.9	246
		7.5	6/6	-2.6	+1.4	28.2 ± 18.6	16.5 ± 4.9	171
		9.0	5/6	-3.9	+1.4	18.0 ± 7.5	16.5 ± 4.9	109
H	Br	11	6/6	-0.9	+2.1	12.7 ± 2.6	10.67 ± 3.01	119
		15	5/6	-0.8	+2.1	15.5 ± 7.0	10.67 ± 3.01	145
		19	6/6	-1.9	+2.1	11.5 ± 2.2	10.67 ± 3.01	108
		20	4/6	-2.1	+2.9	12.7 ± 6.3	10.83 ± 3.31	117

TABLE XXXI

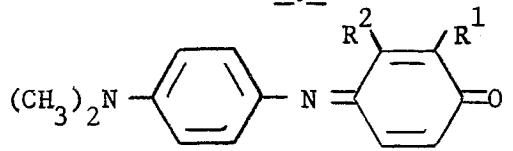
INHIBITION OF ASCITIC SARCOMA 180 BY N,2,6-TRIHALO-1,4-BENZOQUINONE 4-IMINES



Substituents R^1	Substituents R^2	Daily Dose, mg/kg	5-Day Survivors	Ave. Wt. Gain, % Treated	Ave. Wt. Gain, % Controls	Survival Times, Days Ave. ± Standard Error Treated	Survival Times, Days Ave. ± Standard Error Controls	T/C, %
Bl	Cl	15.0	6/6	-0.9	+1.6	21.0 ± 3.6	14.0 ± 3.9	150
		20.0	6/6	-2.2	+1.6	28.0 ± 7.8	14.0 ± 3.9	200
		22.5	5/6	-6.1	+1.1	41.0 ± 21.1	18.8 ± 2.1	218
		25.0	5/6	-1.9	+1.6	27.8 ± 19.9	14.0 ± 3.9	199
		30.0	2/6	-2.9	+1.1	5.5 ± 3.5	18.8 ± 2.1	29
		37.5	0/6	--	+1.1	3.0 ± 0.9	18.8 ± 2.1	16
Br	Br	10	6/6	+0.5	+2.9	15.2 ± 4.9	10.8 ± 3.3	140
		12	5/6	0.0	+2.1	11.2 ± 4.1	10.7 ± 3.0	105
		16	6/6	-0.9	+2.1	15.5 ± 6.2	10.7 ± 3.0	145
		20	6/6	-2.2	+2.1	18.5 ± 10.0	10.7 ± 3.0	173
Cl	Cl	30	5/6	-3.1	+2.9	11.3 ± 4.3	10.8 ± 3.3	105
		40	4/6	-3.0	+2.9	11.8 ± 5.7	10.8 ± 3.3	109
		50	6/6	-2.3	+2.9	14.8 ± 3.9	10.8 ± 3.3	137
		60	4/6	-2.4	+1.6	12.7 ± 7.10	16.2 ± 4.6	78

TABLE XXXII

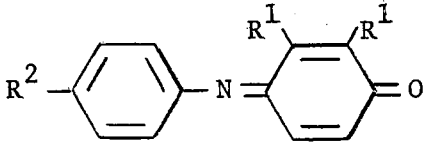
INHIBITION OF ASCITIC SARCOMA 180 BY N,N-DIMETHYLINDOANILINE DERIVATIVES



Substituents R^1	R^2	Daily Dose, mg/kg	5-Day Survivors	Ave. Wt. Gain, % Treated	Ave. Wt. Gain, % Controls	Survival Times, Days Ave. ± Standard Error Treated	Survival Times, Days Ave. ± Standard Error Controls	T/C, T
CH_3	H	4.5	6/6	+2.6	+1.9	12.3 ± 2.7	13.7 ± 3.4	90
		6.0	6/6	-0.1	+1.6	18.0 ± 4.6	14.2 ± 3.3	127
		7.5	6/6	+0.6	+1.9	12.7 ± 3.5	13.7 ± 3.4	93
R_1, R_2 = benzo		150	6/6	-1.4	+1.6	8.5 ± 1.6	14.2 ± 3.3	60
		200	6/6	-0.3	+3.5	8.5 ± 1.5	10.2 ± 3.1	84
		250	6/6	-1.4	+1.6	9.2 ± 2.3	14.2 ± 3.3	65
H	H	18	6/6	+3.0	+3.5	15.2 ± 11.7	12.5 ± 6.2	121
		24	6/6	+2.1	+3.5	10.2 ± 2.2	12.5 ± 6.2	81
		30	6/6	-0.4	+3.5	16.5 ± 11.8	12.5 ± 6.2	132
		40	4/6	-2.7	+2.8	14.8 ± 17.1	14.8 ± 3.7	100
OCH_3	H	6.7	6/6	+1.3	+3.5	15.7 ± 3.1	10.2 ± 3.1	154
		9.0	6/6	+0.8	+3.5	14.5 ± 3.4	10.2 ± 3.1	143
		10.0	6/6	-2.8	+2.1	19.3 ± 4.3	10.7 ± 3.0	181
		11.2	6/6	-1.0	+3.5	16.0 ± 2.1	10.2 ± 3.1	157

