This dissertation has been
microfilmed exactly as received 67-13,711

SINGH, Ishwar, 1941-
SYNTHETIC APPROACHES TOWARD NAPHTHAZARIN DERIVATIVES.

University of Hawaii, Ph.D., 1966
Chemistry, organic

University Microfilms, Inc., Ann Arbor, Michigan

© Ishwar Singh 1967

All Rights Reserved

SYNTHETIC APPROACHES TOWARD NAPHTHAZARIN DERIVATIVES

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF THE

UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

SEPTEMBER 1966

By

Ishwar Singh

Thesis Committee:

Paul J. Scheuer, Chairman
Christopher Gregory
Richard G. Inskeep
Edgar F. Kiefer
Larry L. Schaleger

SYNTHETIC APPROACHES TOWARD

NAPHTHAZARIN DERIVATIVES

By Ishwar Singh

A thesis submitted to the Graduate School of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

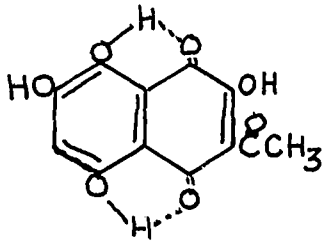
The sea urchin pigments, spinochromes A, C, D, and E (I, II, III, and IV) are derivatives of naphthazarin (V). They constitute a group of compounds which have been studied for a quarter of a century, but whose structures were determined only in recent years. In order to confirm the assigned structures and to make these compounds and their derivatives available for physical and chemical studies these four compounds were synthesized from readily available starting materials. All four synthetic pigments proved to be identical with the natural compounds.

The electronic spectra of some fifty derivatives and synthetic intermediates were measured. The observed shifts in wavelength were correlated with structural parameters.

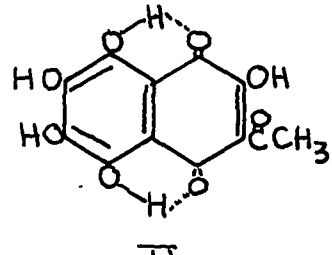
Attempts to explore new synthetic pathways toward naphthazarins and to introduce the acetyl function into the polyhydroxy naphthoquinone nucleus met with little success.

An x-ray crystallographic analysis was undertaken of a dimethyl ether of spinochrome A in order to solve a structural ambiguity which

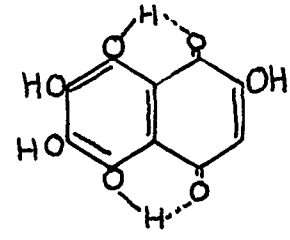
seemed insoluble by other physical and chemical methods. In spite of probable twinning of the crystal the x-ray diffraction studies resulted in the assignment of VI as the most probable structure of spinochrome A dimethyl ether, m.p. 181-182°.



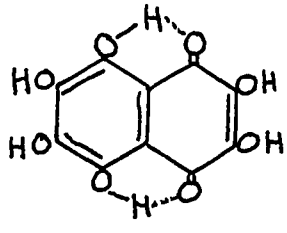
I



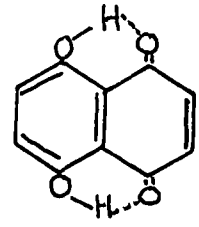
II



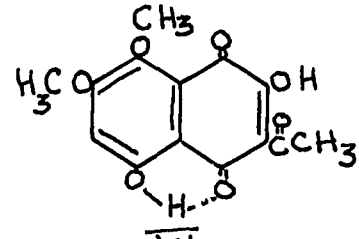
III



IV



V



VI

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Page

LIST OF ILLUSTRATIONS

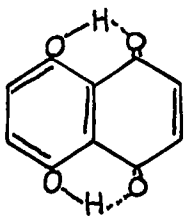
CHAPTER I

INTRODUCTION

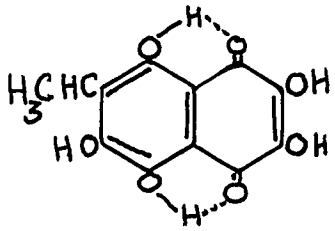
A. SURVEY OF THE LITERATURE

1. Structural Work

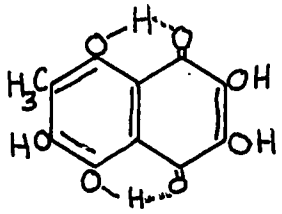
The quinoid pigments are very diverse and widely distributed in nature, but only a few naturally occurring napthoquinone pigments have been isolated from animal sources (1). Most of these pigments are derivatives of naphthazarin (I) and have been isolated from sea urchins.



I



II



III

The first pigment of this class was isolated from the body fluids of Strongylocentrotus lividus by MacMunn in 1885 (2) and was named echinochrome. However, the structure of this enchinochrome, which is now called echinochrome A, was determined by Kuhn, Wallenfels, and Gtauhe in the years 1939-1943 to be 2-ethyl-3,5,6,7,8-penthahydroxy-1,4-napthoquinone (3, 4).

Lederer and Glaser (5) also isolated a pigment from the spines of P. lividus and stated that the compound differed from

echinochrome A by one additional oxygen atom. It was named spinochrome.

Goodwin, Lederer, and Musajo (6) proposed that the pigments isolated from the calcified sections of the sea urchins be called spinochromes, whereas pigments from the ovaries, perivisceral fluid and claeocytes be called echinochromes. Individual members of each group would be distinguished by letter suffixes. This distinction has now been questioned because of isolation of echinochrome A, from calcified regions of sea urchins, by Nishibori (7, 8).

Musajo et al. (9) isolated a pigment from the violet spines of P. lividus and called it spinochrome P, but it was identical with the spinochrome of Glaser and Lederer (5), which is now called spinochrome A. Structure II was proposed for it.

Spinochrome C (VII) was isolated from the spines of A. lixula (Linn) by Glaser and Lederer (10) who suggested that it may be similar to the pigment spinone A (11), which was reported by Kuhn and Wallenfels to occur in the same sea urchin.

