METHODS OF DRUG DESIGN APPLIED TO BENZOQUINONE ANALOGUES AND THEIR ACTIVITIES AGAINST ASCITIC SARCOMA 180 IN SWISS MICE

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CHAPTER I

INTRODUCTION

Chemotherapy is a formidable weapon in man's arsenal for his continuous warfare against cancer. Before a compound can be used clinically, however, it must withstand a battery of tests against several animal tumor systems and against cell cultures. Volumes of data accumulated during the course of such preliminary screenings could be successfully utilized in drug design. Drug design can play a very important role not only in reducing the amount of work involved in modifying a successful therapeutic agent for increased potency but also in propagating new drugs.

Quinones are known to possess some antitumor activity[17]. They have demonstrated other types of biological activity as well[3]. Quantitative structure-activity relationships constitute a major facet of drug design, Hansch-type multiple regression analysis being the foremost[22]. Discriminant analysis, a relatively new qualitative structure-activity method, has been applied to a few systems with success[36].

The de novo method developed by Free and Wilson[18] could be useful in assigning additivity constants to

structural features of compounds belonging to a series and thereby evaluating the contributions of various substituents to the biological activity.

The purpose of this work was, hence, to prepare several benzoquinone analogues, measure their antitumor activities against ascitic Sarcoma 180 tumor system in Swiss mice and apply statistical methods to the data thus obtained with a view to evaluating the potential of these compounds as antitumor agents and putting in perspective the usefulness of the structure-activity methods.

CHAPTER II

HISTORICAL

While it may be naive to expect a miracle drug or a magic bullet that will selectively destroy the malignant cell, chemotherapy remains a promising modality in the cure or at least, the control of cancer. Unavailability and/or prohibitive cost of other methods such as surgery and radiotherapy in many parts of the world have rendered chemotherapy increasingly important in the world health scene. Combination therapy, i.e., the application of chemotherapy along with the other modalities has come to be the most efficient approach against cancer. Excellent reviews depicting the role of drug therapy in the continuous combat against cancer can readily be found in recent literature[5,6,12,51].

The most extensive investigation of plant, microbial and synthetic drugs is operated by the Cancer Chemotherapy National Service Center of the National Cancer Institute, Bethesda, Maryland, USA. In addition, drug companies, specialized government agencies, co-operative agencies and academic institutions are contributing significantly to the development of anti-neoplastic agents. This has resulted in an enormous amount of data pertaining to

hundreds of thousands of potentially anticancer compounds. Unfortunately, this has resulted in fewer than 100 drugs which could be used clinically.

Nonetheless, the seemingly endless search must go on. A realistic approach for drug design and development has been suggested by Alfred Burger[7]. Drug research has been termed a "lucky accident"[9] and "the luck of the draw"[21]. Meanwhile, scientists worldwide are engaged in finding ways and means of rendering the drug research more of a systematic approach than a "hit or miss" proposition. Structure-activity relationships, attempting to correlate the effect of structure modifications on the biological activities are an integral and essential part of such an attempt. They are especially effective in determining the most active compounds belonging to a series as well as in unearthing new compounds potentially more active than the ones under investigation.

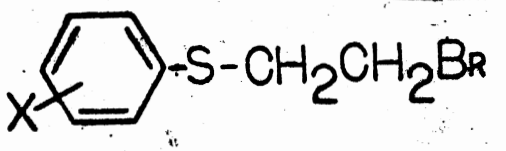
Multiple-parameter regression analysis is the foremost amongst the quantitative structure-activity studies and is popularly known as the Hansch method after its illustrious sire. This involves preparation of several compounds with a parent structure and several substituents and evaluation of their biological activities. Biological activity should be expressible in the form of a molar concentration of compound producing a standard response in a constant time interval. Various properties of the substituents can be quantified in terms of substituent

constants: Hammett-type σ constants for electronic properties[20], Hansch-type π constants for lipophilicity-hydrophobicity[23,26], Swain and Lupton-type[53] $\mathcal{F}$ and R constants for field and resonance effects, and molar refraction for general "steric bulk". Numerical values of these constants for over 200 substituents have been listed[24]. A recent article includes values for 48 additional substituents[25].

Typically the biological response, log (1/C), to the drug can then be expressed as the following equation:

$$\log (1/C) = k_1\sigma + k_2\pi + k_3\pi^2 + k_4 MR + k_5\mathcal{F} + k_6 R + k_7 \quad (1)$$

where C is the concentration or dose of a drug that will produce a given response. By the use of a multiple regression analysis all the constants can be evaluated; the equation (1) is called a structure-activity-relationship (SAR). A typical example will be toxicities of (2-bromoethylthio)benzenes against eggs of Tetranychus telarius (L)[27].



$$-\log LC_{50} = -2.18\pi'^2 + 1.69\pi' - 1.45\sigma + 4.38 \quad (2)$$

(n = 8; s = 0.164; r = 0.968)

where LC_{50} = lethal concentration, 50%

n = number of compounds

s = standard deviation

r = multiple regression coefficient.

It is then conceivable that the LC_{50} of a compound bearing a new substituent could be calculated by substituting the values for the various parameters in equation (2). A comprehensive treatise is available on this subject[55].

Before one could arrive at a standard response, it is necessary to have detailed biological information in the form of dose-response curves. This is not generally accesible to medicinal chemists from the vast amount of screening data present in the literature. Even when dose-response curves are available or have been determined, the estimated concentration suffers from inherent interpolation errors. There is yet another statistical tool available which removes the necessity of a specific concentration value. This method, known as discriminant analysis, requires only that compounds belonging to a chemical series be classified into two or more distinct groups based on some form of biological activity[34,35].

Instances where disciminant analyses have been used successfully in structure-activity-relationship studies are scarce. Martin et al.[36] have used it to study the relationship between structure and the inhibition of monoamine oxidase by aminotetralins and aminoindans. The usefulness of disciminant analysis in the study of synthetic antitumor compounds has been demonstrated by Hodenett and co-workers[28,29,45].

Once again, a series of compounds with a parent structure and various substituents has to be prepared and

the biological activities evaluated. It is then necessary to classify the compounds into two or more groups on some biological basis. Substituent properties are expressed in a manner similar to that used in the Hansch method and subjected to discriminant analysis. Results of such an analysis, usually in the form of discriminant functions, will indicate which of the structural variations are most important in determining whether or not a compound is active.

An example is found in the case of fifteen naphthoquinones and their activities against ascitic sarcoma 180 tumor system in Swiss mice[45]. The classification functions obtained for moderately active compounds:

$$-28.0(7.60)\pi + 9.81(5.30)MR + 9.44(3.70)\pi^2 - 455(3.57)E_{\frac{1}{2}} - 71.1$$

for active compounds:

$$-18.6(7.60)\pi + 7.30(5.30)MR + 6.30(3.70)\pi^2 - 392(3.57)E_{\frac{1}{2}} - 52.8 \qquad (3)$$

where π is the hydrophobic parameter developed by Hansch, MR is the molar refractivity and $E_{\frac{1}{2}}$ is the polarographic half-wave potential. The numbers given in parentheses are F-values with the degrees of freedom 1,10 for a given variable. By comparing these values with the standard F distribution, one can determine at what level of statistical significance that variable alone can differentiate between the two groups. Only if this significance level is above a particular value is the variable in question

allowed to enter the classification function. Each of the classification functions describes the cases belonging to that group. A discriminant function capable of assigning a compound to either group is readily derived by taking the difference between the corresponding coefficients. Such a function placed all the fifteen compounds into their proper groups at a confidence level of more than 95% ($F = 4.38 > F_{4,10,0.95} = 3.48$). The signs of the coefficients indicate whether a particular parameter should be increased or decreased to obtain enhanced biological activity. By substituting numerical values for the substituent parameters for a new compound in the discriminant function, one can evaluate the probability that the compound will belong to either group.

The Free-Wilson additivity model does not use parameters to describe the various physiocochemical properties of substituents[18]. An excellent review describing the method and comparing it to the Hansch method is available[14]. According to the Free-Wilson method, a series of de novo constants is derived using only the biological activities of compounds and the following basic assumption: a particular substituent will play a constant role in determining the over-all biological activity of the molecule. It may contribute to, or detract from, the over-all biological activity, but it must always play the same role.

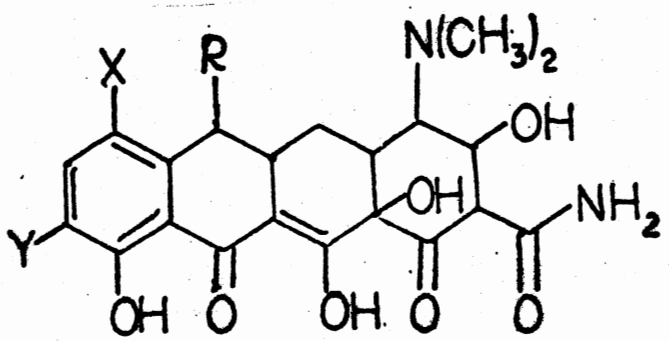
This basic assumption is checked by the statistical

parameters which result from the solution of a matrix, which expresses the above assumption in the form of the following equation for each compound,

$$\text{Biological activity} = \mu + \sum G_i X_i \qquad (4)$$

where μ is the average biological activity and $G_i X_i$ represents the contribution of the i^{th} group at the i^{th} position to the biological activity. In constructing the matrix, X takes the value of 0 or 1 to indicate the absence or the presence of the group in a given molecule. The matrix thus represents a series of equations in multiple unknowns, one equation per compound. The solution of the least square matrix yields the values for the de novo substituent constants for every substituent at each position.

An example of the successful application of this method is presented by Free and Wilson[18] using the in vitro inhibitory potencies of ten disubstituted against Staphylococcus aureus. The analogs included the following:



where R = H or CH_3; X = Br, Cl, or NO_2; and Y = NO_2, NH_2 or CH_3CONH.

Eighteen compounds are possible with these structural

variations but only ten were tested. The matrix used is shown in Table I, and the results are shown in Table II. The model accounted for 90.6% of the total variation of the biological activity. The results suggest that the best compound in the series would have R = H, X = Cl and Y = NH_2. The estimated biological activity would be 443 and the actual response was 525. The discrepancy might be due to biological variation in the in vitro procedure or the presence of a nonadditive component in the series, since the model accounts for 90% of the total variation, the latter possibility can be discounted.

Biological functions of quinones have been recognized for a long time[37]. Antitumor activities of naturally occurring and synthetic quinones have been catalogued by Driscoll et al.[17]. Extensive work on the antitumor characteristics of certain synthetics has been done by Sartorelli and co-workers[31,32,33] and Driscoll[13,17]. Some of the naturally occuring quinones like anthracyclines, mitomycins, streptonigrin and lapachol have also exhibited antineoplastic activity[17,19,47,49]. Some of these are shown in Figure 1. They are known to possess anti-bacterial, antifungal and enzyme inhibition properties as well[3].

About two hundred quinonoid compounds have been screened by NCI for antitumor activities, but, nitrogen analogs of quinones have not been so extensively studied. Several benzoquinones analogs have been studied and their

TABLE I

BIOLOGICAL ACTIVITY OF TEN TETRACYCLINES

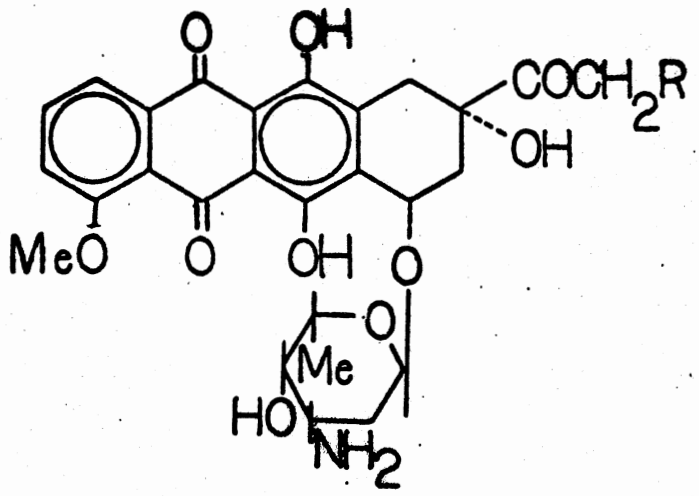
Compound Identification								Biological Activity
R		X			Y			
H	CH_3	NO_2	Cl	Br	NO_2	NH_2	CH_3CONH	
1		1			1			60
1			1		1			21
1				1	1			15
1			1			1		525
1				1		1		320
1		1				1		275
	1	1				1		160
	1	1					1	15
	1			1		1		140
	1			1			1	75

TABLE II

CONTRIBUTION OF STRUCTURAL CHANGES TO ACTIVITY OF TETRACYCLINES[a]

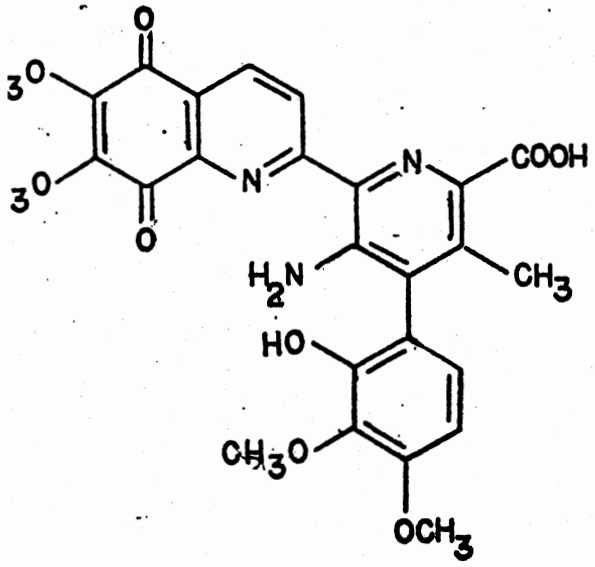
Side Chain Positions					
R		X		Y	
a H	75	b Cl	84	c NH_2	123
a CH_3	-112	b Br	-16	c CH_3CONH—	18
		b NO_2	-26	c NO_2	-218

[a]The solution includes these restrictions: -6a H + 4a CH_3 = 0; 2b Cl + 4b Br + 4b NO_2 = 0; and 5c NH_2 + 2c CH_3CONH— + 3c NO_2 = 0. The over-all average was 161.

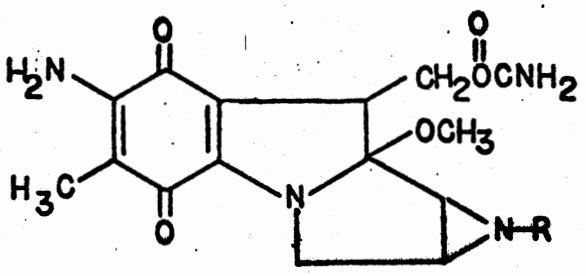


Adriamycin, R = OH

Daunomycin, R = H



Streptomycin



Mytomycin, R = H

Porfiromycin, R = CH_3

Figure 1. Some Naturally Occurring Quinones Showing Potential Antitumor Activity

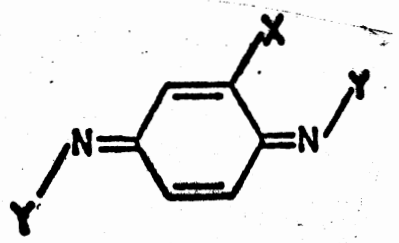
biological activities evaluated[4,28]. The mechanism of action is apparently different for each mode of activity complicated by the difference in structures. However, the redox capacity of the structurally different quinones seems to be the key factor in electron transport and oxidative phosphorylation processes[37].

CHAPTER III

EXPERIMENTAL

Preparation of Compounds

The system of compounds chosen for this study is

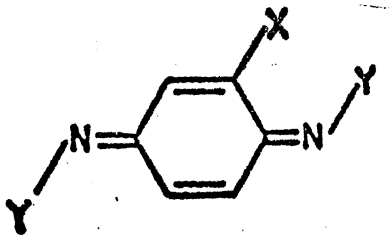


where Y = Cl or Br, and X = H, Cl, CH_3, NO_2 or SO_3Na. The IUPAC name for these is 2-substituted-N,N'-dihalo-2,5-cyclohexadien-1,4-diimines. For convenience's sake, these are referred to as 2-substituted-N,N'-dihalo-1,4-benzoquinonediimines. In Table III, these compounds are assigned numbers by which they are designated throughout the thesis.

Compounds 2, 3, 4, 8, 9, and 10 were stabilized by adding buffering agents such as Na_2HPO_4, K_2HPO_4, or AcONa in order to maintain a pH of the compounds between 5-8[52]. Compounds 4 and 5 were mixed with various arylamines to yield differing shades of color and were used for dyeing or printing polypropylene fibers[43]. Melting points have been reported only for compounds 1, 2, and 3[42]. Melting

TABLE III

2-(SUBSTITUTED)-N,N'-DIHALO-1,4-BENZOQUINONEDIIMINES



Compound No.*	Y	X
1	Cl	H
2	Cl	Cl
3	Cl	CH_3
4	Cl	OCH_3
5	Cl	NO_2
6	Cl	SO_3Na
7	Br	H
8	Br	Cl
9	Br	CH_3
10	Br	OCH_3
11	Br	NO_2
12	Br	SO_3Na

*The compound numbers given in this table are used to designate the compounds throughout this thesis.

points reported for these are suspect because it is not clear whether the compounds were isolated, purified, and dried thoroughly. On the other hand, the melting points reported in this thesis should be considered more reliable. Reasonable elemental analysis and proof of structures through spectral analysis render credibility to this claim.

Detailed spectral and other chemical information on these compounds are provided herein for the first time.

N,N'-Dibromo-1,4-benzoquinonediimine[8]

Bromine (32g, 0.2 mole) was dissolved in a solution of 16.7 g of sodium hydroxide pellets (0.42 mole) in 500 mL of water. This was chilled to 0°C and a solution of 54 g of 1,4-phenylenediamine (0.05 mole) in 300 mL of water was added. The resulting reddish brown precipitate was filtered and washed repeatedly with generous portions of water. The precipitate was dried under vacuum. The crude product was dissolved in 80 mL of warm benzene, shaken with anhydrous $CaCl_2$ and filtered. Addition of 60 mL of petroleum ether with chilling yielded 8.7 g of yellow solid, mp 99°C. The theoretical yield was 13.1 g. The melting point for this compound is not published.

N,N'-Dibromo-2-methoxy-1,4-benzoquinonediimine

Bromine (32g, 0.2 mole) was dissolved in a solution of 16.7 g (0.42 mole) of sodium hydroxide in 500 mL of

water. 2-Methoxy-1,4-phenylenediamine sulfate (11.8 g, 0.05 mole) was dissolved in 900 mL of water. The hypochlorite solution was stirred in a 2-liter beaker with ice and the amine solution was added with stirring. The yellow solid which separated was filtered out, washed with water and dried under vacuum. The crude product, upon recrystallization from 1:1 water-ethanol mixture, yielded 4 g of purified product, mp 87-89°C. Theoretical yield was 14.6 g. No published melting point is available for this compound.

Reduction of 2-methyl-4-nitroaniline

2-Methyl-4-nitroaniline (30.4 g, 0.2 mole) was mixed with 118.6 g (1 mole) of mossy tin in a 250-mL flask. Ethanol (2 mL) was added to control the foaming. Con. hydrochloric acid (240 mL) and 300 mL of water were added and the reaction mixture was heated overnight with reflux. The almost colorless solution was decanted and kept in the refrigerator. The resulting glistening light-yellow plates were filtered and dried. The yield was 49.0 g. The crude 2-methyl-1,4-phenylenediamine hexachlorostannate was used in the oxidation step without further purification.