TABLE XXXIII

INHIBITION OF ASCITIC SARCOMA 180 BY INDOPHENOL DERIVATIVES



Substituents R^1	Substituents R^2	Daily Dose, mg/kg	5-Day Survivors	Ave. Wt. Gain, % Treated	Ave. Wt. Gain, % Controls	Survival Times, Days Ave. ± Standard Error Treated	Survival Times, Days Ave. ± Standard Error Controls	T/C, %
H	ONa	15	6/6	-0.4	+1.3	37.3 ± 20.2	15.3 ± 5.8	244
		20	5/6	-2.5	+1.3	24.7 ± 22.0	15.3 ± 5.8	161
		25	6/6	-1.3	+1.3	23.8 ± 3.6	15.3 ± 5.8	155
H	$OCOCH_3$	20	6/6	-0.3	+2.8	19.2 ± 5.6	14.8 ± 3.7	129
		26	6/6	-0.9	+2.8	15.0 ± 4.4	14.8 ± 3.7	101
		32	6/6	-0.2	+2.8	15.5 ± 9.9	14.8 ± 3.7	104
		34	5/6	-2.0	+1.1	13.8 ± 9.6	15.8 ± 1.9	87
		45	3/6	-2.1	+1.1	20.0 ± 21.7	15.8 ± 1.9	126
		56	1/6	-3.2	+1.1	4.7 ± 0.8	15.8 ± 1.9	30

TABLE XXXIV

INHIBITION OF ASCITIC SARCOMA 180--SUMMARY

Compound	Optimum Dose, mg/kg	Optimum T/C, %	Effective Dose, mg/kg	Maximum T/C, %
N,N-Dimethylindoaniline	18	121	18	132
2-Methyl-N,N-dimethylindoaniline	6	127	6	127
2-Methoxy-N,N-dimethylindoaniline	10	181	3	181
1,4-Benzoquinone 4-Oxime	100	102	---	107
2-Methoxy-1,4-benzoquinone 4-Oxime	52	131	40	173
N-Chloro-1,4-benzoquinone 4-Imine	5	133	4.75	245
N-Bromo-1,4-benzoquinone 4-Imine	15	145	12	145
N,2,6-Trichloro-1,4-benzoquinone 4-Imine	50	137	45	137
N-Chloro-2,6-dibromo-1,4-benzoquinone 4-Imine	20	200	12	217
N,2,6-Tribromo-1,4-benzoquinone 4-Imine	20	140	18	173
Indophenol acetate	20	129	18	129
Indophenol sodium salt	15	244	5	244

four survivors out of six animals at the end of five days.

Oxidation-Reduction Potentials

The procedure used for this study was adapted from the work done by Fieser and Fieser.[69] Redox potential measurements were made by titration of a solution of each compound prepared by dissolving 0.1 mmole of the oxidant in 60 ml of solvent (50% alcohol, 0.1 N HCl, and 0.2 N in LiCl).[69] The above solution was prepared just before titration with ascorbic acid as the reducing agent. The ascorbic acid solution was made by dissolving 2.202 g (0.0125 mole) of ascorbic acid in 500 ml of the same solvent. All titrations were done at 24.8 ± 0.05° under nitrogen with a Beckman Research pH meter using the millivolt scale. Electrodes used were bright platinum and the reference electrode was a saturated calomel electrode.

The ascorbic acid solution was added slowly to the solution of the compound with magnetic stirring. Potential readings were made after each addition of 0.25 to 0.50 ml. The potential reading at one-half titration plus the potential of the saturated calomel electrode (0.2464 v) gave the redox potential. Duplicate determinations of this potential in all cases agreed within 1-5 mv. and the values reported in Table XXXV are averages. Since the redox potential here measured is valid only for the conditions of the experiment (solvent, hydrogen ion concentration, and salt concentration) it is referred to as a conditional redox potential.