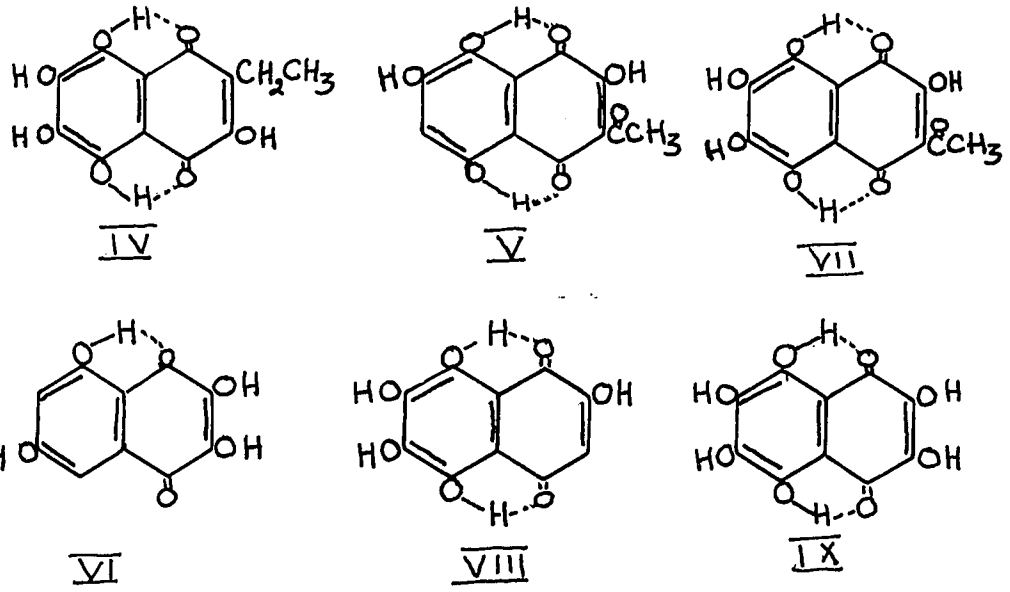
Since 1939 four sea urchin species were investigated by Kuroda and coworkers. They described about a dozen pigments and designated them spinochrome Ak_1, Ak_2, M, M_1, M_2, B, B_1, B_2, B_3, F, and F_1 (12, 13, 14). Structure III was proposed for Spinochrome Ak or D by Kuroda (12) on the basis of elemental analyses of its derivatives. However, only the structure of spinochrome N had been proven rigorously through 1963 (15). Postulated structures of spinochromes based on insufficient or conflicting evidence,

coupled with the fact that the European workers did not consult the Japanese workers when they advanced proposals for nomenclature of these pigments, left the whole field of spinochrome chemistry in a confused state.

During 1964, however, two publications removed many uncertainties in this field. The first was a communication by Gough and Sutherland (16), who isolated spinochrome B from the spines of Salmacis sphaeroides. They proved its identity with spinochrome N (VI) by comparison of the nuclear magnetic resonance (NMR) spectra of the trimethyl ethers of these spinochromes. The structure of spinochrome N had already been proven to be 2,3,5,7-tetrahydroxy-1,4-napthoquinone by Smith and Thomson (15). Gough and Sutherland further showed the identity of spinochrome B with previously reported spinochromes, Ak_1, P, B_1, M_2.

Also in 1964, Scheuer's group (17) isolated a spinochrome from six species of Hawaiian sea urchins in five genera and found that the same pigment occurred as the major pigment in the spines of Echinometra oblonga Blainville and Colobocentrotus atratus Linn., and as a minor constituent in the spines of Tripneustes gratilla Linn., Echinothrix diadema Linn., E. calamaris Pallis, and Diadema paucispinum Agassiz. They proved its structure to be 2,7-dihydroxy-3-acetylnaphthazarin, and demonstrated its identity with spinochrome M. In another communication (18) Scheuer's group showed that spinochrome M was also identical with A and that another pigment, spinochrome C, was identical with F. As a

result of recent researches, therefore, the total number of pigments has been reduced to six pigments of unquestioned identity. They are echinochrome A (IV) and spinochromes A (V), B (VI), C (VII), D (VIII), and E (IX). Further work (19) in this laboratory has resulted in the isolation of further spinochromes which occur in trace amounts in E. diadema and E. calamaris.



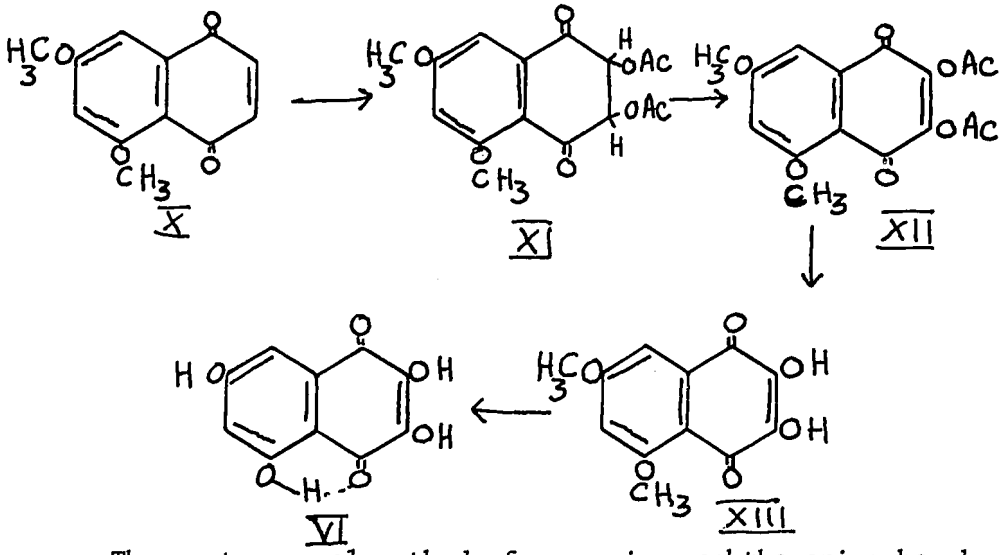
2. Previous Synthetic Approaches

When this research was begun, syntheses of echinochrome A (4) and of spinochromes B (15) and D (24) had been reported. Echinochrome A was synthesized by Wallenfels and Gauhe (4) by effecting the condensation of 2-ethyl-1,3,4-trimethoxybenzene with dibenzoyloxymaleic anhydride in an aluminum chloride-sodium chloride melt in only 1.5 per cent yield.