Oxidation of 2-Methyl-1,4-phenylenediamine hexachlorostannate with Clorox

2-Methyl-1,4-phenylenediamine hexachlorostannate (22 g, 0.05 mole) was dissolved in 800 mL of water. A pint

of Clorox was stirred with ice in a 1-liter beaker and the amine-salt solution was added slowly with stirring. A yellow precipitate formed; this was filtered out, washed with water and dried under vacuum. The crude 2-methyl-N,N'-dichloro-1,4-benzoquinonediimine was recrystallized from absolute ethanol. The yield was 7.8 g; mp 184-185°C; published: 160°C[42]. The theoretical yield was 9.45 g.

Oxidation of 2-methyl-1,4-phenylenediamine chlorostannate with hypobromite

2-Methyl-1,4-phenylenediamine hexachlorostannate (11.4 g, 0.025 mole) was dissolved in a solution of 8.4 g (0.21 mole) of sodium hydroxide in 250 mL of water in a 1-liter beaker. To the cooled hypobromite solution was added the amine-salt solution gradually and with stirring. The resulted flesh-colored precipitate was filtered, washed with water and dried under vacuum. The crude 2-methyl-N,N'-dibromo-1,4-benzoquinonediimine was recrystallized from absolute ethanol. The yield was 4.8 g; mp 250°C. The theoretical yield was 6.90 g. The melting point for this compound has not been reported.

N,N'-Dichloro-1,4-benzoquinonediimine

1,4-Phenylenediamine (5.4 g, 0.05 mole) was dissolved in 300 mL of water. A pint bottle of commercial bleach (Clorox, sodium hypochloride 5.25%) was stirred in a 1-liter beaker with ice. The amine solution was added

gradually with vigorous stirring. The resulting dirty yellow precipitate was filtered and rapidly washed with plenty of water and the solid was dried under vacuum. The crude product was recrystallized from absolute ethanol. The yield was 4.6 g; mp 127-129°C; published: 126-128°C[42]. The theoretical yield was 8.75 g.

N,N'-Dibromo-2-chloro-1,4-benzoquinone-diimine

2-Chloro-1,4-phenylenediamine sulfate (24 g, 0.1 mole) was dissolved in water (300 mL). Hypobromite solution was prepared by dissolving 64 g (0.2 mole) of bromine in a solution of 16 g (0.42 mole) of sodium hydroxide in 500 mL of water. To half the hypobromite solution, half the cooled amine solution was added with stirring. The remaining amine solution was similarly oxidized. The resulting yellow precipitate was filtered out, washed with water and dried under vacuum. The crude product was recrystallized from absolute ethanol. The yield was 14.0 g; mp 77-78°C. The theoretical yield was 26.2 g. The melting point for this compound has not been reported.

Preparation of 2-methoxy-N,N'-dichloro-1,4-benzoquinonediimine

2-Methoxy-1,4-phenylenediamine sulfate (12.1 g, 0.05 mole) was dissolved in 300 mL of water. A pint bottle of commercial bleach was stirred with ice in a 1-liter

beaker. The amine-salt solution was added slowly with stirring to the chilled bleach solution and the resulting dirty yellow solid was filtered out, washed with water, and dried under vacuum. The crude product was recrystallized from ethanol. The yield was 7.9 g; mp 90°C. The theoretical yield was 10.25 g. The melting point for this compound has not been published.

Oxidation of 1,4-phenylendiamine-2-sulfonic acid by sodium hypobromite solution

1,4-Phenylenediamine-2-sulfonic acid (9.4g, 0.05 mole) was dissolved in 75 mL of water. Bromine (32 g, 0.2 mole) was dissolved in a solution of 8 g (0.2 mole) of sodium hydroxide in 45 mL of water. The amine solution was added slowly with stirring to the chilled hypobromite solution. Sodium chloride (30 g) was added to the yellow-colored reaction mixture and cooled in the refrigerator overnight. The resulting solid was filtered out, washed with water and dried under vaccum and the crude sodium N,N'-dibromo-1,4-benzoquinonediimine-2-sulfonic acid was recrystallized from ethanol. The yield was 4.5 g; mp 254-256°C. The theoretical yield was 18.3 g. No published melting point for this compound is available in the literature.

Preparation of 2-nitro-N,N'-dichloro-1,4-benzoquinonediimine[34]

Calcium hypochlorite (25g, 0.17 mole) was dissolved in a 300 mL round-bottomed flask. Sodium carbonate (13.5 g, 0.17 mole) and 3.5 g (0.089 mole) of sodium hydroxide were dissolved in 25 mL of water. This solution was added to the calcium hypochlorite solution and the mixture shaken for 15 minutes. The filtrate was separated from the milky white solid by filtration, the filter cake washed with and the washings added to the filtrate. The combined filtrate was used as oxidizing agent rather than Clorox, because the volume could be kept low and the concentration was sufficient to effect the oxidation of 2-nitro-1,4-phenylenediamine in spite of the presence of the deactivating nitro group. The hypochlorite solution was stirred with ice in a 1-liter beaker. 2-Nitro-1,4-phenylenediamine (7.65 g, 0.05 mole) was dissolved in 300 mL of water. The amine solution was added slowly with stirring to the chilled hypochlorite solution. The resulting brown solution was filtered out, washed with water and dried under vaccum. The crude product was recrystallized from ethanol. The yield was 9.2 g; mp 250°C. The theoretical yield was 11.0 g. The melting point for this compound has not been reported.

Preparation of 2-nitro-N,N'-dibromobenzoquinonediimine

2-Nitro-1,4-phenylenediamine (7.7 g, 0.058 mole) was dissolved in 75 mL of water. Bromine (32g, 0.2 mole) was added to a solution of 8 g (0.2 mole) of sodium hydroxide in 250 mL of water. The amine solution was added slowly and with stirring to the chilled hypobromite solution. The resulting brown precipitate was filtered, washed with water and dried under vacuum. The crude product was recrystallized from ethanol. The yield was 7.9 g; mp 360°C. The theoretical yield was 17.81 g. The melting point for this compound has not been reported.

Preparation of 2-chloro-N,N'-dichloro-1,4-benzoquinonediimine

2-Chloro-1,4-phenylendiamine (2.5 g, 0.1 mole) was dissolved in 600 mL of water. One liter of commercial bleach was stirred with ice in a 2-liter beaker. The amine solution was slowly added with stirring to the chilled bleach solution. The yellow solid was filtered out, dried and recrystallized from ethanol. The yield was 14 g; the theoretical yield was 20.9 g; mp 69-70°C. Ota[42] reports that the melting point was indefinite and attributes this to polymerization.

Elemental Analysis of Compounds

All the compounds were submitted to M-H-W Laboratories, P.O. Box 15853, Phoenix, AZ.85018 for elemental analysis. The results are summarized in Table IV.

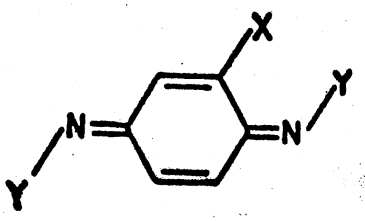
Spectral Analysis of Compounds

The NMR spectra were recorded on an XL-100 (15) NMR spectrometer made by Varian Associates, Palo Alto, California. All ^{1}H spectra were recorded in 5-mm samples at 100.1 Mhz and at 2.35 Tesla. The Pulsed Fourier Transformation accessory for pulsed experiments was a Nicolet TT-100 unit. Tetramethylsilane (TMS) was used as the internal standard and $CDCl_3$ was the solvent unless otherwise specified. The complete spectra are presented in Figures 2-16. Only continuous wave spectra were taken while using solvents other than $CDCl_3$.

Infrared spectra of the compounds were obtained from potassium bromide pellets on a Beckman IR-5A spectrophotometer with sodium chloride optics. The complete spectra are presented in Figures 17-26 and major absorption bands are listed in Table V. The infrared spectra of all ten compounds have two absorption peaks in common which were used as evidence for the quinonoid structure. One peak is a medium to strong and broad band in the region of between 1575 and 1640 cm^{-1} and the other is a relatively weak but sharp peak at 1410-1450 cm^{-1}. Comparison of a

TABLE IV

ANALYSIS OF COMPOUNDS FOR NITROGEN



Compound No.	Y	X	Nitrogen Calcd., %	Nitrogen Found, %
1	Cl	H	16.00	15.86
2	Cl	Cl	13.37	13.28
3	Cl	CH_3	14.81	14.81
4	Cl	OCH_3	13.66	13.32
5	Cl	NO_2	19.09	21.69
7	Br	H	10.61	9.58
8	Br	Cl	9.38	8.92
9	Br	CH_3	9.52	8.62
10	Br	OCH_3	10.07	8.79
11	Br	NO_2	13.59	12.78

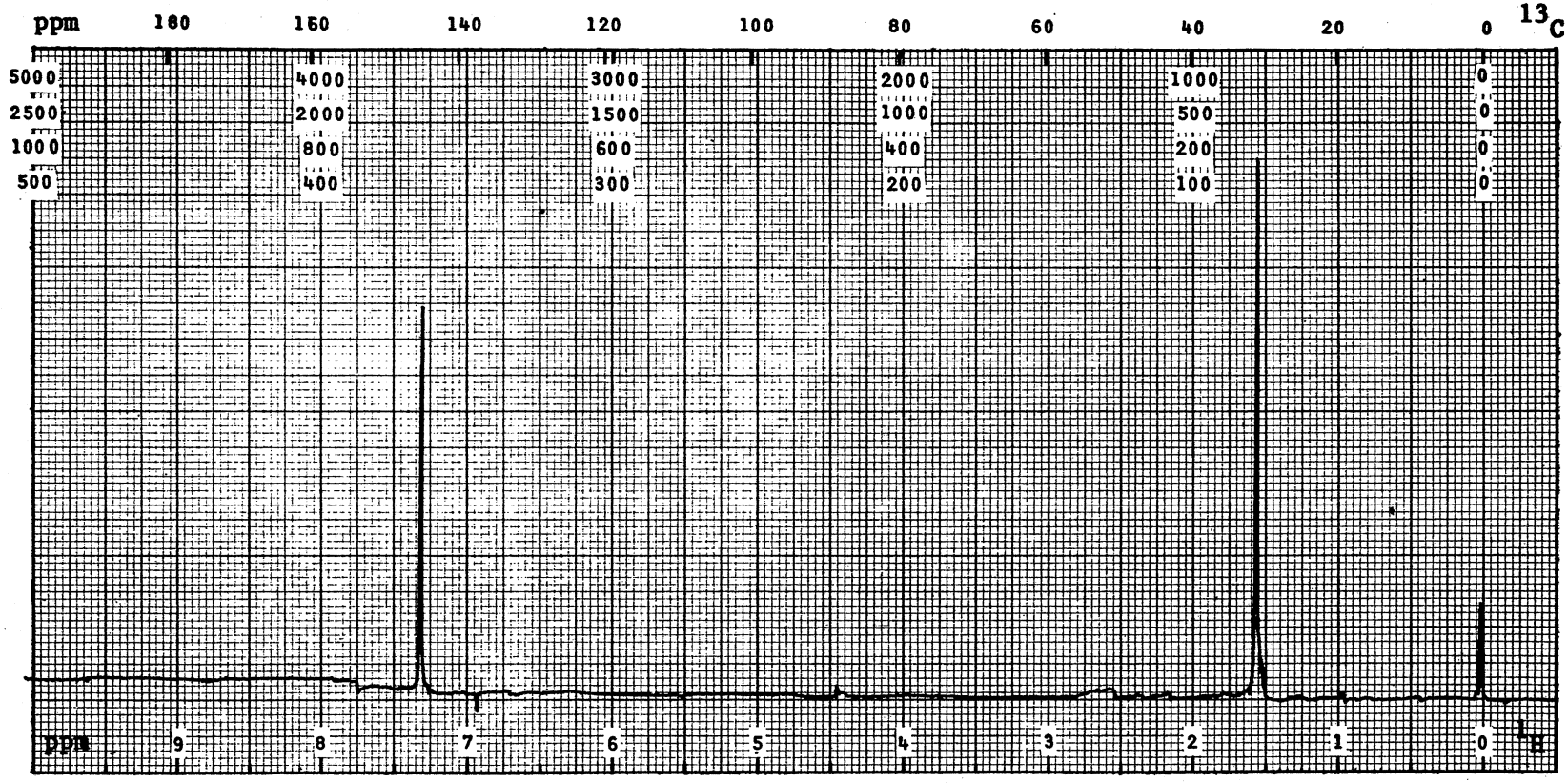


Figure 2. PMR spectrum of $CDCl_3$

PFT _ CW _ ; Solvent. . ; SO. .45251 Hz; SW. .1000 Hz; T. . 30°C; Acq/SA. .28

Size. . K; P2/RF. .20 µs/dB; SF. .100.1 Hz; FB. . Hz; Lock. . ^2H ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . --W/dB; NBW. . -- Hz

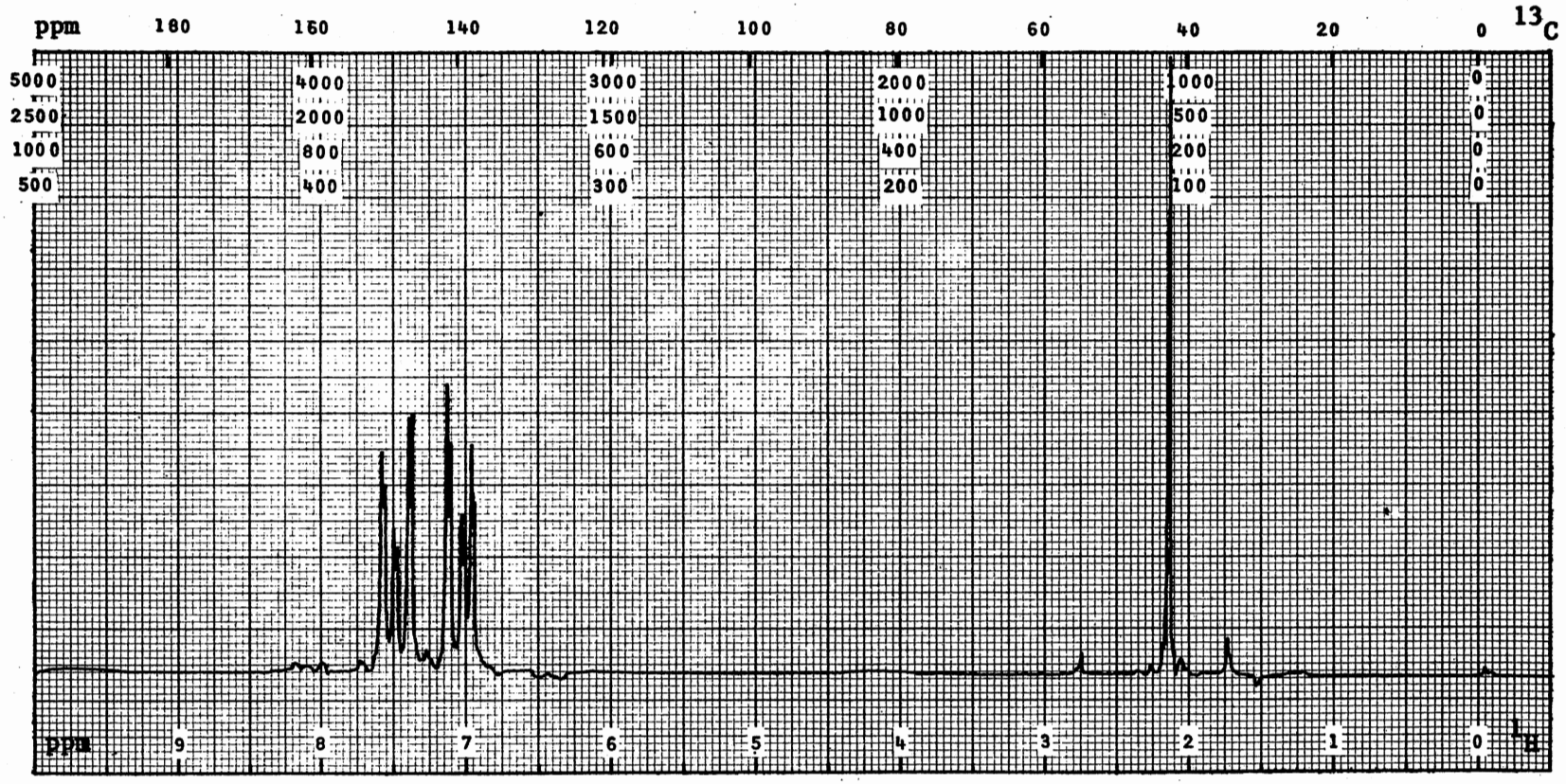


Figure 3. PMR Spectrum of N,N'-dichloro-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. .$DCCl_3$; SO. . 45251Hz; SW. .1000 Hz; T. . 30°C; Acq/SA. . 28

Size. . 8K; P2/RF. . 5 μs/dB; SF. . 100.1Hz; FB. . Hz; Lock. . ^2D ; D5/ST. . 3s

DC. .-- ; Gated Off. . --; Offset. . -- Hz; RF. . -- W/dB; NBW. . -- Hz

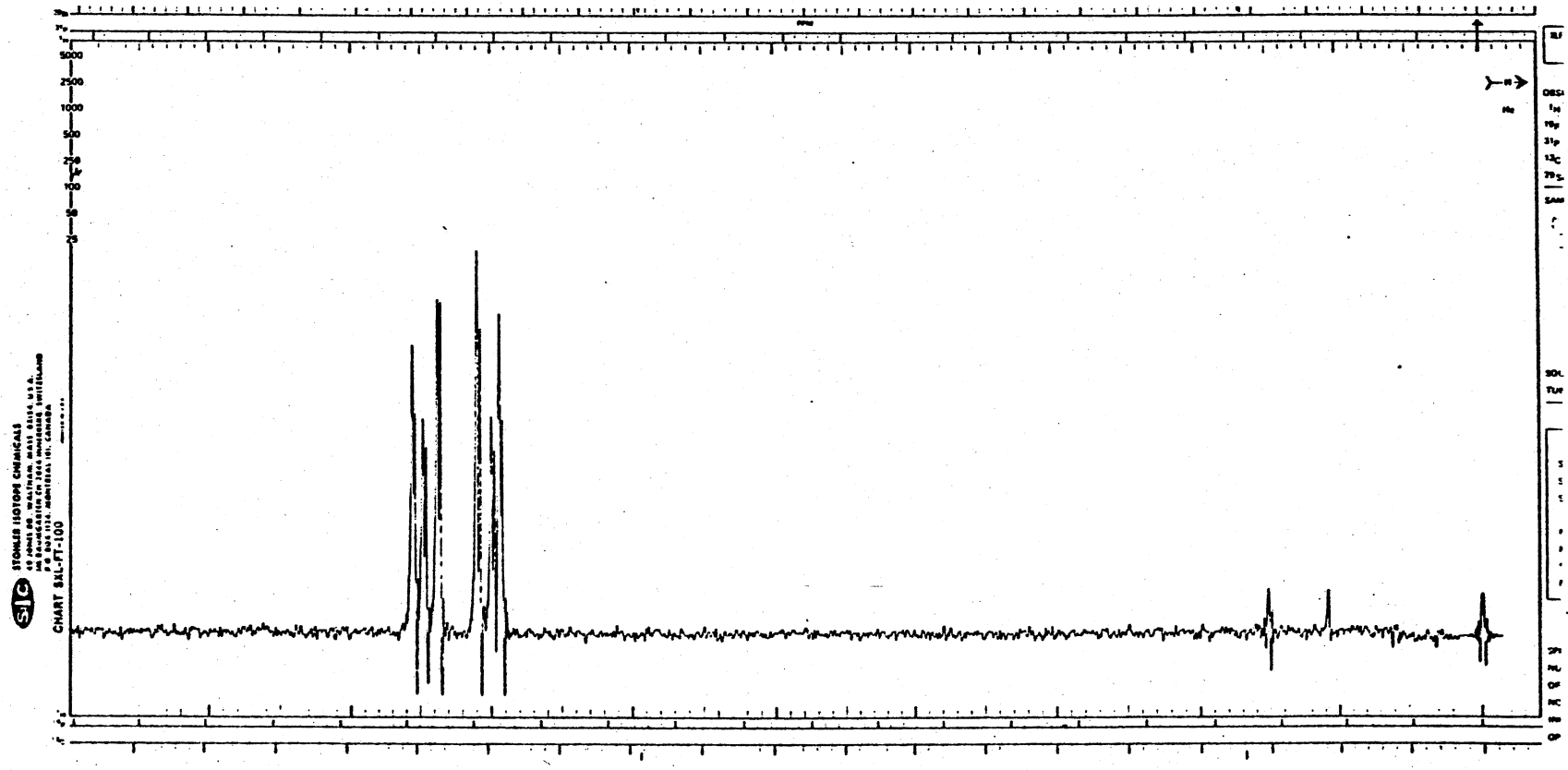


Figure 4. PMR spectrum of N,N'-dichloro-1,4-benzoquinonediimine in CCl_4

PFT No.; SW.1000 Hz; ST.250 sec; SO. 85701 Hz;

Filter. 2 Hz; RF Field. 72 dB; SA. 10.