TABLE XXXV

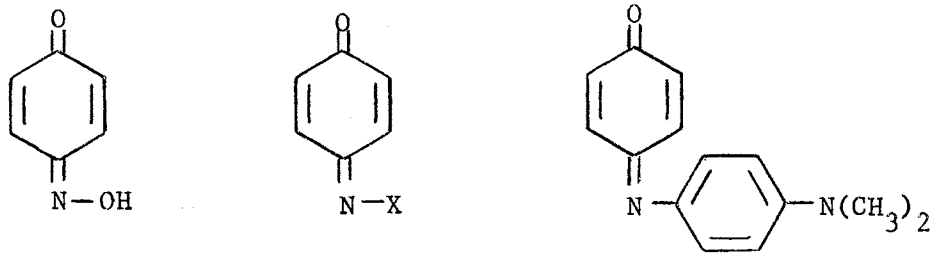
OXIDATION-REDUCTION POTENTIALS OF THE COMPOUNDS

Compound	Conditional Redox Potential, mv.
N,N-Dimethylindoaniline	628
2-Methyl-N,N,dimethylindoaniline	604
2-Methoxy-N,N-dimethylindoaniline	561
2-Chloro-N,N-dimethylindoaniline	658
Benzo-N,N-dimethylindoaniline	493
2-Acetamido-N,N-dimethylindoaniline	580
1,4-Benzoquinone 4-Oxime	544
2-Chloro-1,4-benzoquinone 4-Oxime	516
2-Methoxy-1,4-benzoquinone 4-Oxime	521
3-Chloro-1,4-benzoquinone 4-Oxime	537
3-Bromo-1,4-benzoquinone 4-Oxime	532
3-Iodo-1,4-benzoquinone 4-Oxime	531
1,4-Naphthoquinone 4-Oxime	503
N-Chloro-1,4-benzoquinone 4-Imine	652
N-Bromo-1,4-benzoquinone 4-Imine	518
N-Chloro-2,6-dibromo-1,4-benzoquinone 4-Imine	665
N-2,6-Tribromo-1,4-benzoquinone 4-Imine	678
N-2,6-Trichloro-1,4-benzoquinone 4-Imine	659
N,4,6-Tribromo-1,2-benzoquinone 2-Imine	648
N,4,6-Trichloro-1,2-benzoquinone 2-Imine	720
2-Amino-1,4-naphthoquinone 4-Imine Hydrochloride	541
N,N'-Diacetyl-2-amino-1,4-naphthoquinone 4-Imine	528
Indophenol sodium salt	619
Indophenol acetate	633

CHAPTER IV

RESULTS AND DISCUSSION

Three series of compounds, represented by the following structures, have been prepared and studied as potential tumor inhibitors.



With different substituents on the ring, they were expected to possess a wide range of solubility and electronic properties, which should affect their biological properties.

Although the above compounds have quite different chemical and physical properties and were synthesized by different procedures, they all have one portion of structure in common, a quinoid structure, which is an important factor in their biological activities.

The relative potency of members of a series of drugs is often considered to be a function of differences in hydrophobic, electronic, and steric factors. In the past several years considerable advances have been made in quantifying such relationships.[70] Two basic concepts with respect to quantitative relationships between chemical structure and biological activity have been established. According to the first concept, originating in the work of Bruice, Kharasch, and Winzler[71] and generalized by Free and Wilson[72], the correlation employs a mathematical

model based on the independent and additive contributions of substituents joined to a molecular skeleton which is constant for the entire series. The second method correlates biological activity with thermodynamically defined physico-chemical parameters, that is, in terms of linear free energy relationships of the type of Hammett[73,74] and Taft[75,76] equations, which are used for the quantitative study of the reactivity of organic compounds. This model has been detailed by Hansch et al.[77]

The data obtained from study of the toxicity and ability of these compounds to inhibit ascitic sarcoma 180 in the mouse were analyzed by the Hansch[77] and the Free and Wilson[72] methods. By subjecting the data to multiple linear regression analysis, the contribution of each substituent to the toxicity and the antitumor activity of these compounds was determined, but because of the limited number of compounds in the test no significant correlations could be made. However, from the qualitative point of view, it is apparent that among the three series of compounds studied, the N-haloquinone imines are more active against ascitic sarcoma 180 than the indoaniline derivatives. The latter in turn are more active in this system than the quinone oximes.

Of the compounds tested against ascitic sarcoma 180, 10 had T/C values over 125%, indicating significant antitumor activity. They displayed this inhibitory activity at dose levels of 10 - 50 mg/kg. Unfortunately the toxic levels were close to the effective doses.

The N-haloquinone imines which show the greatest inhibitory effect also are least stable in water at pH 7. These compounds decompose in water with half-lives of 3.7 to 40 hours. The oximes and indoanilines have half-lives under these conditions of 5 - 20 days and 8 - 33 days respectively. The rate of decomposition seems to be related in a favor-

able manner to the tumor-inhibiting abilities.

The oxidation-reduction potentials may be related to the ability of the compounds to inhibit ascitic sarcoma 180 in mice, but no reliable correlations were found to connect these two properties. However, it should be noted that the more inhibitory N-haloquinone imines have redox potentials under these conditions of 652 - 720 mv., whereas the oximes and the indoanilines have values of 503 - 544 mv. and 493 - 628 mv. respectively.

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