Synthesis of spinochrome D was reported by Thomson et al.,

proving the structure of spinochrome D to be VIII rather than III, the earlier structure proposed by Kuroda.

Spinochrome B (VI), which is a juglone derivative, was synthesized under the designation N by Smith and Thomson (15) in 1961. Starting with 5,7-dimethoxy-1,4-naphthoquinone, the dihydrodiacetate XI was formed, which on air oxidation in basic solution yielded the quinone XIII. Treatment of this compound in an aluminum chloride-sodium chloride melt resulted in the desired product VI.



The most general method of preparing naphthazarins has been the condensation of quinols with maleic anhydride in fused sodium chloride-aluminum chloride (20). Most known naphthazarins were made by this means, but the method was not suitable for the synthesis of naphthazarins containing one substituent in each ring, or two substituents in one ring and one in the other, since mixtures resulted which were difficult to separate. Thus, toluquinol and citraconic anhydride afforded a mixture of 2,6- and 2,7- dimethylnaphthazarin. However, in an attempted synthesis

of chloro naphthazarin from chloromaleic anhydride and hydroquinone, a mixture of dichloronaphthazarins, trichloronaphthazarin and naphthazarin resulted (21). In an attempt to prepare 2,3-dihydroxynaphthazarin by condensing dibenzoyloxymaleic anhydride with quinol Bruce and Thomson isolated only 2,5-dihydroxyacetophenone (22).

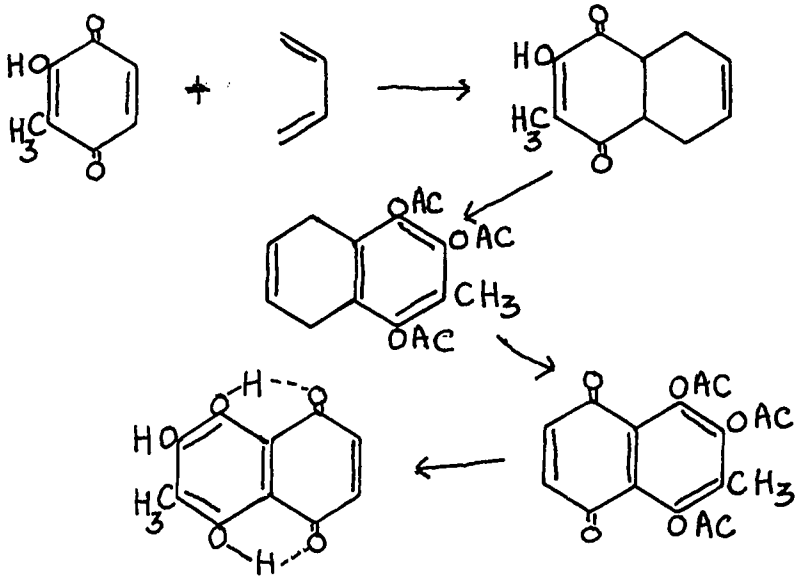
The oldest method of preparing naphthazarin itself consists of the action of sulfur and fuming sulfuric acid on 1,5-dinitronaphthalene (23). Bruce and Thomson prepared methylnaphthazarin, 2,6-dimethyl-, and 2,6-dichloronaphthazarin in quite low yields from the appropriate dinitro compounds. Chang prepared 2,6-dimethoxynaphthazarin from 1,5-dimitro-2,6-dimethoxynapthalene in 3 percent yield (17).

Another very tedious method is the conversion of suitably substituted tetralone derivatives. This has been successfully applied by Thomson et al. (24) in his synthesis of spinochrome D and for juglone derivatives (25).

Another general method concerns the introduction of various substituents into the naphthazarin molecule. This method appears advantageous but appears to be difficult, e.g. addition of acetic anhydride requires ten days (26). However, a modified procedure which is based on a Thiele-Winter reaction (27), has been quite useful. Naphthazarin is oxidized to the diquinone, which reacts with acetic anhydride in the presence of boron trifluoride or sulfuric acid to yield 2,5,8-triacetoxynaphthoquinone and, after hydrolysis, naphthopurpurin. When naphthazarin bears a substituent

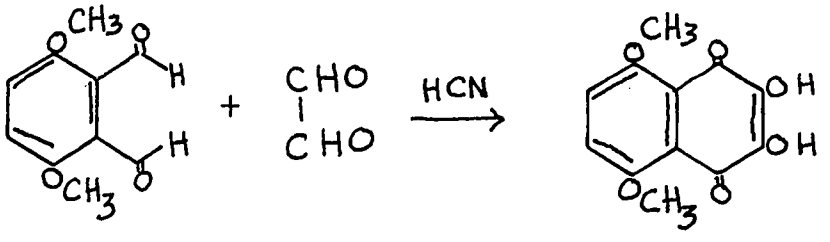
which is not affected by lead tetraacetate, the above method results in a mixture of hydroxynapthazarins, which could be separated chromatographically (28).

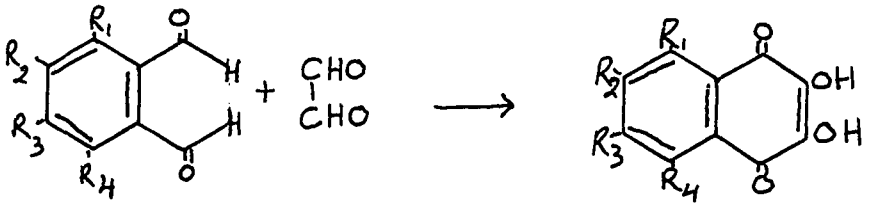
Another interesting approach has been the application of the diene synthesis (29). The synthesis of 3-hydroxy-2-methylnaphthazarin, for example, was achieved by the following pathway.