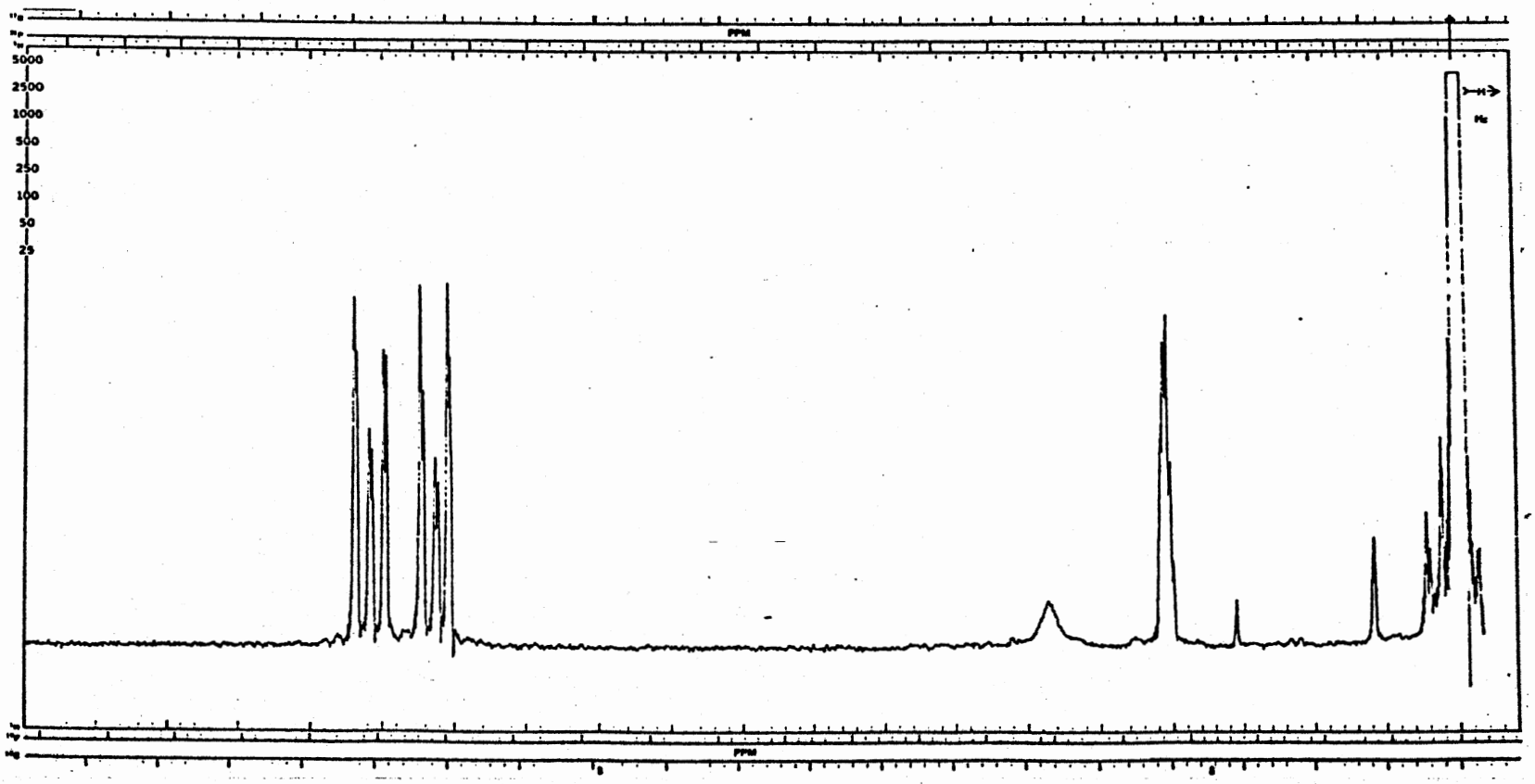


Figure 5. PMR spectrum of N,N'-dichloro-1,4-benzoquinonediimine in CD_3COCD_3

PFT. No.; SW. 1000 Hz; ST. 250 sec; SO. 86292 Hz;

Filter. 2 Hz; RF Field. 69 dB; SA 2.0.

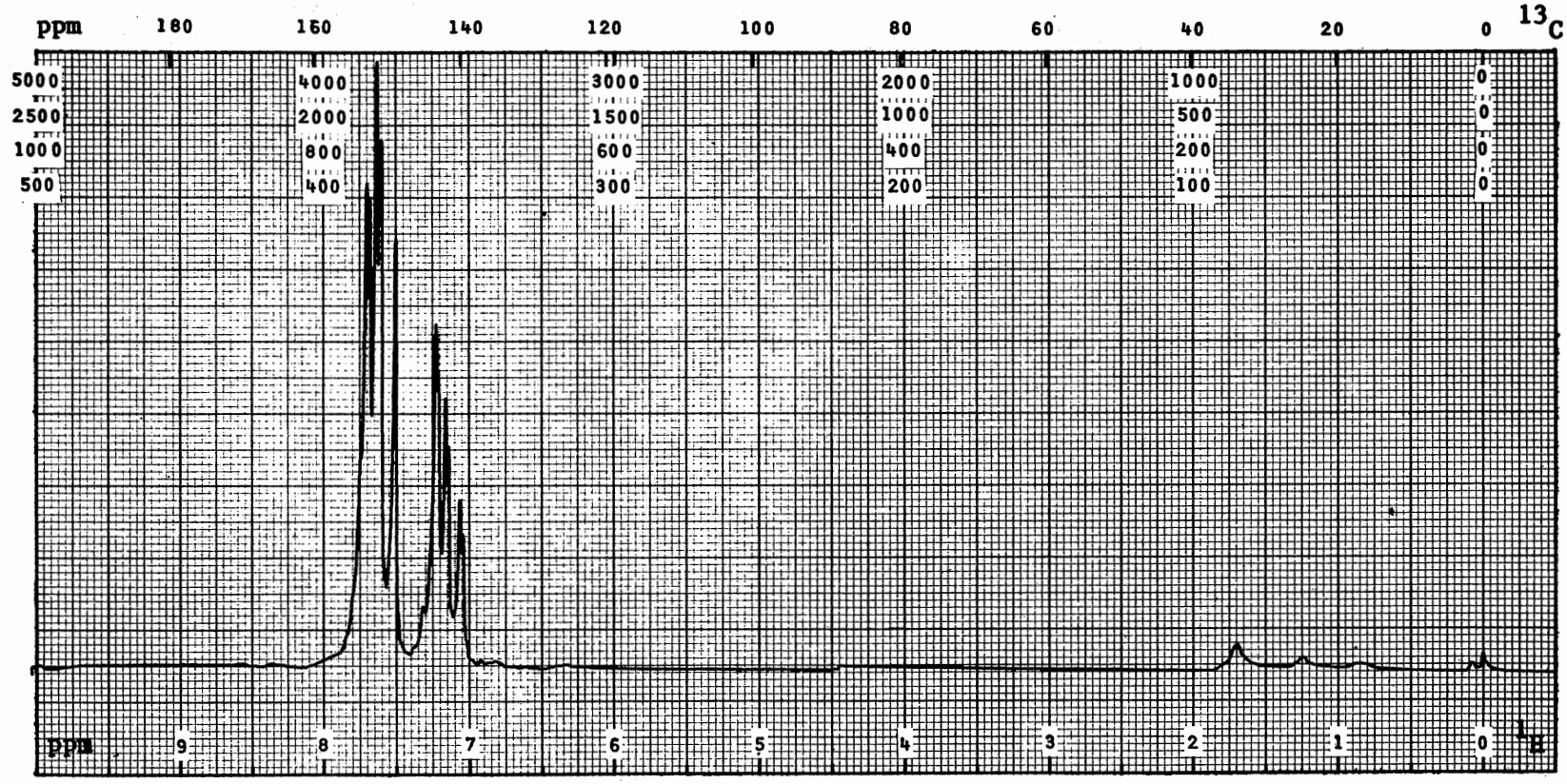


Figure 6. PMR spectrum of 2-chloro-N,N'-dichloro-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. .$CDCl_3$; SO. . 45251 Hz; SW. . 1000 Hz; T. . 30 °C; Acq/SA. . 28

Size. . 8 K; P2/RF. . 5 µs/dB; SF. 100.1 MHz; FB. . Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . -- W/dB; NBW. . -- Hz

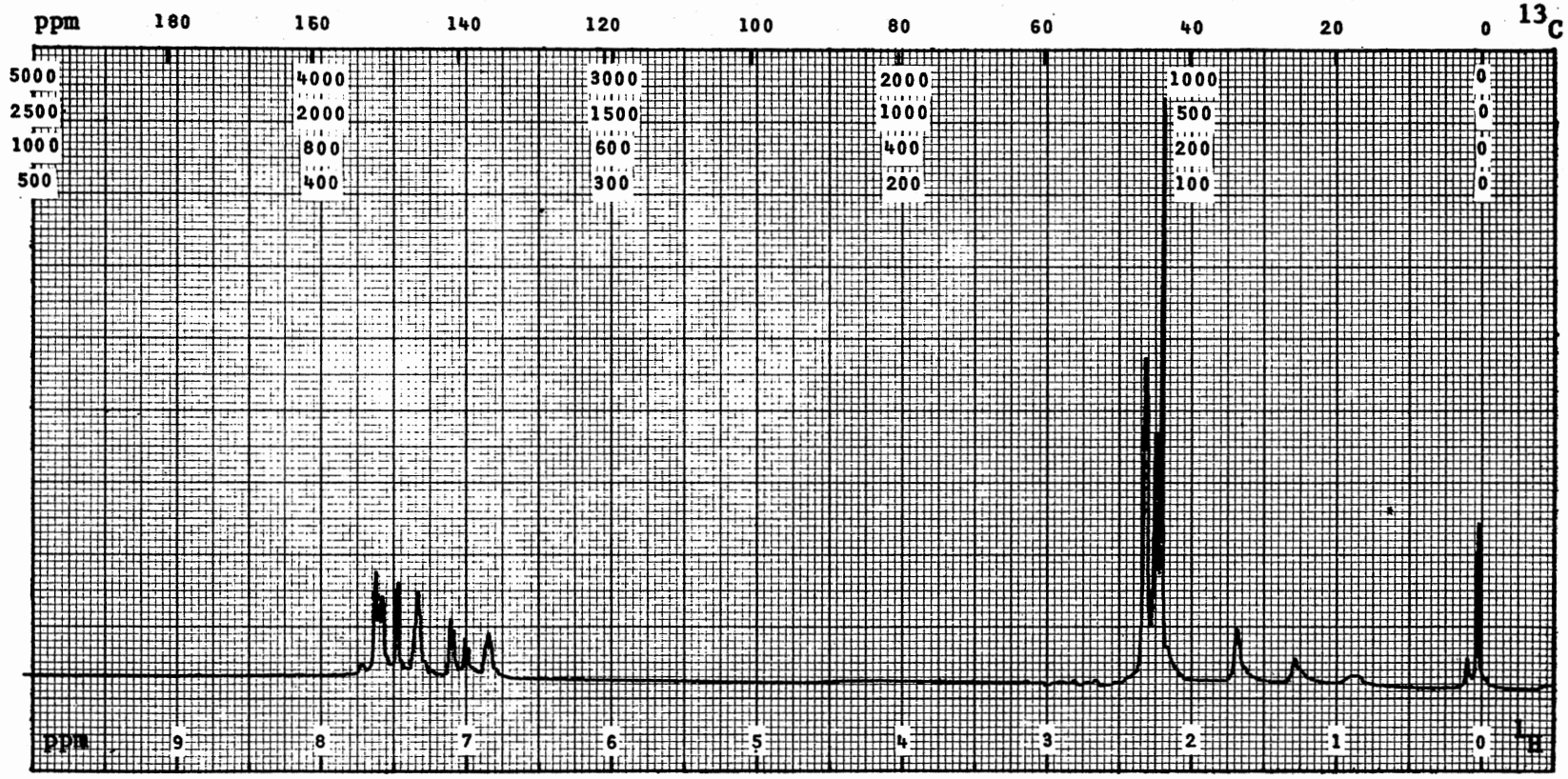


Figure 7. PMR spectrum 2-methyl-N,N'-dichloro-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. . 45251 Hz; SW. .1000 Hz; T. . 30 °C; Acq/SA. . 28

Size. . 8 K; P2/RF. . 5 µs/dB; SF. .100.1 MHz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . -- W/dB; NBW. . -- Hz

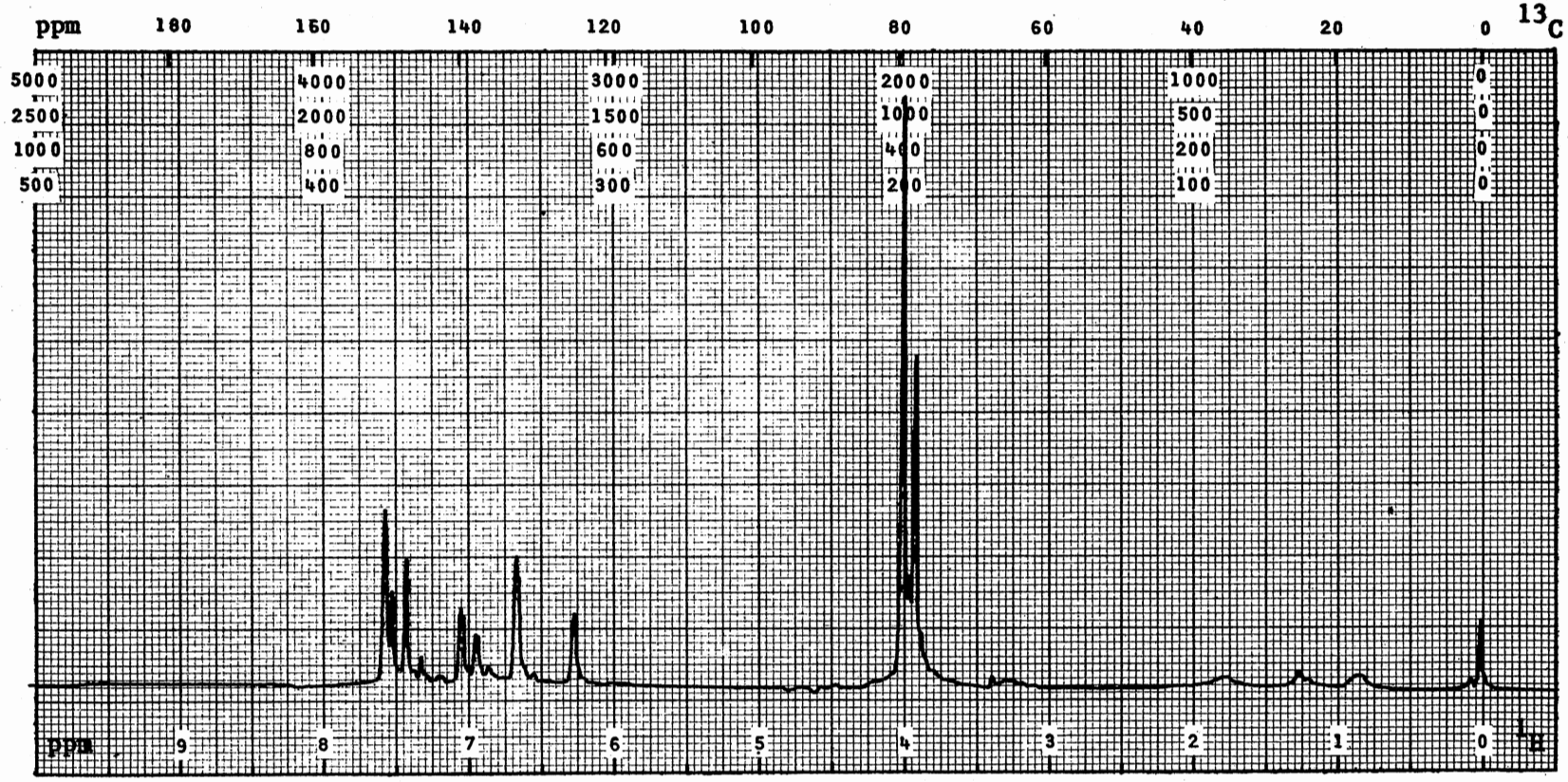


Figure 8. PMR spectrum of 2-methoxy-N,N'-dichloro-1,4-benzoquinonediimine in $CDCl_3$

PFT ✓ CW _ ; Solvent. . $CDCl_3$; SO. 45251 Hz; SW. 1000 Hz; T. . 30 °C; Acq/SA. . p

Size. . K; P2/RF. . 70 µs/dB; SF. . 100.1 Hz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . -- s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . -- W/dB; NBW. . -- Hz