But the scope of this method is limited because of the unaccessibility of suitably substituted butadiene derivatives.

A very restricted ingenious method used by Weygand (30) is an extension of the benzoin condensation, as follows:





$R_1, R_2, R_3, R_4 = H, OCH_3$ or Alkyl

B. STATEMENT OF THE PROBLEM

At first glance it seems surprising that structural determination and synthesis of such relatively simple compounds as the spinochromes should be beset with difficulties. On closer examination, however, one finds that the high ratio of oxygen to carbon in these compounds leads to problems in purification and presents obstacles toward normal derivatization. On the other hand, these compounds have interesting physical and chemical properties such as their ability to chelate selectively with metals (31) and their high oxidation-reduction potentials. Lack of such data is no doubt caused by their occurrence in nature in small concentration (generally less than 0.1 per cent of dried animal) and by the absence of satisfactory synthetic routes. The additional fact that structural proof by synthesis is often needed to remove any elements of doubt remaining after a structure proof by degradation, emphasizes the need for synthetic studies. It seemed therefore appropriate to investigate synthetic approaches to these compounds in the hope to make them readily available for physical and chemical study. Systematic identification of the products obtained would be carried out largely by spectral

correlations.

CHAPTER II

EXPERIMENTAL SECTION

GENERAL STATEMENT

All melting points reported herein were recorded from a Fischer-Johns hot stage block and are uncorrected.

All infrared (IR) spectra were measured on samples in potassium bromide disks on a Beckman IR-5 automatic recording spectrophotometer. The potassium bromide was of infrared quality (Harshaw Chemical Company).

Relative intensities are defined as follows: b (broad), vs (very strong), s (strong), infl. (inflection), w (weak), and sh (shoulder).

All ultraviolet (UV) spectra were recorded on a Cary-14 recording spectrophotometer. Absolute methanol and chloroform were the neutral solvents, and potassium hydroxide in absolute methanol was the basic solvent.

Nuclear magnetic resonance (NMR) spectra were observed with a model A-60 Analytical N.M.R. Spectrophotometer (Varian Associates, Palo Alto, Cal.). All values are expressed in parts per million (ppm) referred to tetramethyl-silane (TMS) having S = 0.

Separation of the naphthazarins and their purefication was achieved using colum chromatography (CC) and thin-layer chromatography (TLC). Adsorbent used for CC was deactivated silica gel (DSG) which was prepared as follows: Silica gel (B of A 80-200 mesh, chromatography grade) was washed with 0.5 N hydrochloric acid and filtered and dried with suction. The resulting deactivated silica

gel (DSG) was spread on porcelain trays and air-dried in a fume hood.

The plates for TLC were prepared using a Desaga/Brinkmann Standard applicator (Brinkmann Instruments, Inc.). A mixture consisting of a slurry of 30 g of silica gel G (E. Merck, Germany) was stirred in a mortar and applied to a series or combinations of plates (20 x 20 and/or 20 x 5 cm), which were then allowed to dry at room temperature for at least six hours and finally stored in a wooden cabinet.

All the combustion analyses reported herein were carried out at Berkeley Analytical Laboratory, Berkeley, Cal.

A. DIENE SYNTHESES

1. p-Benzoquinone and Furan

p-Benzoquinone was purified by steam distillation and furan by distillation.

a. At room temperature without solvent.--A mixture of 0.13 g of p-benzoquinone and 0.10 g of furan was allowed to stand at room temperature; furan was evaporated; and the residue was identified as p-benzoquinone; an excess of furan was added, and this solution was again allowed to stand for several hours. When the furan was evaporated, the residue was unreacted p-benzoquinone.

b. Refluxing in ether, benzene, or absolute alcohol -- Equimolar mixtures of p-benzoquinone and furan were refluxed under argon in these solvents under various conditions, but only starting material was recovered.

c. In a sealed tube. -- An equimolar mixture of benzoquinone and furan was sealed in a pyrex tube and was heated for several hours on a steam bath or at 110° C in an oil bath, but there was no reaction.

2. 2-Acetylquinone

A mixture of 1.0 g 2,5-dihydroxyacetophenone, 1.5 g anhydrous magnesium sulfate, 10 ml dry benzene, and dried silver oxide (from 5.5 g of silver nitrate) was shaken for 30 min and then filtered through sintered glass. The solid was washed with benzene and the combined solutions were evaporated under reduced pressure to yield 894 mg (91%) of 2-acetyl-1,4-benzoquinone, m.p. 60-64°; sublimation at 1 mm pressure yielded 796 mg of orange crystals, m.p. 66-67° (Literature (32) m.p. 65.5-66.5°).

3. 2-Acetoxyfuran

2-Acetoxyfuran was prepared from 2,5-diacetoxy-2,5-dihydrofuran, which in turn was prepared by the method of Clauson-Kaas (33).

A mixture of 2,5-diacetoxy-2,5-dihydrofuran (10.0 g), dibutyl phthalate (25 ml) and 2-napthalenesulfonic acid monohydrate (0.05 g) was heated in a Claisen flask under 2 mm pressure for 45 min; the temperature of the heating bath (Wood's metal) was raised slowly from 100 to 190°. The crude distillate (9.5 g, b.p. 28-65°/2 mm) was redistilled through a small packed column to give 2-acetoxyfuran, b.p. 30-45°/3 mm.

4. p-Benzoquinone and 2-Acetoxyfuran; 2-Acetyl-p-benzoquinone

and Furan or 2-Acetoxyfuran

p-Benzoquinone (0.25 g) and 2-acetoxyfuran (0.3 g) were dissolved in 10 ml ether and allowed to stand at room temperature (30° C) for four h. The solvent was evaporated and starting materials were recovered. Refluxing the same mixture in ether, benzene, or alcohol did not result in any reaction. Similarly, reactions of 2-acetyl-p-benzoquinone and furan and of 2-acetoxy-furan with 2-acetylbenzoquinone resulted in no reaction.

5. Crotonaldehyde and p-Benzoquinone

A mixture of 540 mg of p-benzoquinone and 350 mg of crotonaldehyde was dissolved in 10 ml of benzene. To this solution was added a solution of one drop piperidine in 1 ml benzene. It was allowed to stand for 1 h. The dark solution was evaporated under vacuum to a dark tarry material. The residue thus obtained was triturated with chloroform and boiled for a few minutes with charcoal and filtered through a sintered glass funnel. The solution was concentrated and was put on a column of deactivated silica gel and eluted with benzene. The green fluorescent solution arising from a yellow hand was evaporated; the product was crystallized from alcohol giving 95 mg of a product, m.p. 283-286° (subl.). The compound was characterized as anthraquinone by a mixture melting point with an authentic sample of anthraquinone and by comparison of their infrared and ultraviolet spectra. Similar reaction of crotonaldehyde and 2-acetyl-p-benzoquinone resulted in the formation of 2,5-dihydroxyacetophenone.

B. INTRODUCTION OF AN ACETYL GROUP INTO NAPHTHALENE DERIVATIVES

1. 2-Acetyl-1,7-dihydroxynaphthalene

Method A. Three gram of freshly fused powdered zinc chloride was dissolved in a mixture of 2 ml glacial acetic acid and 1 ml acetic anhydride. To this mixture was added 1 g of 1,7-dihydroxynaphthalene and this solution was heated to boiling for 30 min. After cooling, it was poured over ice to decompose the complex and to dissolve the zinc chloride. The dark tarry product was extracted with chrloroform, boiled with charcoal, filtered, and the filtrate evaporated. The residue was dissolved in sodium hydroxide, filtered, and acidified with dil HCl. The dirty yellow precipitate were filtered and dried, m.p. 183-186°. It was sublined at a temperature 100-110°/0.05 mm to yield 250 mg yellow needles, m.p. 2/4-2/5°, (20% yield).

Anal. Calcd. for $C_{12}H_{10}O_3$: C, 71.30; H, 4.94.

Found: C, 71.43; 71.53; H, 5.27, 5.39.

Method B. One gram of 1,7-dihydroxynaphthalene was dissolved in 5 ml glacial acetic acid. To this solution was added 6 ml of a solution of 45% boron trifluoride in ether. This solution was refluxed for 10 min. At that point the color of the solution was orange-green and the solvent was removed under vacuum. To the residue was added ice-cold water and the solution was left to stand for a few hours. The orange-red precipitate was filtered under suction, which on air drying, gave 1.3 g of reaction product. It was dissolved in iN sodium hydroxide

precipitated with dil hydrochloric acid, filtered under suction, and crystallized from ethanol, m.p. 214-215°. No depression in m.p. was observed on admixture with compound resulting from Method A. The product from above reaction produced one spot on a thin-layer plate of deactivated silica gel using chloroform as the solvent.

2. 2-Acetyl-1,5-dihydroxynaphthalene

Two gram of 1,5-dihydroxynaphthalene was taken up in 6 ml glacial acetic acid. To this solution was added 6 ml of a 45% solution of boron trifluoride in ether. After refluxing for 20 min the solution was concentrated under vacuum and the residue decomposed with crushed ice. The resulting solid was filtered under suction and dried (1.9 g). It was crystallized from methanol, yellow needles m.p. 266-267°. There was no depression in melting point on admixture with a sample of 2-acetyl-1,5-dihydroxynaphthalene prepared by the method of Spruit (34).

3. 2-Acetyl-1,8-dihydroxynaphthalene

One gram of 1,8-dihydroxynaphthalene-4-sulfonic acid was suspended in 4 ml glacial acetic acid. Gaseous boron trifluoride was passed through this solution until it became yellow while the temperature rose to 105°. This solution was heated at 90° for about 10 min. The solvent was removed under vacuum. The dark colored residue was taken up in methanol, boiled with powdered charcoal, and filtered. On concentration of the solution, yellow needles crystallized out, m.p. 95-96°, yield 200 mg (16%).

(Literature (35) m.p. 102-103°.)

4. 1-Hydroxy-5-methoxynaphthalene

Ten gram of 1,5-dihydroxynaphthalene was taken up in 110 ml 10 N sodium hydroxide and 20 ml water in a 3-necked flask, provided with a good mechanical stirrer, a reflux condenser, and a dropping funnel. Seventy-five milliliter of dimethyl sulfate was added slowly over about 40 min. The temperature of the flask was kept at 70° for about 30 min. Ten milliliter water and 10.0 ml 10N sodium hydroxide were added to the above solution to destroy excess dimethyl sulfate. The solution was allowed to cool. A brown insoluble residue was filtered and the filtrate was acidified with hydrochloric acid. The resulting light brown precipitate was filtered, crystallized from methanol, m.p./38° yield, 4.1 g). (Literature (44) m.p. 140°.)

5. 1-Hydroxy-2-acetyl-5-methoxynaphthalene

Method A. One gram of 1,5-dihydroxy-2-acetylnaphthalene was taken up in 1 ml 10 N sodium hydroxide and 2 ml water in a 25 ml 3-necked flask, fitted with a reflux condenser and a dropping funnel. A stream of nitrogen was passed through the flask during the reaction. Dimethyl sulfate (0.75 ml) was dropped slowly over 15 min and the flask was heated to 80-85° for about 15 min. Two milliliter of 5 N sodium hydroxide was added to the above solution to destroy excess dimethyl sulfate. The solution was diltered after cooling and the filtrate was acidified with ice-cold hydrochloric acid. The ensuing light yellow precipitate was

filtered and crystallized from 80% ethanol-water, yellow flakes m.p. 107-108°, yield 300 mg.

Anal. Calcd. for $C_{13}H_{12}O_3$: C, 72.22; H, 5.55

Found: C, 72.21; H, 5.59.