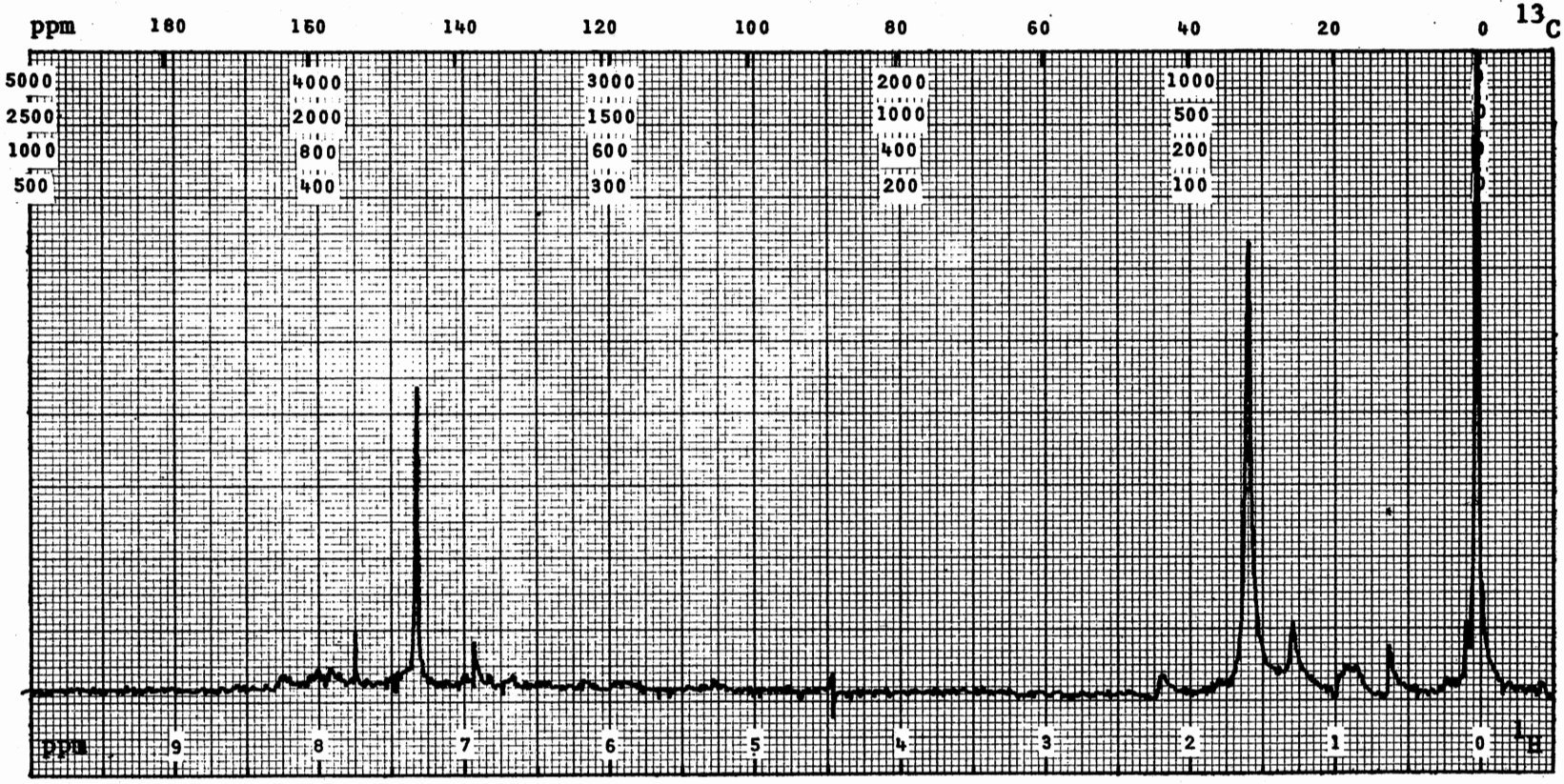


Figure 9. PMR spectrum of 2-nitro-N,N'-dichloro-1,4-benzoquhonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. . 45251 Hz; SW. .1000 Hz; T. . 30 °C; Acq/SA. . 28

Size. . 8 K; P2/RF. . 5 µs/dB; SF. .100.1 MHz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . Hz; RF. . W/dB; NBW. . Hz

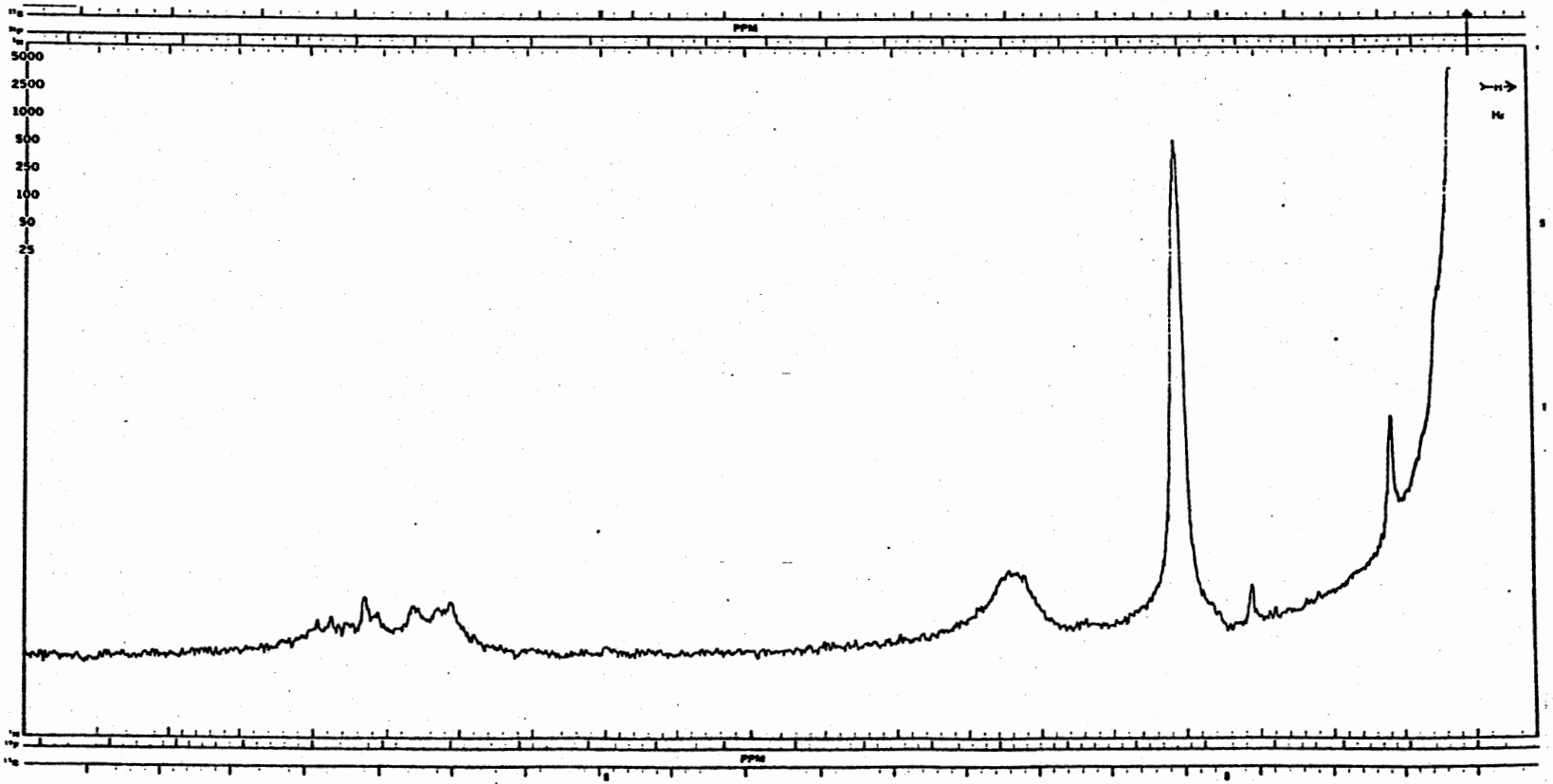


Figure 10. PMR spectrum of 2-nitro-N,N'-dichloro-1,4-benzoquinonediimine in CD_3COCD_3

PFT. No.; SW. 1000 Hz; ST. 250 sec; SO. 86291 Hz;

Filter. 2 Hz; RF Field. 69 dB; SA 3.2.

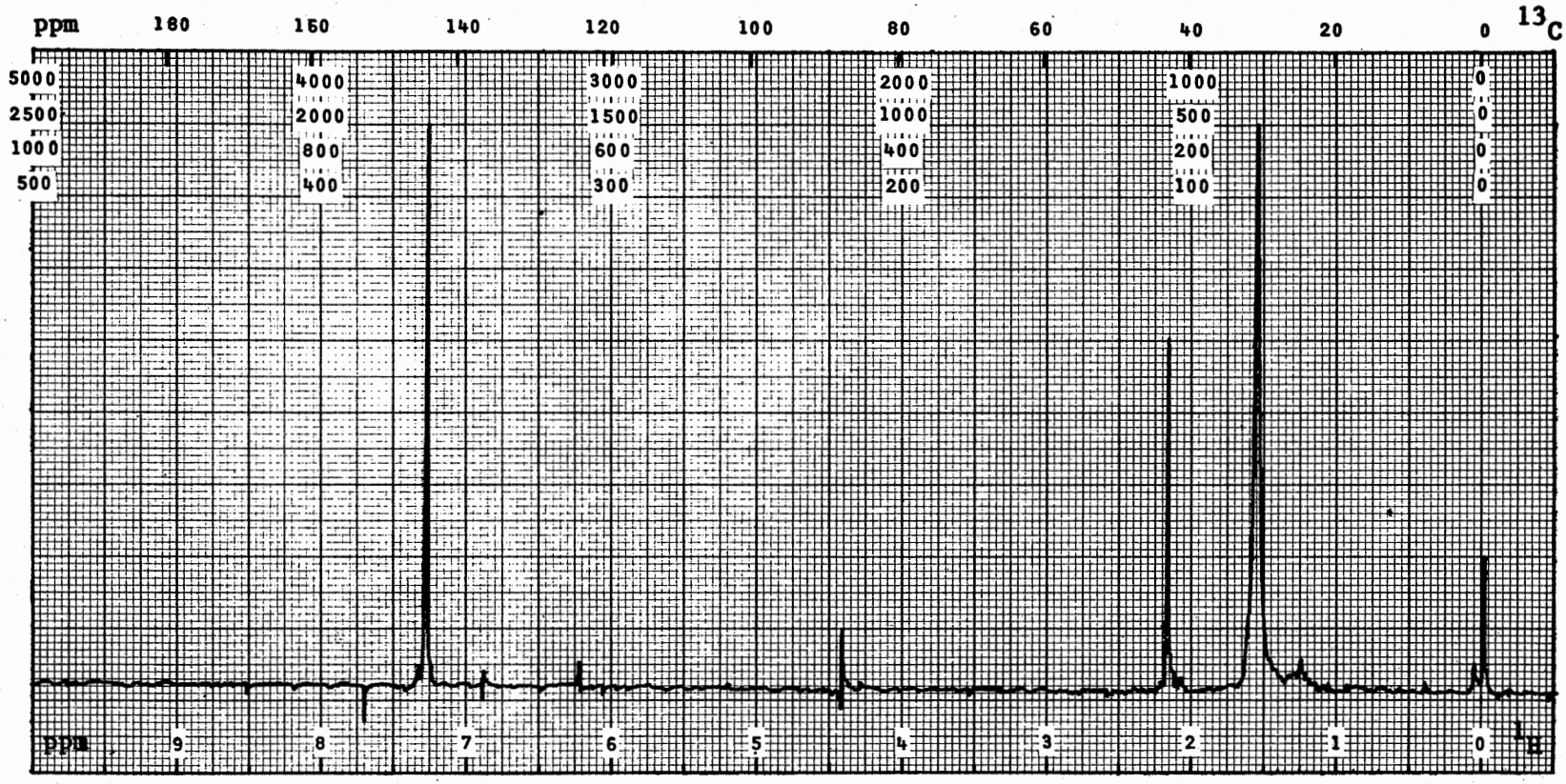


Figure 11. PMR spectrum of N,N'-dibromo-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. .45251 Hz; S_W. . 1000Hz; T. . 30 °C; Acq/SA. . 28

Size. .8 K; P2/RF. . 5 µs/dB; SF. .100.1MHz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . --W/dB; NBW. . -- Hz

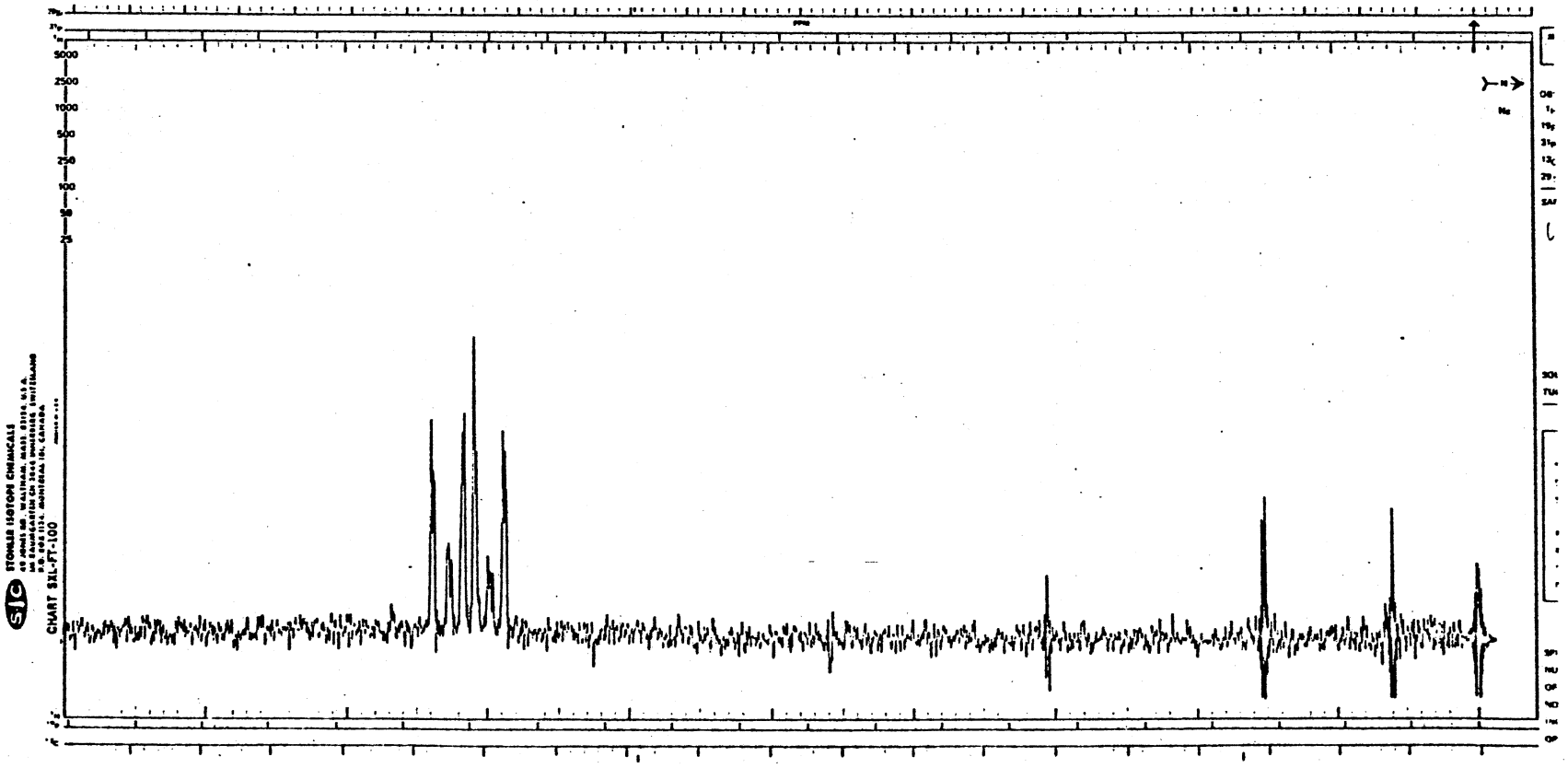


Figure 12. PMR spectrum of N,N'-dibromo-1,4-benzoquinonediimine in CCl_4

PFT. No.; SW. 1000 Hz; ST. 500 sec; SO. 83761 Hz;

Filter. 1 Hz; RF Field 73 dB; SA. 32.

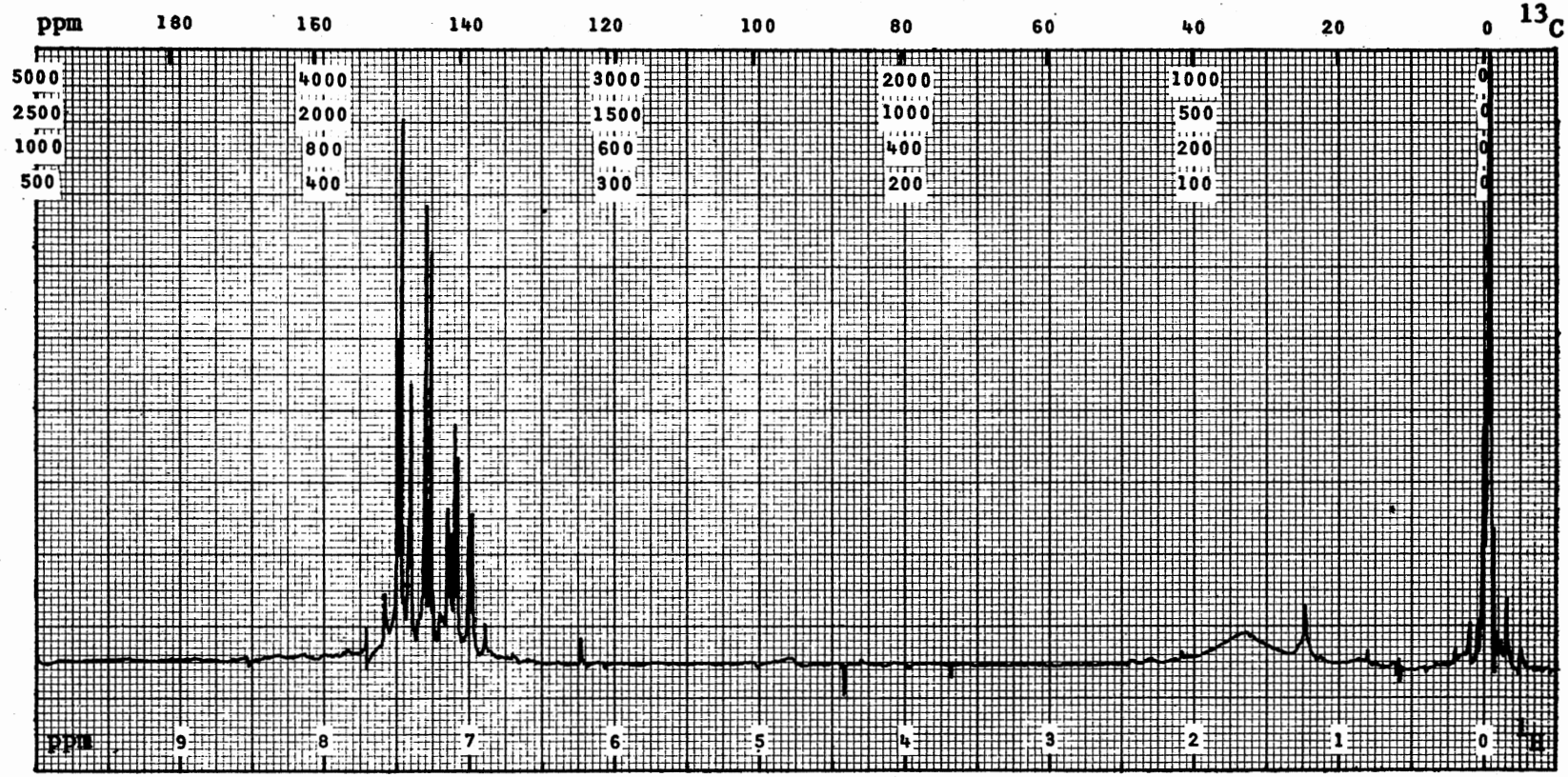


Figure 13. PMR spectrum of 2-chloro-N,N'-dibromo-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. .45251 Hz; S_W. .1000 Hz; T. . 30°C; Acq/SA. .