Method B. Five hundred milligram of 1-hydroxy-5-methoxy-naphthalene was taken up in 3 ml acetic anhydride. Through this solution boron trifluoride gas was passed until the solution turned greenish-yellow and the temperature rose to 90°. After heating for 5 min, the solution was concentrated under vacuum and ice-cold water was added to decompose excess acetic anhydride. After standing for one hour this solution was filtered under suction and it was crystallized from dilute methanol, yield 285 mg (71% yield) m.p. 107-108°. No depression in melting point was noted on admixture with a sample of the product from Method A.

Infrared absorption maxima and NMR data of substituted 2-acetylnaphthalene are given in Tables I and II.

TABLE I. INFRARED ABSORPTION MAXIMA OF SUBSTITUTED 2-ACETYLNAPHTHALENES IN POTASSIUM BROMIDE

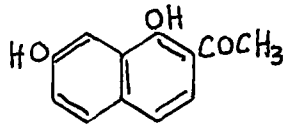
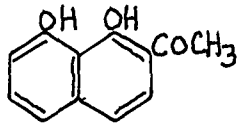
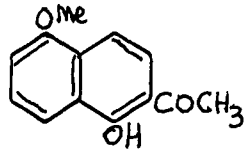
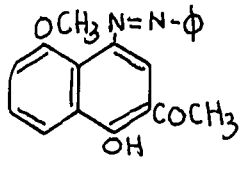
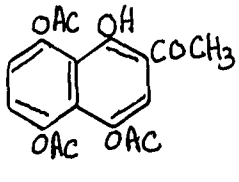
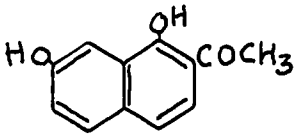
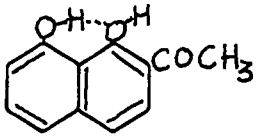
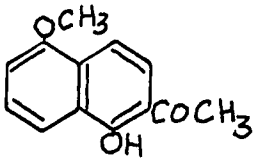
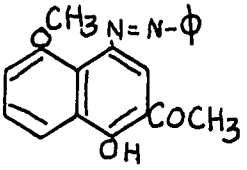
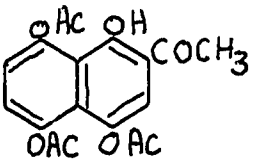
Compound	Absorption Maxima, μ
	3.05, 5.7, 6.22, 6.62, 6.85, 7.00 infl, 7.29, 7.52, 7.68, 8.00, 8.15, 8.23 sh, 8.4, 8.65, 9.29, 10.02, 10.65w, 11.22, 11.9, 12.01, 12.6, 12.80, 12.96, 13.80, 14.7
	2.98, 3.42w, 6.13, 6.3, 6.52, 6.73, 7.01, 7.3, 7.45, 7.6, 7.78w, 7.9, 8.05, 8.33, 8.6, 9.3, 9.6, 9.85 infl., 10.1 infl., 11.2w, 12.03, 13.00, 13.86, 14.4, 15.75w
	3.1w, 3.41w, 6.16, 6.27, 6.35 infl, 6.65, 6.85, 7.05, 7.19, 7.31, 7.51, 7.89, 8.1, 8.28, 8.41, 8.6w, 8.31, 9.3, 9.4, 9.8, 10.1, 10.52, 10.95, 11.25, 12.10, 12.23, 12.65, 13.28, 14.2, 14.49, 15.15
	6.15, 6.3w, 6.36w, 6.68, 6.86, 7.00, 7.20, 7.3 infl 7.52 infl, 7.95, 8.05 infl, 8.2, 8.45, 8.75, 9.09 9.45, 9.6, 9.75, 10.13, 10.7, 10.9, 11.00, 11.50, 11.9sh, 12.1, 12.38, 12.96, 13.4, 14.55, 14.95w
	5.69S, 6.13S, 6.3, 6.61, 7.00w, 7.19, 7.3S, 7.55w, 8, 8.2 to 8.35 broad band, 8.78, 8.94w, 9.6w, 9.83, 10.25w, 10.45w, 10.71S, 11.1S, 11.35, 11.6w, 11.9w, 12.1w, 12.4w, 12.91w, 13.55w, 14.11, 14.81w

TABLE II. NUCLEAR MAGNETIC RESONANCE DATA OF SUBSTITUTED 2-ACETYLNAPHTHALENES IN DEUTERIOCHLOROFORM

Compound	Signals, δ
	*C_1-OH, 13.98; C_2-$CoCH_3$, 2.72; C_3-H, 8.68 C_8-H 7.48; C_4-H, C_5-H and C_6-H 7.28-7.77; (Complex multiplet).
	C_1-OH 16.06; C_2-$CoCH_3$, 2.66; C_8-OH, 9.8; C_3-H, C_4-H, C_5-H, C_6-H and C_7-H, 6.8-7.6 (Complex multiplet).
	C_1-OH, 13.86; C_2-$CoCH_3$, 2.66; C_5-OMe, 3.99; C_3-H, C_4-H, C_6-H, C_7-H and C_8-H, 6.86-8.1 (Complex multiplet).
	C_1-OH, 14.00; C_2-$CoCH_3$, 2.68; C_5-OMe, 3.83; C_3-H, C_6-H, C_7-H, C_8-H and phenyl protons, 7.24-8.32 (Complex multiplet).
	C_1-OH, 14.56; C_2-$CoCH_3$, 2.58; C_3-H, 7.3; C_4-OAC, C_5-OAC, C_8-OAC, 2.36; C_6 and C_7-H, 7.15-7.20 (unresolved AB quartet).

*Taken in acetone-d_6.

C. COUPLING OF 1-NAPTHOLS WITH DIAZOTIZED ANILINE

1. 1-Hydroxy-2-acetyl-4-diazoanilino-5-methoxynaphthalene. -- Aniline (44 mg) was dissolved in 2.5 ml 2 N hydrochloric acid and diazotized by addition of 33 mg of sodium nitrite. To this solution was added with thorough mixing a solution of 100 mg of 1-hydroxy-2-acetyl-5-methoxynaphthalene in 15 ml of water, 2 ml 2 N sodium hydroxide, and 200 mg sodium carbonate. The solution was acidified with conc hydrochloric acid, cooled in ice, and filtered under suction. The carrot-red precipitate was crystallized from dil ethanol as long needles, m.p. 127-128°, yield 105 mg (67%).