Size. . 8K; P2/RF. . -- µs/dB; SF. .100.1MHz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . --; Gated Off. . -- ; Offset. . -- Hz; RF. . --W/dB; NBW. . -- Hz

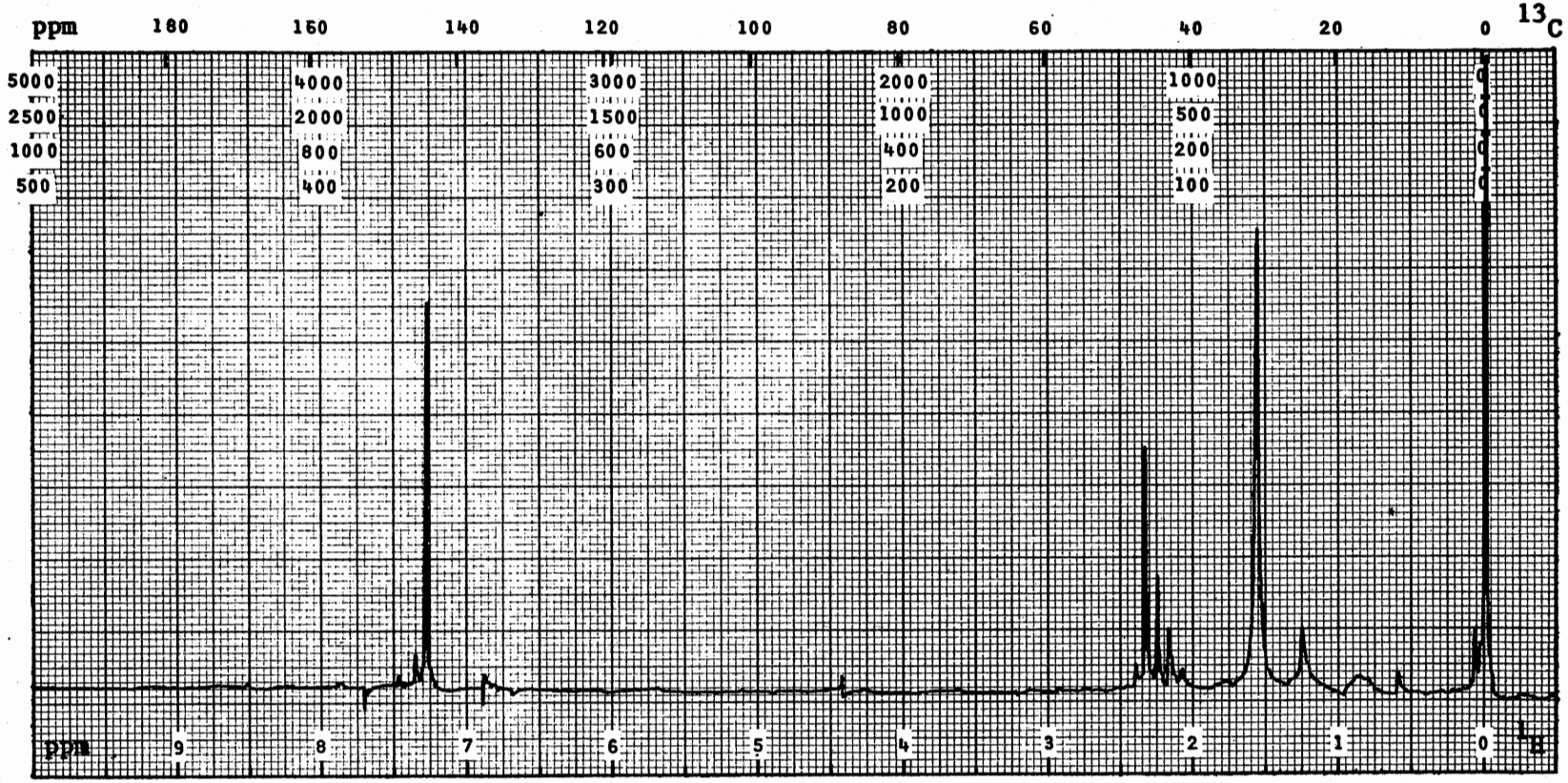


Figure 14. PMR spectrum of 2-methyl-N,N'-dibromo-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. .45251 Hz; SW. .1000 Hz; T. . 30 °C; Acq/SA. . 28

Size. .8 K; P2/RF. . 5 µs/dB; SF. .100.1MHz; FB. .__ Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . __; Gated Off. . __ ; Offset. . __ Hz; RF. . __W/dB; NBW. . __ Hz

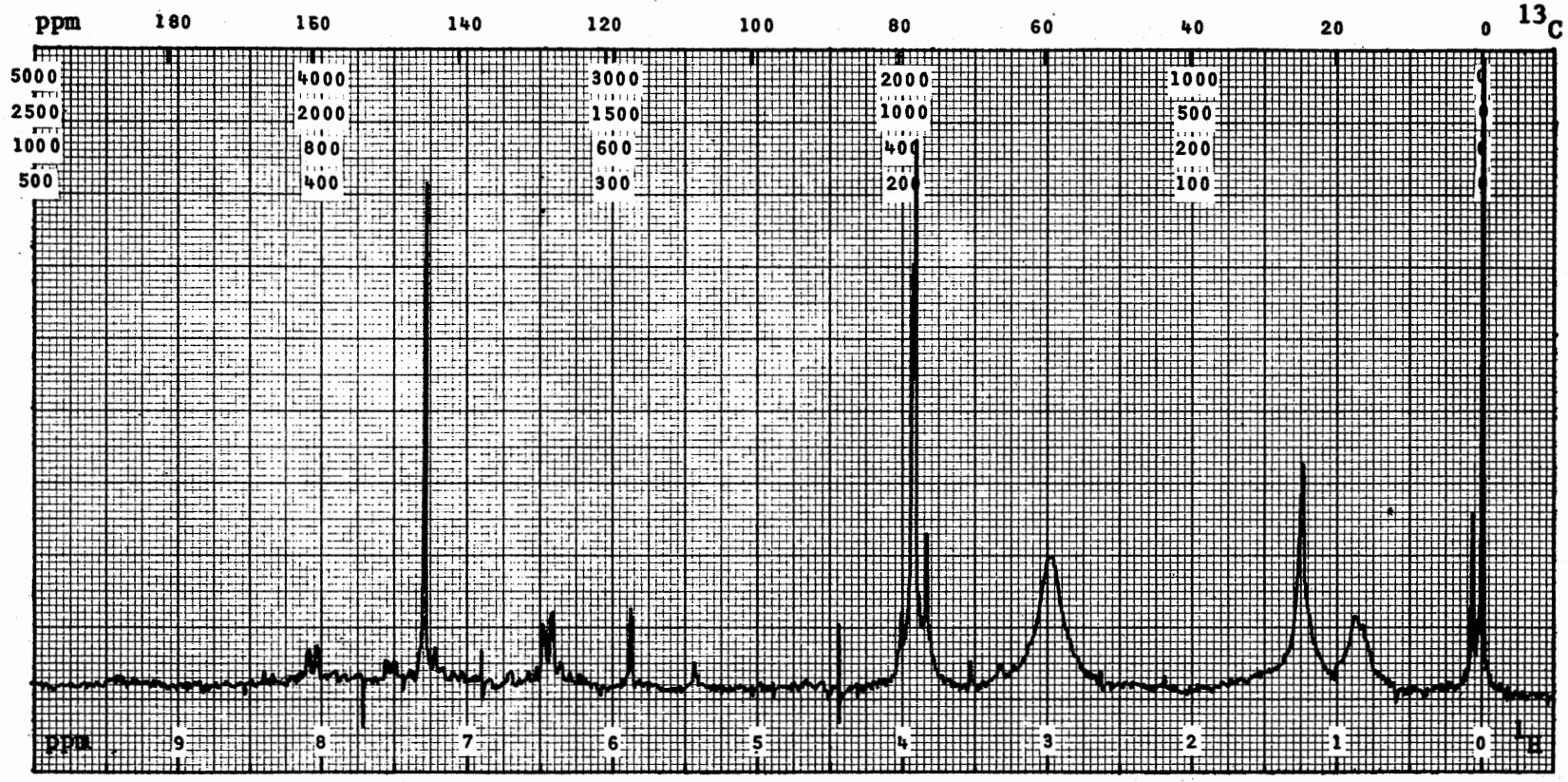


Figure 15. PMR spectrum of 2-methoxy-N,N'-dibromo-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. 45251 Hz; SW. . 1000 Hz; T. . 30 °C; Acq/SA. . 28

Size. . 8 K; P2/RF. . 5µs/dB; SF. 100.1 MHz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . -- W/dB; NBW. . -- Hz

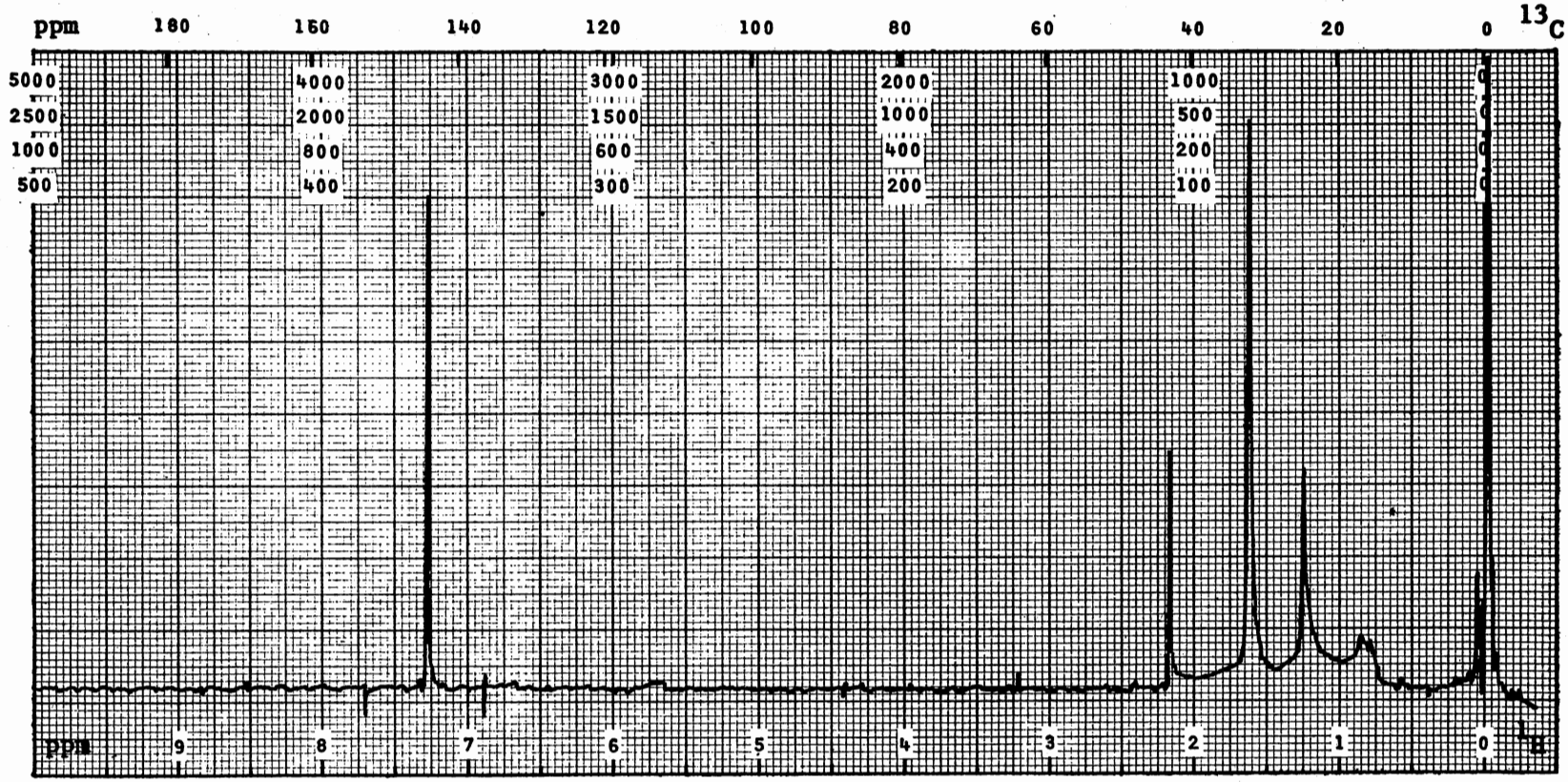


Figure 16. PMR spectrum of 2-nitro-N,N'-dibromo-1,4-benzoquinonediimine in $CDCl_3$

PFT _ CW _ ; Solvent. . $CDCl_3$; SO. 45251 Hz; SW. . 1000 Hz; T. . 30 °C; Acq/SA. . 28

Size. . 8 K; P2/RF. . 5 µs/dB; SF. 100.1 MHz; FB. . -- Hz; Lock. . ^2D ; D5/ST. . 3 s

DC. . -- ; Gated Off. . -- ; Offset. . -- Hz; RF. . -- W/dB; NBW. . -- Hz

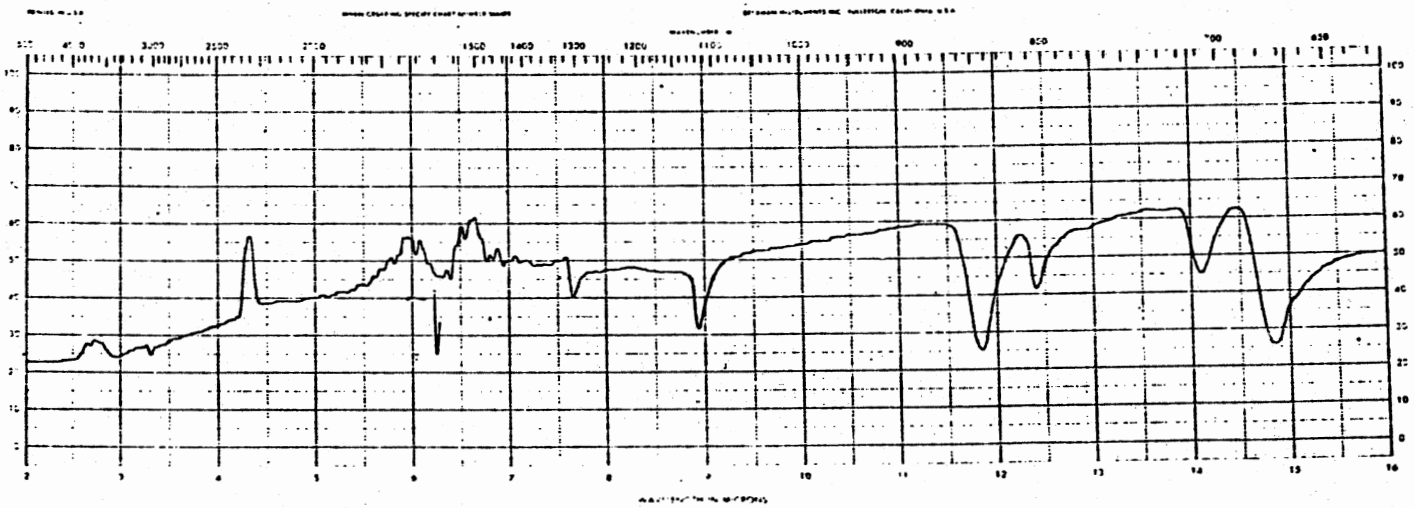


Figure 17. IR spectrum of N,N'-dichloro-1,4-benzoquinonediimine - KBr pellet

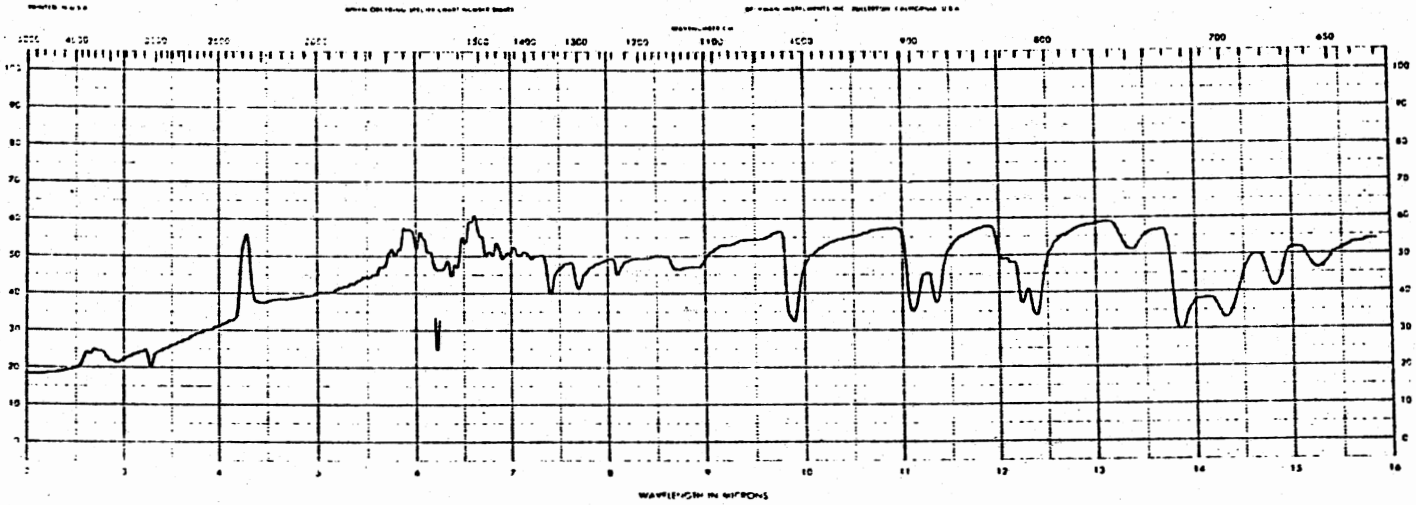


Figure 18. IR spectrum of 2-chloro-N,N'-dichloro-1,4-benzoquinonediimine - KBr pellet

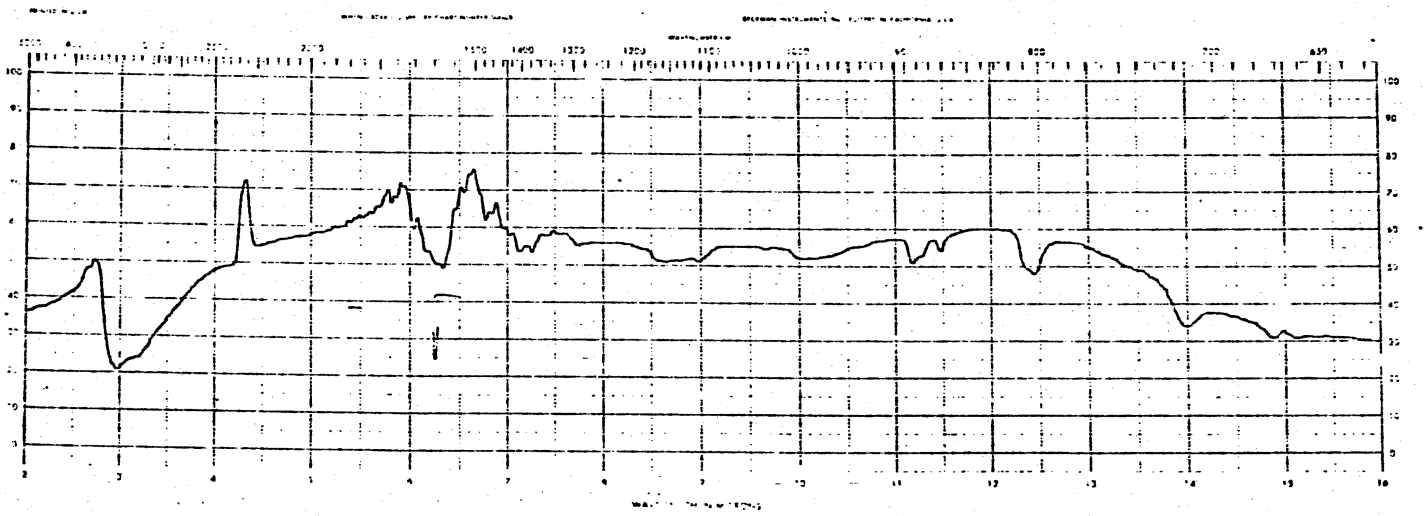


Figure 19. IR spectrum of 2-methyl-N,N'-dichloro-1,4-benzoquinonediimine - KBr pellet

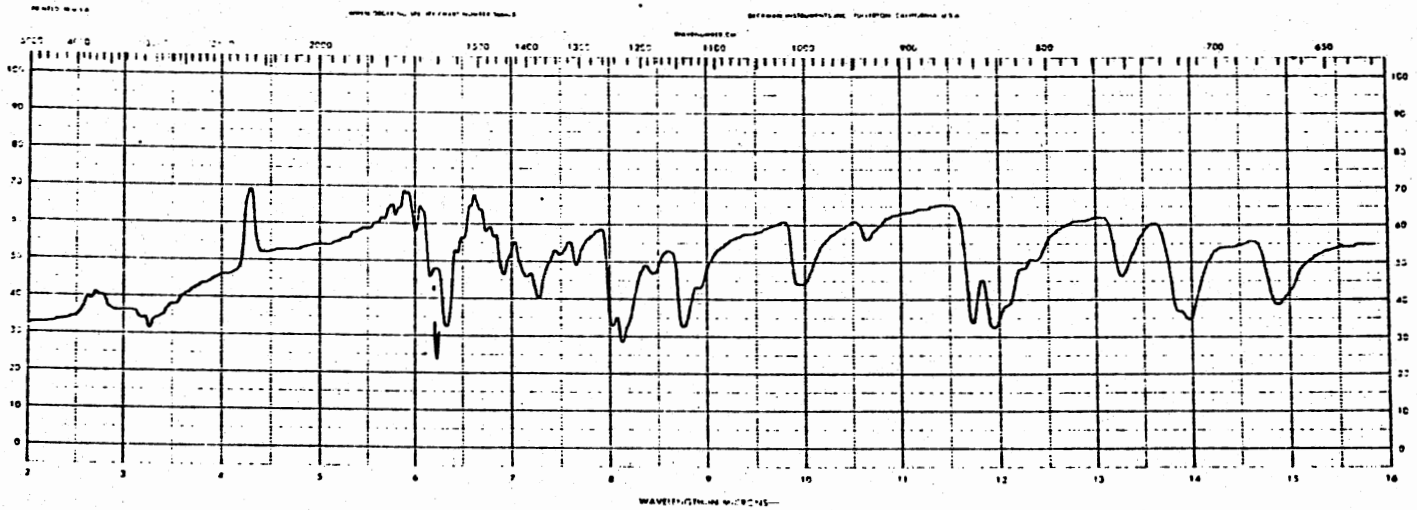


Figure 20. IR spectrum of 2-methoxy-N,N'-dichloro-1,4-benzoquinonediimine - KBr pellet

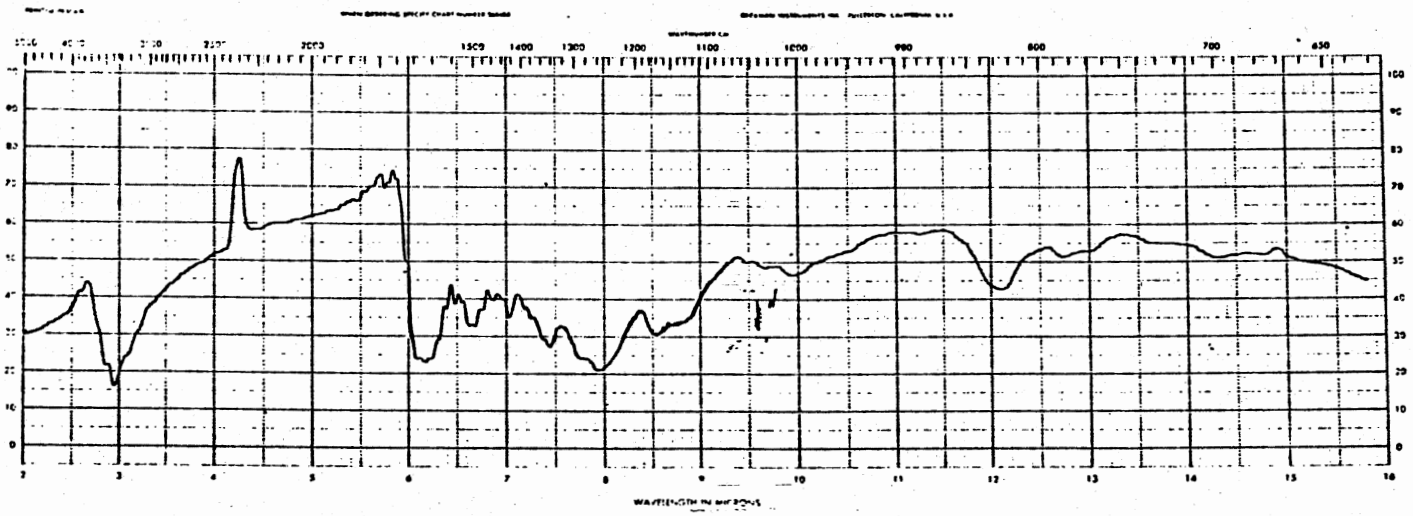


Figure 21. IR spectrum of 2-nitro-N,N'-dichloro-1,4-benzoquinonediimine - KBr pellet

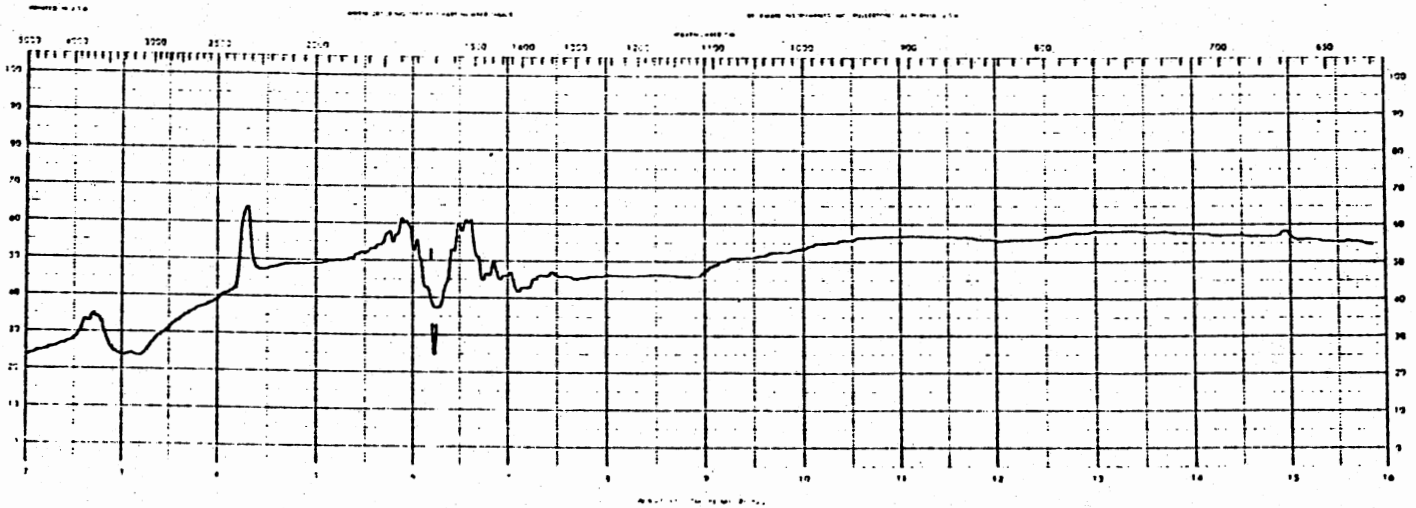


Figure 22. IR spectrum of N,N'-dibromo-1,4-benzoquinonediimine - KBr pellet

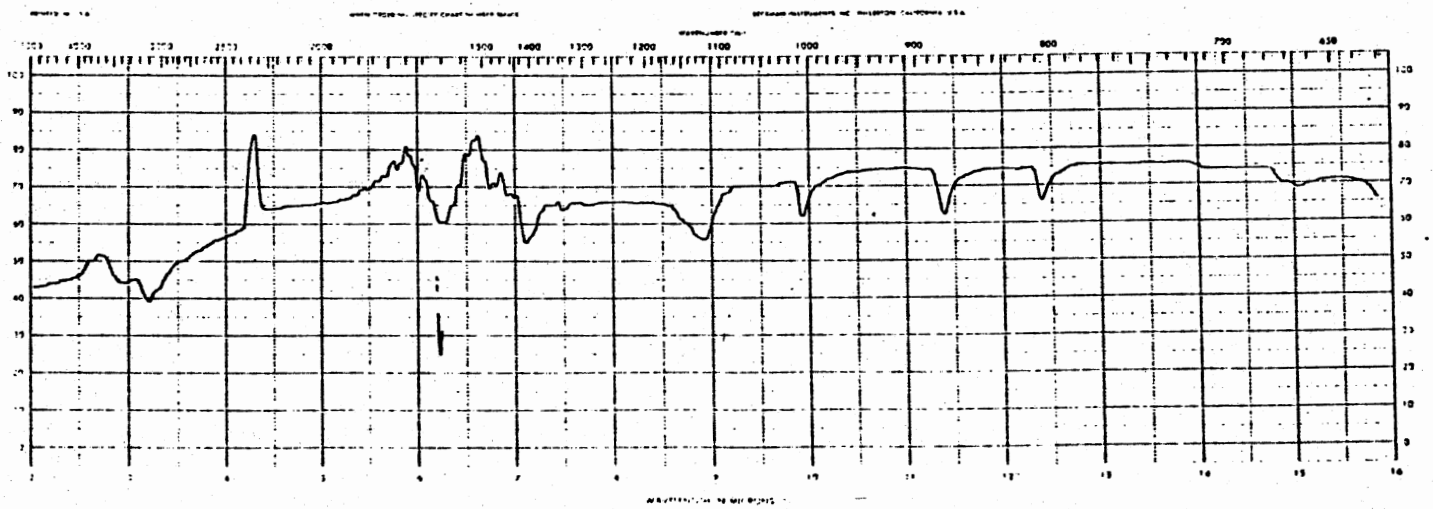


Figure 23. IR spectrum of 2-chloro-N,N'-dibromo-1,4-benzoquinonediimine - KBr pellet

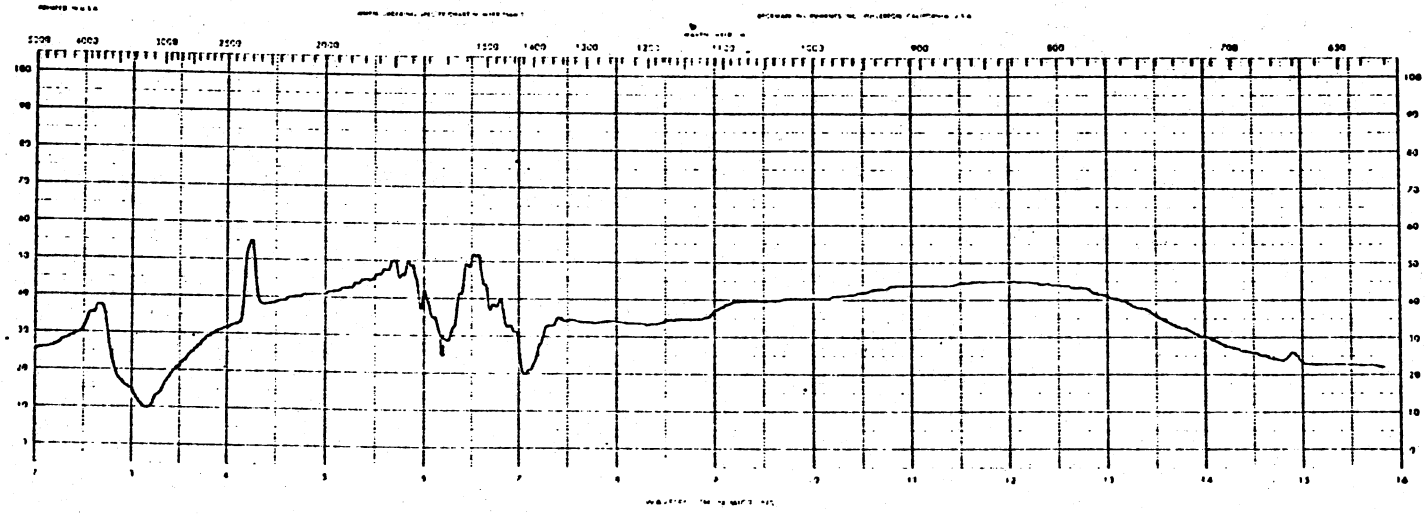


Figure 24. IR spectrum of 2-methyl-N,N'-dibromo-1,4-benzoquinonediimine - KBr pellet

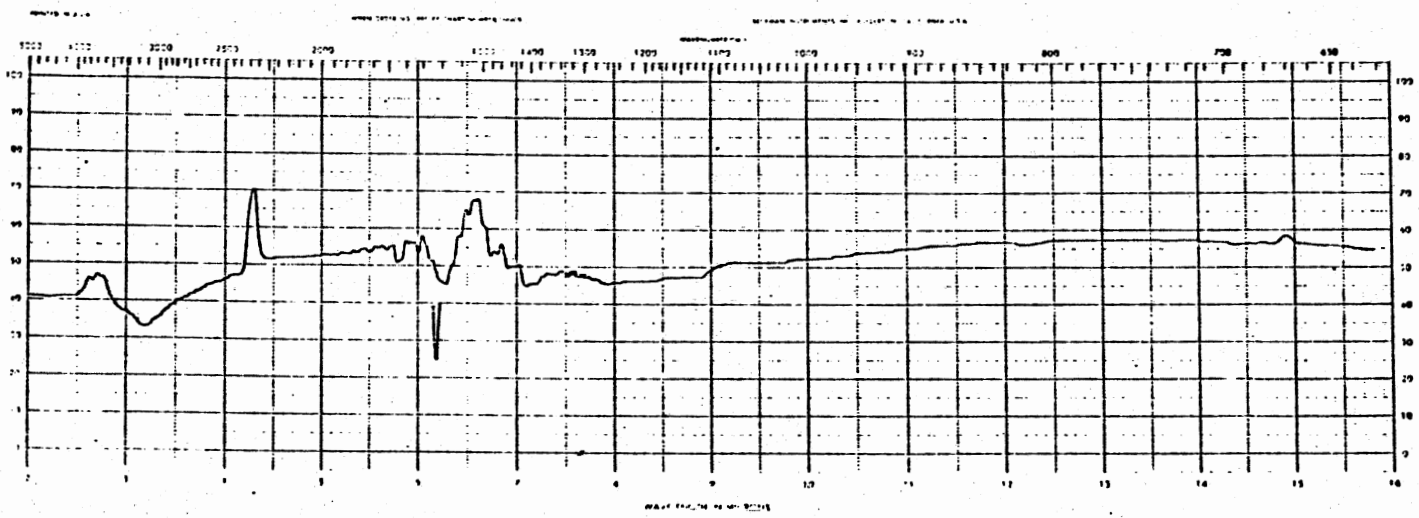


Figure 25. IR spectrum of 2-methoxy-N,N'-dibromo-1,4-benzoquinonediimine - KBr pellet

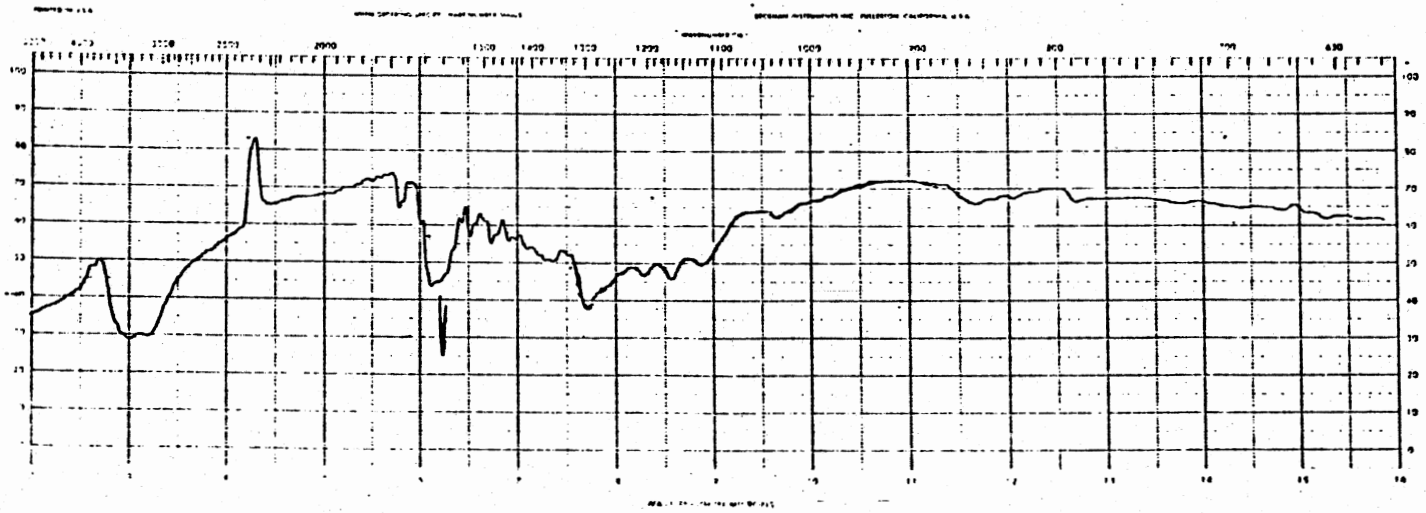
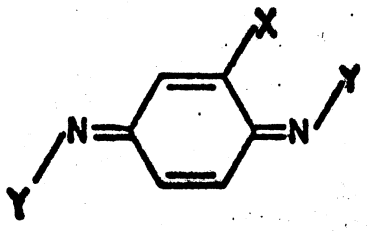


Figure 26. IR spectrum of 2-nitro-N,N'-dibromo-1,4-benzoquinonediimine - KBr pellet

TABLE V

INFRARED SPECTRAL ASSIGNMENTS
FOR THE TITLE COMPOUNDS



Y	X	C=C Stretching, cm^{-1}	C=N Stretching, cm^{-1}
Cl	H	1595	1448
	Cl	1595	1410
	CH_3	1595	1420
	OCH_3	1590	1410
	NO_2	1640	1450
Br	H	1595	1440
	Cl	1600	1452
	CH_3	1575	1420
	OCH_3	1575	1405
	NO_2	1615	1425

published IR spectrum of benzoquinone-1,4-diimine[54] and that of N,N'-dicyclohexyl-2,5-dimethyl-1,4-benzoquinonediimine[48] suggests that these should be attributed to C=C stretching and C=N stretching modes respectively. The absence of bands characteristic of aromatic structure at 1600-2000 cm^{-1} and 667-1200 cm^{-1} lend credence to the fact that these compounds are, in fact, quinonoid.

The interpretations of NMR spectra were complicated by the lack of suitable solvents for these compounds. In order to have a uniform basis for the comparison of all the compounds, pulsed spectra were obtained in 98.5% $CDCl_3$ regardless of the solubility of the compounds. For those compounds that were insoluble in $CDCl_3$ and/or that were interfered with by the absorption due to the undeuterated solvent, NMR spectra were taken using CCl_4 or acetone-d_6 as solvents. In the case of unsubstituted compounds, A_2B_2-type spectra are observed. For example, in the spectrum of N,N'-dichlorobenzoquinone-1,4-diimine, Figure 2, two triplets are observed, the chemical shifts being 7.1δ and 7.6δ. The three doublets centered around 7.1δ represent the C-2 and C-6 protons being coupled with C-3 and C-5 protons plus the effect of syn-anti isomerism. The mirror-image doublets centered around 7.6δ are due to the C_3 and C_5 protons. The coupling constants are $J_{AA'}$ = 8.5 cps, $J_{BB'}$ = 11.5 cps, $J_{AB'} = J_{A'B}$ = 2.0 or 1.0 cps and $J_{AB} = J_{A'B'}$ = 2.0 or 1.0 cps. N,N'-Dibromo-1,4-benzoquinonediimine, Figure 12 gave an identical splitting

pattern.