Anal. Calcd. for $C_{18}H_{14}N_2O_3$: C, 71.24; H, 5.03; N, 8.75
Found: C, 71.14; H, 5.27; N, 8.54.

2. 1,5-Dihydroxy-2-acetyl-4-diazoanilinonaphthalene

This compound was prepared from 1 g 1,5-dihydroxynaphthalene by the above method. It was crystallized from methanol, red needles, m.p. 207-208°, 0.7 g (40% yield).

Anal. Calcd. for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15
Found: C, 70.06; H, 4.56; N, 8.85.

D. INTRODUCTION OF AN ACETYL GROUP INTO NAPHTHAZARINS

1. 2-Acetylnaphthazarin

a. 1,4,5,8-Tetraacetoxynaphthalene

Naphthazarin was prepared by the reaction of sulfur and fuming sulfuric acid with 1,5-dinitronaphthalene. When the product was purified on a column of deactivated silica gel and

crystallized from chloroform, it was found that naphthopurpurin was a previously undetected product of this reaction.

Method A. Five hundred milligram of naphthazarin was taken up in 6 ml acetic anhydride. To this was added 50 mg zinc dust or zinc powder and 20 mg potassium acetate in a 25 ml round bottom flask. The mixture was refluxed gently for 30 min until the red color had disappeared. The pale yellow solution was filtered while hot and the residue was washed with hot acetic acid. The solution of acetic acid and acetic anhydride was concentrated under reduced pressure. To the residue was added 10 ml water with cooling. The yellowish white precipitate was filtered under suction and crystallized twice from acetic acid, white needles, m.p. 280-281°. The yield of the crude product was 200 mg. (Literature (20) m.p. 278-279°.)

NMR spectrum in deuteriochloroform: δ 2.36 ($4CH_3$-CO_2-),
7.12 (4 aromatic H)

Method B. Two hundred milligram of naphthazarin was taken up in 10 ml acetic anhydride and 20 mg anhydrous potassium acetate. The solution was reduced with hydrogen at room temperature and atmospheric pressure using 10 mg of 10% palladium-on-charcoal, until 1 mole of hydrogen was absorbed and the red color was completely discharged. This took about 1.5 h. This solution was refluxed for 30 min under a stream of oxygen-free nitrogen. The acetic anhydride was removed under pump pressure after

filtering through a sintered funnel to remove the catalyst. Water was added to the residue and the mixture was cooled. The resulting white precipitate was filtered and dried, 210 mg (72%). It was crystallized from methanol or dil acetic acid, m.p. 280°. Mixture melting point with the compound prepared by Method A showed no depression. The n.m.r. spectra were identical.

b. 2-Acetyl-1-hydroxy-4,5,8-triacetoxynaphthalene

One hundred milligram of 1,2,5,8-tetraacetoxynaphthalene was dissolved in 5 ml acetic anhydride boron and trifluoride gas was passed through this solution until it was deep yellow and the temperature rose to 110°. The solution was allowed to cool and after 1 hr the excess acetic anhydride was removed under vacuum. Water was added to the residue and the resulting yellow precipitate was filtered under suction and dried, 90 mg (90%) yield). This material was dissolved in a minimum amount of chloroform and was placed on a short column of deactivated silica gel (pretreated with 0.5 N hydrochloric acid). The column was first eluted with benzene and a very fast moving orange-colored fraction was collected. This solution was evaporated and the residue, 5 mg, collected. This substance is further described in section 1 of part D. Neither benzene nor chloroform eluted additional material. The major band could be eluted with ethyl acetate. The solvent was evaporated and the residual material was sublined at 120°/0.05 mm. It was crystallized from ethyl acetate to furnish, light yellow needles, 50 mg (50%) of pure

substance, m.p. 209-210°.

Anal. Calcd. for $C_{18}H_{16}O_8$: C, 60.00; H, 4.44

Found: C, 59.98, 59.96; H, 4.78, 4.80.

c. Acetylnaphthazarin

Method A. Twenty-five milligram of 1-hydroxy-2-acetyl-4,5,8-triacetoxynaphthalene was dissolved in 5 ml 1 N sodium hydroxide solution under nitrogen. The reddish-orange solution was then exposed to air for a few min, which caused its color to turn green. This green solution was acidified immediately with ice-cold dil hydrochloric acid and immediately extracted with chloroform, which was dried over anhydrous sodium sulfate for a few hours. Chloroform was removed under vacuum at a water bath temperature of 35°. The mixture was dissolved in a minimum amount of benzene, placed on a column of deactivated silica gel and eluted with benzene; a purple band was collected and the benzene solution was evaporated under vacuum at room temperature. The compound thus obtained was crystallized from petroleum ether (60-90°), dark purple needles 5 mg, m.p. 106°.

Anal. Calcd. for $C_{12}H_8O_5$: C, 62.06; H, 3.44

Found: C, 61.48; H, 3.90.

Method B. Twenty-five milligram of 1-hydroxy-2-acetyl-4,5,8-triacetoxynaphthalene was dissolved in 5 ml methanol and 5 ml dil hydrochloric acid. The reaction mixture was heated at 70-75° for about 30 min and the solvent was removed under vacuum at a water bath temperature of 70-75°. The residue thus obtained was

chromatographed on deactivated silica gel (pretreated with 0.5 N hydrochloric acid) and eluted with benzene. The first band consisted of naphthazarin, (2.5 g), which was confirmed by comparison of melting points, infrared spectra, and R_f-values. The second band furnished acetylnaphthazarin (2 mg). This was followed by starting material (7 mg), which was eluted with ethyl acetate.