In the case of 2-substituted compounds, a complex pattern is observed which can be construed as due to an ABX system. The proton on C-3 has a chemical shift different than that of C-5 and C-6 protons. Typically, this consists of 15 lines, 2 quartets (that sometime overlap) for C_5 and C_6 protons, from lines for C-3 proton and three weak combination lines. This pattern is considerably simplified for the nitro- and chloro-substituted compounds. Thus, the NMR spectra are consistent with the quinonoid structure for these compounds.

Biological Studies

Toxicity Studies

It is imperative to determine the acute host toxicity of a compound before its antitumor potential can be evaluated. LD_{50}, the dose required to kill one-half the test animals within four days, serves as a good indicator of the toxicity of the compound to the host animal.

The protocol to determine LD_{50} consists of administering a compound intraperitoneally (i.p.) at a given dose level to a set of six mice weighing between 17 and 20 g and recording the number of deaths every 24 hrs. The solutions were prepared fresh by weighing a given amount of the compound and adding to it the necessary amount of salt to make it biologically isotonic. The mixture was

ground to a very fine powder and suspended in a proper amount of distilled water. Appropriate volume of the suspension were then administered to the mice. Weights of the mice were recorded on the day of administration of the compound and on the fourth day (72 hrs after the i.p. injection). Significant weight losses and/or excessive mortalities would indicate a trial at a lower dose level, while higher dose levels would be warranted by the absences of weight losses and/or deaths. The experiment is repeated at varying dose levels until a value for LD_{50} is found.

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

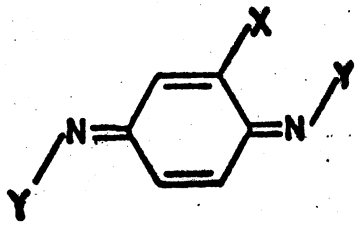
Inhibition of Ascitic Sarcoma 180

Ascitic Sarcoma 180 cells used in this study were originally obtained from Frederick A. French of Mt. Zion Hospital, San Francisco, California, through living mice carriers. These were maintained by transplanting 1×10^6 cells to 4 healthy mice each week.

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 approximately 1 ml of the ascitic fluid was withdrawn using 1-ml disposable syringe with a 25-gauge needle and diluted with 20 ml of biologically isotonic saline solution. The number of viable sarcoma

TABLE VI

ACUTE HOST TOXICITIES OF 2-Substituted-N,N'-DIHALOBENZOQUINONE-1,4-DIIMINES



X	Y	LD_{50}(mg/kg)	Molecular Weight	LD_{50}(mmoles/kg)
H	Cl	175	176.0	0.99
Cl	Cl	47	210.5	0.22
Me	Cl	245	190.0	1.29
MeO	Cl	75	206.0	0.36
NO_2	Cl	280	221.0	1.27
SO_3Na	Cl	400	255.1	1.57
H	Br	200	265.0	0.75
Cl	Br	150	295.5	0.51
Me	Br	380	279.0	1.36
MeO	Br	175	295.0	0.59
NO_2	Br	180	310.0	0.58
SO_3Na	Br	700	324.1	2.16

cells was determined using a hemocytometer after staining a sample of the ascitic fluid with tryphan blue dye. The dead cells were stained blue and ignored in the counting procedure. After the fluid had been diluted with a calculated amount of saline solution, 0.2 ml of the fluid containing a ca. 1 x 10^6 sarcoma 180 cells were injected intraperitoneally into a set of control and test animals each containing six female Swiss mice weighing between 17 and 20 g. The day the tumor cells were implanted is designated day 0.

On days 1, 2 and 3, the test animals were injected with a dose of the test compound and the control animals with isotonic saline solution. The initial dose levels were approximately one-third the LD_{50} for a single injection. The test suspensions were prepared fresh in a manner analogous to that described in the section dealing with toxicity studies. Weights of the mice were taken on day 0 and on day 4. The animals were observed for a period of sixty days and any deaths duly recorded. Any mice surviving the sixty-day period were considered cured if an autopsy failed to reveal any evidence for the presence of tumor cells.

Mean survival times for the treated and the control animals were computed by averaging the survival times of the corresponding six animals. The effectiveness of a compound at a given dose level in inhibiting the growth of the sarcoma 180 tumor system is expressed as the ratio of

the mean survival time of the treated animals and that of the control animals, T/C. A T/C value of at least 125% is necessary for the effectiveness to be statistically significant. Values lower than 100% indicate acute chemical toxicity.

Percent increase in life span values (ILS) can be computed by subtracting 100 from the % T/C values. Dose response curves were computed by plotting % ILS versus dosage. These were subjected to linear least squares analysis and dosage values for effecting 25% and 50% ILS were estimated and are designated ED_{25} and ED_{50} respectively. Biological data are summarized in Table VII. Optimum dose is the dose at which maximum T/C was observed. Therapeutic Index (TI) is the ratio of LD_{50} and ED_{25}.

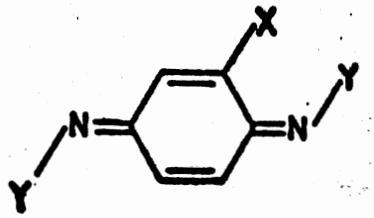
Stability Studies

Relative stabilities for the compounds in aqueous solution were determined spectrophotometrically. A buffer solution was prepared by dissolving 1.21 g (0.01 mole) of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) in distilled water contained in 1-liter volumetric flask. Enough concentrated hydrochloric acid and distilled water were added to bring the pH to 7.0 and the volume to 1 liter.

A small amount of the compound was dissolved in a drop of absolute ethanol and diluted with enough Tris buffer to obtain a ultraviolet spectrum. The instrument was a Cary-14 uv spectrophotometer. The cuvettes were

TABLE VII

INHIBITION OF ASCITIC SARCOMA 180



Y	X	Maximum T/C	Optimum Dose mg/kg	ED_{25}, mg/kg	ED_{50}, mg/kg	TI
Cl	H	2.25	20	7.81	9.89	22.44
Cl	Cl	2.43	15	6.03	7.6	7.79
Cl	CH_3[d]	3.09	60	44.74	46.58	5.48
Cl	OCH_3	1.83	20	5.01	9.17	14.97
Cl	NO_2[e]	1.66	50	38.24	44.91	7.32
Cl	SO_3H	1.77	100	86.69	92.99	4.61
Br	H	1.69	25	18.47	21.94	10.83
Br	Cl[a]	2.59	40	13.45	17.01	11.15
Br	CH_3	1.54	125	108.16	122.17	3.51
Br	OCH_3[b]	1.45	70	65.97	71.92	7.65
Br	NO_2[c]	2.58	45	20.31	22.40	8.86
Br	SO_3H	1.36	250	195.15	268.92	3.59

a. 1 cure each at 40 and 20 mg/kg
b. 1 cure at 70 mg/kg
c. 1 cure each at 45, 35 and 25 mg/kg
d. 2 cures at 60 mg/kg
e. 1 cure each at 50 and 40 mg/kg

1 cm in thickness. Optical densities were measured at appropriate time intervals to provide sufficient data to determine the half-life period of the quinonediimines under hydrolytic conditions. The solutions were maintained in a constant temperature bath at 24.8±0.1°C.

Treatment of Kinetic Data

A first order reaction, A products, can be descibed by the equation

$$\alpha_i = \exp(-kt_i) \tag{5}$$

where α_i is the fraction of reaction at time t_i and k is the rate constant. α_i is defined in terms of the concentration of the reactant by

$$\alpha_i = \frac{[A_i]}{[A_o]} \tag{6}$$

where $[A_i]$ and $[A_o]$ are concentrations of A at times t_i and 0 respectively. Then α_i ranges from α_i=0 when t=0 to α_i=1 where t=∞ (assuming A =0). Because of the dimensionless nature of α_i, the concentration terms can be replaced by some measurable physical property, in this instance, optical density, OD.

$$|OD_i - OD_\infty| = C[A_i] \tag{7}$$

where C is constant and the modulus signs recognize the fact that OD may increase or decrease with time. Expressing α in terms of OD gives

$$\alpha_i = (|OD_i - OD_\infty|) / (|OD_o - OD_\infty|) \tag{8}$$

and, combining equations (5) and (8) and taking logarithms

$$\ln (|OD_i - OD_\infty|) = \ln (|OD_o - OD_\infty|) - kt_i \quad (9)$$

The rate constants can then be determined from the measured values of OD_i, i=1,...,n and OD_∞ and plotting $\ln (|OD_i - OD_\infty|)$ against t_i without the need to know OD_o.

However, an accurate measurement of OD_∞ is not always possible because of one or more of the following: precipitation, further reaction of the products at long times and the desirability of an estimate of the rate constant before the completion of an especially slow reaction. While several methods are available for the estimation of OD_∞ and discussed by Holt and Norris[30], the direct search algorithm of Davies, Swann, and Campey[1], is used because of its simplicity and speed of convergence. The Fortran program was kindly supplied by Holt and Norris and was utilized slightly modified.

The results of the above search are summarized in Tables VIII-XV. The rate constant thus obtained, k, is used to estimate the half-life period, $t_{\frac{1}{2}}$, which is given by

$$t_{\frac{1}{2}} = 0.693/k \quad (10)$$

The half-life periods are listed in Table XVI.

Structure-Activity Relationship Studies

Hansch Correlations

Numerical values for substituent constants used in this analysis were from the list published by Hansch and

TABLE VIII

THE HYDROLYSIS OF N,N'-DICHLORO-1,4-BENZOQUINONEDIIMINE

Data Set 1 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0367A

Minimum Required Stepsize = 3.67×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.01536279

Final Estimates:

First Order Rate Constant = $0.016 \pm 0.005 HR^{-1}$

Initial Optical Density = 1.24 ± 0.065A

Infinity Optical Density = 0.48A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Optical Density A(T) Predicted	Ln (A(T)-A(INF)) Experimental	Ln (A(T)-A(INF)) Predicted
0.87	1.21	1.22	-0.3091	-0.2939
2.13	1.18	1.21	-0.3522	-0.3143
3.58	1.18	1.19	-0.3622	-0.3377
19.7	1.07	1.03	-0.5241	-0.5985
23.7	1.03	0.999	-0.5904	-0.6630
31.2	0.979	0.940	-0.7030	-0.7844
50.3	0.773	0.819	-1.241	-1.092
55.6	0.686	0.791	-1.599	-1.179
72.0	0.484	0.719	-9.329	-1.444

TABLE IX

THE HYDROLYSIS OF 2-CHLORO-N,N'-DICHLORO-1,4-BENZOQUINONE-DIIMINE

Data Set 2 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0353A

Minimum Required Stepsize = 3.53×10^{-5}A

Step Sizez Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.02589126

Final Estimates:

First Order Rate Constant = $0.010 \pm 0.001 HR^{-1}$

Initial Optical Density = 0.009 ± 0.041A

Infinity Optical Density = 0.43A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Predicted	Ln (A(T)-A(INF)) Experimental	Predicted
0.75	1.53	1.57	0.0974	0.1346
5.8	1.51	1.52	0.0782	0.0843
23.1	1.45	1.34	0.0212	-0.874
29.3	1.24	1.29	-0.2102	-0.1484
43.0	1.16	1.18	-0.3087	-0.2846
45.9	1.19	1.16	-0.2792	-0.3127
76.0	0.939	0.976	-0.6824	-0.6106
93.0	0.815	0.892	-0.9639	-0.7797
119.	0.830	0.786	-0.9253	-1.042

TABLE X

THE HYDROLYSIS OF 2-METHYL-N,N'-DICHLORO-1,4-BENZOQUINONE-DIIMINE

Data Set e Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0446A

Minimum Required Stepsize = 4.46×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.0004049675

Final Estimates:

First Order Rate Constant = $0.01 \pm 0.0004 HR^{-1}$

Initial Optical Density = 1.38 ± 0.006A

Infinity Optical Density = 0.29A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Predicted	Ln (A(T)-A(INF)) Experimental	Predicted
0.08	1.39	1.37	0.0919	0.0827
2.67	1.31	1.33	0.0240	0.0372
5.42	1.28	1.28	-0.0046	-0.0113
10.5	1.19	1.19	-0.1094	-0.1009
27.5	0.968	0.963	-0.3939	-0.4006
94.6	0.498	0.498	-1.5877	-1.585

TABLE XI

THE HYDROLYSIS OF 2-METHOXY-N,N'-DICHLORO-1,4-BENZOQUINONE-DIIMINE

Data Set 4 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0133A

Minimum Required Stepsize = 1.33×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.0001293646

Final Estimates:

First Order Rate Constant = $0.027 \pm 0.002 HR^{-1}$

Initial Optical Density = 0.821 ± 0.005A

Infinity Optical Density = 0.53A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Predicted	Ln (A(T)-A(INF)) Experimental	Predicted
0.63	0.82	0.81	-1.2413	-1.2656
2.17	0.79	0.80	-1.3394	-1.3067
4.25	0.78	0.78	-1.3665	-1.3621
9.83	0.75	0.75	-1.5006	-1.5109
96.1	0.55	0.55	-3.8170	-3.8126

TABLE XII

THE HYDROLYSIS OF 2-METHOXY-N,N'-DICHLORO-1,4-BENZOQUINONE-DIIMINE

Data Set 5 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0398A

Minimum Required Stepsize = 3.97×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.002162764

Final Estimates:

First Order Rate Constant = $0.029 \pm 0.002 HR^{-1}$

Initial Optical Density = 1.49 ± 0.03A

Infinity Optical Density = 0.52A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Predicted	Ln (A(T)-A(INF)) Experimental	Predicted
0.75	1.47	1.47	-0.0503	-0.0456
23.0	1.04	1.02	-0.6483	-0.6855
47.2	0.72	0.77	-1.5707	-1.3817
67.0	0.67	0.66	-1.8585	-1.9535

TABLE XIII

THE HYDROLYSIS OF 2-CHLORO-N,N'-DIBROMO-1,4-BENZOQUINONE-DIIMINE

Data Set 6 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0188A

Minimum Required Stepsize = 1.88×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.001751659

Final Estimates:

First Order Rate Constant = $0.52 \pm 0.034 HR^{-1}$

Initial Optical Density = 1.23 ± 0.030A

Infinity Optical Density = 0.62A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Predicted	Ln (A(T)-A(INF)) Experimental	Predicted
0.78	1.05	1.02	-0.8263	-0.88666
0.92	0.98	1.00	-0.9979	-0.96053
1.03	0.95	0.97	-1.1007	-1.0186
1.72	0.87	0.86	-1.3758	-1.3826
1.78	0.86	0.86	-1.4161	-1.4143
1.92	0.84	0.84	-1.4932	-1.4882
2.13	0.82	0.81	-1.5719	-1.5990
2.20	0.82	0.81	-1.5963	-1.6359
4.22	0.68	0.68	-2.7702	-2.7017

TABLE XIV

THE HYDROLYSIS OF 2-METHYL-N,N'-DIBROMO-1,4-BENZOQUINONE-DIIMINE

Data Set 7 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0128

Minimum Required Stepsize = 1.28×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.0035955

Final Estimates:

First Order Rate Constant = $0.027 \pm 0.011 HR^{-1}$

Initial Optical Density = 0.88 ± 0.068A

Infinity Optical Density = 0.61A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Optical Density A(T) Predicted	Ln (A(T)-A(INF)) Experimental	Ln (A(T)-A(INF)) Predicted
0.53	0.89	0.88	-1.2673	-1.3002
23.5	0.68	0.75	-2.6367	-1.9275
30.3	0.60	0.72	-6.0325	-2.1140
43.3	0.70	0.69	-2.3903	-2.4701
48.6	0.66	0.68	-2.9077	-2.6148
77.7	0.66	0.64	-2.9642	-3.4089
90.9	0.63	0.63	-3.6651	-3.7686

TABLE XV

THE HYDROLYSIS OF 2-METHOXY-N,N'-DIBROMO-1,4-BENZOQUINONE-DIIMINE

Data Set 8 Analysed By Direct Search Over Infinity Optical Density

Initial Stepsize = -0.0172A

Minimum Required Stepsize = 1.72×10^{-5}A

Step Sizes Are Reduced By 0.0500

Process Converged After 18 Function Evaluations

Minimum Weighted Sum Of Squares = 0.02829888

Final Estimates:

First Order Rate Constant = $0.025 \pm 0.013 HR^{-1}$

Initial Optical Density = 1.271 ± 0.095A

Infinity Optical Density = 0.92A

Comparison Of Data Predicted Using Optimized Infinity Optical Density

Time T HR	Optical Density A(T) Experimental	Predicted	Ln (A(T)-A(INF)) Experimental	Predicted
0.75	1.32	1.26	-0.9057	-1.0748
5.08	1.09	1.22	-1.7415	-1.1822
19.2	1.02	1.13	-2.3304	-1.5326
22.2	1.01	1.12	-2.3512	-1.6090
46.2	1.01	1.03	-2.4162	-2.2038
68.5	0.83	0.98	-2.3779	-2.7578
95.6	0.98	0.95	-2.8091	-3.4300

TABLE XVI

HALF-LIFE PERIODS OF N,N'-DIHALO-1,4-BENZOQUINONEDIIMINES UNDER HYDROLYTIC CONDITIONS

Compound No.	Rate Constant, $k \times 10^2$, Hr^{-1}	Half-life Period $T_{\frac{1}{2}}$, Hrs
1	1.62	42.7
2	0.99	69.7
3	1.76	39.1
4 (Trial 1)	2.67	25.9
(Trial 2)	2.88	24.0
8	52.76	1.3
9	2.7	25.3
10	2.48	27.8

co-workers[24]. In addition, parachor values were calculated for all the compounds[2,46]. Hydrophobic fragmental constants (HF) introduced by Nys and Rekker[40,41] were used as a measure of hydrophobicity-lipophilicity. The various constants are listed in Table XVII.

The dependent variables used were $\log(1/ED_{25}M)$ and $\log(1/LD_{50}M)$ where $ED_{25}M$ and $LD_{50}M$ are ED_{25} (effective dose, 25%) and LD_{50} (lethal dose, 50%), expressed in mmoles/kg of mouse.

The multiple regression was performed using the stepwise procedure available in Statistical Analysis System (SAS) programs package. The program is documented in a clear and usable manner[50]. The statistical results were interpreted according to Draper and Smith[16]. Both the sulfonic acid compounds were omitted from the analysis because of their questionable purities. The set of dependent variables offered as input to the program consisted of the eight substituent parameters, their squared values and all possible cross products of two variables taken at a time.

Discriminant Analysis

The BMD07M Stepwise Discriminant Analysis program, revised December 1975, developed at the Health Sciences Computing Facility, University of California at Los Angeles was used to perform all the discriminant analysis

TABLE XVII

PARAMETERS USED IN HANSCH CORRELATIONS
and discriminant analyses

Compound No.[a]	σ	π	$\tilde{\pi}$	R	MR	MW	HF	PAR	I[b]
1	0.0	0.0	0.0	0.0	1.03	175.0	0.193	332.2	0
2	0.23	0.71	0.41	-0.15	6.03	209.4	0.930	371.9	0
3	-0.17	0.56	-0.04	-0.13	5.65	189.0	0.702	372.2	0
4	-0.27	-0.02	0.26	-0.51	7.87	205.0	0.244	392.0	0
5	0.78	-0.28	0.67	0.16	7.36	220.0	-0.089	399.4	0
7	0.0	0.0	0.0	0.0	1.03	262.0	0.193	357.8	1
8	0.23	0.71	0.41	-0.15	6.03	296.4	0.930	397.5	1
9	-0.17	0.56	-0.04	-0.13	5.65	276.0	0.702	397.8	1
10	-0.27	-0.02	0.26	-0.51	7.87	292.0	0.244	417.6	1
11	0.78	0.28	0.67	0.16	7.36	307.0	-0.089	425.0	1

a. Compounds are designated by the numbers assigned to them in Table III.
b. Indicator constants used to differentiate between functional groups (i.e.) N,N'-dichloro- and N,N'-dibromo-1,4-benzoquinonediimines.

computations in this study*. Classification functions are established for each group by selecting the independent variables one by one in a stepwise manner. The variable with the greatest partial F value is entered at each step. If the F value for a particular variable drops below a desired level of significance as other variables are added, that variable is eliminated from the classification function. The procedures used in this program and the options available are clearly documented[15]. Some of these are summarized herein:

> . . . The program first calculates and prints the overall mean for each variable, the group mean for each group, the standard deviation for each variable in each group, the within and total cross-product matrices, the within-group covariance matrix, and the within-groups correlation matrix.
>
> In the first step of the stepwise procedure for selection of variables for the classification functions, the variable with the greatest partial F value is entered. This variable is the one that alone can best describe the difference between the groups. In each subsequent step, one variable is added or removed from the classification functions according to the following procedures.
>
> (a) If one or more variables that have already been entered into the classification functions have an F value to remove that is smaller than the value set for the program (normally 0.005 unless modified by the user), then the variable with the smallest F value will be removed.
>
> (b) If no variable is removed from the classification functions is chosen by one of three alternative procedures. We have chosen to use throughout this work option (1) that selects the variable with the greatest F value to enter. This variable is the one that together with the variables already entered in the classification functions provides the best discrimination between the groups. At each step a classification function is derived for each group, the compounds

---

*The Health Sciences computing facility is sponsored by NIH special Research Resources Grant RR-3.

are classified into each group based on the classification functions just derived, and the numbers of cases correctly and incorrectly classified are printed.

A classification function for each group k of n members is calculated as

$$c_{kj} = (n - g) \sum_{i=1}^{r} \bar{x}_{kj} a_{ij} \qquad (11)$$

i=1,2,...,r; k=1,2,...,g

where c_{kj} is the coefficient for variable v_j of group k, g is the number of groups used for the analysis, r is the number of variables used, i and j indicate the variables, a_{ij} is the determinant of the inverse within-group cross-products matrix, and $\bar{x}_{kj}$ is the group mean for variable j. A constant term, c_{ko}, is calculated for each classification function.

$$c_{ko} = -\tfrac{1}{2} \sum_{j=1}^{r} c_{kj} x_{kj} \qquad (12)$$

k=1,2,...,g

For purposes of predicting the classification of each case (or compound), the mth classification function, s_{lmk}, can be evaluated for case k of group 1

$$s_{lmk} = c_{mo} + \sum_{j=1}^{r} c_{mj} x_{lkj} \qquad (13)$$

where c_{mo} is the constant term in the classification function for group m, c_{mj} is the coefficient for variable j in the classification function for group m, and x_{lkj} is the value of variable j for case k of group 1. The posterior probability, P_{lmk}, of case k in group 1 having come from group m can be calculated

$$P_{lmk} = \frac{P_m \exp(s_{lmk})}{\sum_{i=1}^{g} P_i \exp(s_{lik})} \qquad (14)$$

where P_m is the prior probability of group m, P_i is the prior probability of group i, and s_{lik} is the function evaluated for case k of group i. This program calculates the posterior probability of each compound (case) belonging to each group on the basis of the classification functions developed in the last step. The stepwise procedure terminates at a predetermined number of steps or when no variable

meets the criteria (F value) set for entry or removal[43, p.369].

The substituent constants used in the discriminant analysis are also listed in Table XVII. The compounds are ranked according to their values for ED_{25}, LD_{50} and TD in Table XVII. The compounds were divided into two groups containing 5 compounds each. Group 1 with low values will represent active compounds in the case of ED_{25}, toxic compounds in the case of LD_{50} and inactive compounds in the case of TI.

Free-Wilson Additivity Correlations

Once again, Compounds 6 and 12 were omitted from these correlations because of lack of purity. The fully constructed structural matrix used is shown in Table XIX. Instead of using the full matrix and applying the restricting conditions, the method of Cammarata[10] was used. Unsubstituted N,N'-dichloro-1,4-benzoquinonediimine was considered the basic compound for the whole set.

This means that μ in equation 4 (p.9) takes the value of the biological activity for N,N'-dichloro-1,4-benzoquinonediimine rather than represent the average biological activity for the whole set of compounds. Consequently, the constants I_1 and I_3, indicating the chloro atom on N and the hydrogen atom at the 2-position respectively, are superfluous.

It is no longer necessary to apply the restrictive

TABLE XVIII

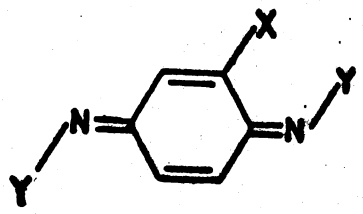
RANK OF COMPOUNDS BY DIFFERENT INDICES OF BIOLOGICAL ACTIVITIES*

	ED_{25}	LD_{50}	TI
	5	2	9
Group 1	4	4	3
Low Values	2	8	5
	1	10	10
	8	1	2
	11	11	11
Group 2	7	7	7
High Values	3	3	8
	10	5	4
	9	9	1

*The compounds are designated by numbers assigned to them in Table III

TABLE XIX

FULLY CONSTRUCTED STRUCTURAL MATRIX
USED IN THE FREE-WILSON
ADDITIVITY MODEL



Compound No.	Compound Identification						
	Y		X				
	Cl	Br	H	Cl	CH_3	OCH_3	NO_2
1	1	0	1	0	0	0	0
2	1	0	0	1	0	0	0
3	1	0	0	0	1	0	0
4	1	0	0	0	0	1	0
5	1	0	0	0	0	0	1
7	0	0	1	0	0	0	0
8	0	0	0	1	0	0	0
9	0	1	0	0	1	0	0
10	0	1	0	0	0	1	0
11	0	1	0	0	0	0	1

conditions that $I_1 + I_2 = 0$ and $I_3 + I_4 + I_5 + I_6 + I_7 = 0$. The resulting simplified matrix which was used in this study is shown Table XX. The numberical value of each constant indicates the contribution to the antitumor activity of a particular substituent at a particular position. These values were obtained by using the stepwise procedure available in the Statistical Analysis System (SAS) programs package.

TABLE XX

SIMPLIFIED STRUCTURAL MATRIX USED IN THIS STUDY*

Compound No.	Compound Identification				
	Y	X			
	Br	Cl	CH_3	OCH_3	NO_2
	I_1	I_2	I_3	I_4	I_5
1	0	0	0	0	0
2	0	1	0	0	0
3	0	0	1	0	0
4	0	0	0	1	0
5	0	0	0	0	1
7	1	0	0	0	0
8	1	1	0	0	0
9	1	0	1	0	0
10	1	0	0	1	0
11	1	0	0	0	1

*The above matrix was derived from the fully constructed matrix presented in Table XIX

CHAPTER IV

RESULTS AND DISCUSSION

Biological Studies

The values for LD_{50} indicate that N,N'-dichloro-1,4-benzoquinonediimines are more toxic than the corresponding bromo analogs. Chlorine atom at the 2-position imparts high toxicity. Consistent with the general axiom of coincidence of chemical toxicity with effectiveness in drug therapy, the dichlorodiimines are more active against sarcoma 180 as evidenced by their having higher T/C values, lower optimal doses, and lower effective doses than the bromo counterparts.

The fact that the dichloro compounds have higher therapeutic indices than the corresponding dibromo compounds implies that the high activities at low dose levels more than compensate for their high toxicities.

Stability Studies

In the case of 2-methoxy-N,N'-dichloro-1,4-benzoquinonediimine, where two trials were conducted, the rate constant was reproducible within 7.8%. Among the seven compounds for which half-life periods in hydrolysis are presented in Table XV, the chloro analogs are more stable

than the bromo compounds. It is supposed that they undergo hydrolysis to produce the corresponding dioximes. Biological data available on monohaloimines and monooximes indicate that the former are more effective against ascitic sarcoma 180. This should explain the smaller effectiveness of dibromoimines which are less stable in aqueous solutions. The incompleteness of the stability studies preclude their inclusion in any quantitative structure-activity studies.

Chemical Studies

Elemental analysis and spectral data essentially establish the quinonoid structure of the compounds in this study. However compounds 6 and 12, which contain the SO_3Na group, contain NaCl used to aid in their precipitation. Their low toxicities and ineffectiveness against sarcoma 180 tumor system could be caused by the presence of sodium chloride. Hence, these compounds have been excluded from all the structure activity studies.

Hansch Correlations

When the ten 2-substituted-N,N'-dihalo-1,4-benzoquinonediimines were subjected to Hansch-type multiple regression analysis, the indicator constant was forcibly included in all the correlations. When $\log(1/ED_{25}M)$ was the independent variable, the following structure-activity relationship was obtained.

$\log(1/ED_{25}M) = -0.032MW + 2.842\pi + 2.383I + 6.96$ (15)

(n = 10; s = 0.06; r = 0.89)

The multiple correlation coefficient r is 0.89 and r^2 has a value of 0.80 which means that 80% of the variable space been "explained" by the regression equation. The F value of 8.01 for the whole analysis is greater than standard F distribution of $F_{3,8,0.99} = 7.59$, implying that the correlation is significant at 99% level. Further more, the null hypothesis that the coefficients of π and MW are zero are rejected with a confidence level of 98.77% and 94.62% respectively.

This relationship would imply that field effect and molecular weight play a significant role in the antitumor activity of these compounds.

In the case of LD_{50}, the following relationship was obtained:

$\log(1/LD_{50}M) = 0.0019\,MW\pi + 1.4936\,\pi - 0.0462I + 0.0852$ (16)

(n = 10; s = 0.0232; r = 0.864)

r^2 has a value of 0.746 meaning that 74.6% of variable space is explained by the correlation; $F = 5.889 > F_{3,6,0.95} = 4.74$. Once again the null hypothesis that the cofficients are zero is rejected.

Based on equations 15 and 16, $\log(1/ED_{25}M)$ and $\log(1/LD_{50}M)$ were calculated for all the ten compounds. Reasonable agreement between experimental and calculated values are found as shown in Table XXI.

An inspection of the QSARs suggests ways of designing

TABLE XXI

RESULTS OF HANSCH CORRELATIONS - A COMPARISON OF THE PREDICTED AND EXPERIMENTAL ACTIVITIES

Compound No.	Log $(1/ED_{25}M)$			Log $(1/LD_{50}M)$		
	Experimental	Predicted	Residual[a]	Experimental	Predicted	Residual[b]
1	1.35	1.36	-0.01	0.0	0.09	-0.09
2	1.54	1.42	0.12	0.65	0.49	0.16
3	0.63	0.80	-0.17	-0.11	-0.07	-0.04
4	1.61	1.14	0.47	0.31	0.19	0.12
5	1.71	1.82	-0.11	-0.10	0.04	-0.14
7	1.15	0.96	0.19	0.12	0.04	0.08
8	1.34	1.02	0.32	0.30	0.51	-0.21
9	0.41	0.40	0.01	-0.14	-0.12	-0.02
10	0.65	0.74	-0.09	0.22	0.19	0.03
11	1.41	1.42	-0.01	0.23	0.10	0.13

a. Net residual = 0.77; b. Net residual = 0.02

new compounds with better antitumor activity. The increase of $\mathcal{F}$ and MW will result in the decrease of $ED_{25}M$ and hence the increase in potency. This would decrease the value for $LD_{50}M$ as well, yielding drugs with low toxicity. Toxicity can also be reduced by selecting substitutents such that the values for π and σ are numerically large but with opposite signs. Electron-withdrawing groups have large values for σ. If these are chosen so that they have large $\mathcal{F}$, large formula weights, and high water solubility (large negative values for π), the resulting compounds will be effective and non-toxic.

Discriminant Analysis

Discriminant analysis was able to successfully classify the ten compounds into active and moderately active groups based on ED_{25} at a statistically significant level. $\mathcal{F}$ and molecular weight were able to classify all the compounds correctly at a confidence level of 97.5% ($F = 7.82 > F_{2,7,0.975}=6.54$).

The classification functions obtained were:

for active compounds

$$-15.33(11.47)\mathcal{F} + 0.16(9.09)MW - 15.59$$

for moderatively active compounds

$$-30.05(11.47)\mathcal{F} + 0.24(9.09)MW - 29.93 \qquad (17)$$

where the numbers in parantheses are F to enter with degrees of freedom being 1, 7 for the corresponding variables. Substituents with large values for $\mathcal{F}$ should be

given consideration for future study.

The following results were obtained when the benzoquinonediimines were divided into (a) toxic group (low values of LD_{50}) and (b) non-toxic group (high values of LD_{50}), each group consisting of five compounds and subjected to discriminant analysis.

The classification functions were:

for toxic compounds

$-176.7(7.50)\sigma + 376.2(15.71)R + 1.93(8.02)PAR - 954$

for non-toxic compounds

$-158.3(7.50)\sigma + 325.6(15.71)R + 1.73(8.02)PAR - 1149$

The analysis was significant at the 95% level ($F = 6.26$ $F_{3,6,0.95}=4.74$). All the ten compounds were classified correctly. Substituents with large values for R and parachor and large negative values for σ will result in lower toxicity.

The benzoquinonediimines were then divided into two groups based on their therapeutic index, five compounds with low values as moderatively active and five compounds with high values as active and subjected to discriminant analysis. A four variable set of σ, π, MR, and parachor classified all the ten compounds correctly. However, the analysis was not significant even at 75% level.

Free-Wilson Additivity Studies

The results of the Free-Wilson analysis depicting the contributions of various substituents to ED_{25}, LD_{50} and TI

are shown in Table XXII. The constant term in each case would represent the activity of the unsubstituted N,N'-dichlorobenzoquinonediimine and a comparison of these with the experimental values are presented in Table XXIII.

Nearly eighty-four percent of the variation in ED_{25} is explained by the additivity model as evidenced by the value of 0.915 for the multiple correlation coefficient r, the value of r^2 being 0.838. The analysis is significant at the 90 percent level, for $F = 4.14 > F_{5,4,0.90}=4.05$.

The additivity model explains seventy-eight percent of the variations in LD_{50}, since $r = 0.885$ and $r^2 = 0.783$. The analysis is significant at the 84 percent level, since $F = 2.89 > F_{5,4,0.75}=2.07$.

Only seventy-one percent of the variations in TI are explained by this model because $r = 0.843$ and $r^2 = 0.71$. The analysis is significant at 74 percent level, because $F = 1.97 \simeq F_{5,4,0.75} = 2.07$.

The effect of replacing the chloro atoms at N and N' positions by bromo atoms is indicated by the values of the <u>de novo</u> constant I_1 in Table XXII. The substitution adds to the effective and toxic dosages but lowers the therapeutic index. The advantage of low toxicities of the dibromo compounds is offset by the larger doses needed for antitumor activities. The nitro substituent is the most effective, but confers some toxicity. The chloro atom is the most toxic and the second most effective next only to the nitro group. The therapeutic index results

TABLE XXII

CONTRIBUTION OF STRUCTURAL CHANGES TO BIOLOGICAL ACTIVITIES

Position	Substituent	Constant	Contribution to ED_{25}	LD_{50}	TI
X	Br	I_1	30.04	47.6	-15.44
Y	Cl	I_2	-3.40	-89.0	-7.16
	CH_3	I_3	63.34	125.0	-12.14
	OCH_3	I_4	22.36	-50.0	-5.33
	NO_2	I_5	-4.96	42.5	23.18

TABLE XXIII

COMPARISON OF PREDICTED AND EXPERIMENTAL BIOLOGICAL ACTIVITIES OF N,N'-Di-CHLORO-1,4-BENZOQUINONEDIIMINE

Biological Activity	Predicted	Experimental
ED_{25}	-1.89	7.81
LD_{50}	163.7	175.0
TI	24.35	22.44

suggest, however, that the nitro group is the most effective.

Recommendations for Future Study

Nitrogen analogues of naphthoquinones and anthraquinones should be synthesized and their biological potential should be evaluated. A series of 4-substituted-N,N'-dihalo-1,2-benzoquinonediimines should also be examined for their antitumor activity. Discriminant analysis should be applied to the volume of antitumor data available in the literature with a view to test its reliability as a qualitative structure activity relationship.

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APPENDIX

SYNTHESIS OF RELATED COMPOUNDS

Preparation of 1,4-Naphthoquinone Dioxime

In a 250-mL beaker, 5 g (0.032 mole) of 1,4-naphthoquinone (practical), 6 g (0.09 mole) of hydroxylamine hydrochloride and 200 mL of 95% ethanol were stirred for 24 hrs. The reaction mixture was poured into a mixture of ice and water. The crude precipitate formed was filtered out and recrystallized from ethanol-water mixture. The yield was 2.7 g. The theoretical yield was 6.02 g. mp 204-205°C dec. Published: 207°C[39].

Preparation of 2,3-Dibromo-1,4-naphthoquinone Dioxime

1,4-Naphthoquinone dioxime (2 g, 0.01 mole) was dissolved in 30 mL of glacial acetic acid. This solution was cooled to room temperature and 2 g (0.013 mole) of bromine was added. The reaction mixture was stirred overnight and poured into ice-water mixture. The resulting red-brown precipitate was filtered out, washed with water and recrystallized from ethanol water mixture. The yield was 1.8 g. mp 195°C. The theoretical yield was 3.46 g.

The melting point for this compound has not been reported.

Oxidation of 1,2-Phenylenediamine by Hypobromite

In a 1-liter beaker, 5.4 g (0.05 mole) of 1,2-phenylenediamine was dissolved in 300 mL of water. Bromine (32 g, 0.2 mole) was dissolved in a solution of 16.7 g (0.4 mole) of sodium hydroxide in 500 mL of water and stirred with ice. To the chilled hydrobromite solution was added the amine solution slowly and with stirring. The precipitate formed was dried under vacuum. It was recrystallized from ethanol. The yield was 7 g. mp 250°C. The theoretical yield was 13.2 g. No melting point has been reported for this compound.

Oxidation of 1,2-Phenylenediamine by Bleach

In a 500 mL beaker, 5.4 g (0.05 mole) of 1,2-phenylenediamine was dissolved in 300 mL of water was added to 500 mL of commercial bleach chilled in a 1-liter beaker slowly and with stirring. The resultant yellow precipitate was filtered, washed with water and dried under vacuum. The crude N,N'-dichloro-1,2-benzoquinone-diimine was recrystallized from ethanol. The yield was 6.2 g. The theoretical yield was 8.75 g. mp 250°C. The melting point for this compound has not been reported.

Oxidation of 4-Chloro-1,2-phenylene-diamine

4-Chloro-1,2-phenylenediamine (7.2 g, 0.05 mole) was dissolved in 300 mL of water in a 500 mL beaker. To a chilled commercial bleach solution (500 mL) was added the amine solution slowly and with stirring. The resulting precipitate was filtered, washed with water and dried under vacuum. The crude product was recrystallized from ethanol. The yield was 6.9 g. The theoretical yield was 10.48 g. mp 250°C. The melting point for this compound has not been published.

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