2. 2,6-diacetylnaphthazarin

a. 1,4,5,8-Tetrahydroxy-2,6-diacetylnaphthalene

One hundred milligram of 1,4,5,8-tetraacetoxynaphthalene was added to 6 ml acetic acid. Boron trifluoride gas was passed through it until the acetate had dissolved completely and the color of the solution changed to orange-red. About 5 ml of the solvent was removed under vacuum, and to the rest of the solution cold water was added. The orange-red precipitate was filtered under suction and dried, 50 mg (34% yield). The material was purified by chromatography and a yellow fluorescent band was collected. Benzene was removed and the orange precipitate was crystallized from 95% ethanol as orange needles, m.p. 255-256°. NMR spectrum of 1,4,5,8-tetrahydroxy-2,6-diacetylnaphthalene in deuteriochloroform:

C_2- and C_6-$COCH_3$, δ 2.70; C_3- and C_7-H, 7.2;

C_1- and C_5-OH, 13.10.

b. 2,6-Diacetylnaphthazarin

Lead tetraacetate was added slowly to a solution 5 mg

1,4,5,8-tetrahydroxy-2,6-diacetylnaphthalene in 5 ml benzene until the solution became light yellow. This solution was filtered and the filtrate was evaporated. Methanol was added to the residue and it was heated for 10 min. The concentrated solution was put on a column of deactivated acid-washed silica gel and eluted with chloroform. The purple solution was concentrated and chromatographed again, this time eluted with benzene. Benzene was removed and the residue was crystallized from methanol, purple needles, 2 mg, m.p. 250-251°. NMR spectrum in deuteriochloroform:

C_1 - and C_4 - OH, δ 12.57; C_2 - and C_6 - $COCH_3$, 2.60;

C_3 and C_7 - H, 7.08.

3. 3-Acetyl-2,6 and 7-hydroxynaphthazarins

a. Reductive acetylation of 2,5,8-triacetoxy-1,4-naphthoquinone

Method A. -- 2,5,8-Triacetoxy-1,4-naphthoquinone was prepared from naphthazarin by oxidation with lead tetraacetate followed by a Thiele reaction on the resulting diquinone, as described by Thomson (27).

Four hundred milligram of this compound was taken up in 6 ml acetic anhydride; zinc dust (100 mg) and 25 mg potassium acetate were added. The mixture was refluxed for 20 min under nitrogen. The solution was concentrated under vacuum and water was added. The aqueous solution was extracted with chloroform and the extract was decolorized with charcoal. Chloroform was removed under vacuum and the resulting white-yellowish precipitate was

crystallized from ethanol as light yellowish needles, m.p. 184-185°, 140 mg (34% yield).

Anal. Calcd. for $C_{18}H_{16}O_9$: C, 57.44; H, 4.51

Found: C, 57.01; H, 4.37.

NMR spectrum of 1-hydroxy-2,4,5,8-tetraacetoxynaphthalene in deuteriochloroform:

C_2-$OCOCH_3$, 2.3; C_4-, C_5, and C_8-$OCOCH_3$, 2.4;

C_6-, and C_7-H, 7.21; C_3-H, 7.35.

The infrared spectrum of 1-hydroxy-2,4,5,8-tetraacetoxynaphthalene showed the following maxima at 2.9 w, 5.69 s, 6.21, 7.00, 7.59 w, 8.4 s, 8.7, 8.59 w, 9.42, 9.58 sh, 9.85 s, 10.2, 11.03, 11.72 w, 13.92 w, 14.2 .

Method B. Fifty milligram of 2,5,8-triacetoxy-1,4-naphthoquinone was taken up in 10 ml acetic anhydride and 5 mg 10% palladium-on-charcoal was added to this solution. On hydrogenation at room temperature the color of the solution changed to light yellow from dark red. This solution was refluxed for 15 min under nitrogen. It was filtered through a sintered glass funnel. The filtrate was evaporated on a water bath under reduced pressure. To the residue water was added and the mixture was cooled. The white precipitate was filtered under suction and crystallized as white needles from an ethanol-water mixture, m.p. 179°. (Literature (20), m.p. 179°)

NMR spectrum of 1,2,4,5,8-pentaacetoxynaphthalene in deuteriochloroform:

C_3-H, 7.33; C_6-H, and C_7-H, 7.22; C_2-$OCOCH_3$, 2.30; C_1-$OCOCH_3$, C_4-$OCOCH_3$, C_5-$OCOCH_3$, and C_8-$OCOCH_3$, 2.4.

b. Reaction of 1,2,4,5,8-pentaacetoxynaphthalene with boron trifluoride-acetic anhydride

Fifty milligram of 1,2,4,5,8-pentaacetoxynaphthalene was taken up in 5 ml acetic anhydride. Boron trifluoride gas was passed through until the solution became dark yellow, and about 3 ml of solvent was removed under vacuum. Water was added to the rest of the solution and cooled. Sodium hydroxide (1N, 5 ml) was added to it and allowed to stand for 10 min. This solution was acidified with ice-cold hydrochloric acid and the solution was extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate for three hr. This solution was filtered under suction and concentrated to about 3 ml. It was applied to 0.5 mm thick-layer plates of 0.5 N hydrochloric acid-treated silica gel G. These plates were placed into chromatogram developing tanks containing chloroform as the developing solvent. It required three plates to spot the above solution. The three bands were extracted separately with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate. The solutions were filtered and evaporated under reduced pressure.

Band I was crystallized from isooctane, dark purple needles m.p. 160-161°. It was identical in all respects with 2-hydroxy-3-acetylnaphthazarin from a natural source (19).

Band II was confirmed to be the starting material. The third purple band was crystallized from isooctane, purple needles, (6 mg), m.p. 179-180°. Direct comparison of ultraviolet spectra, R_f-values, and infrared spectra of this compound with those of 2-hydroxy-6-acetylnaphthazarin from a natural source (19) confirmed the identity of the two compounds.

The fourth red band was crystallized from isooctane to give 5 mg of 2-hydroxy-7-acetylnaphthazarin.
NMR spectrum in deuteriochloroform: C_7-$COCH_3$, δ 2.70.

4. Spinochrome A

a. 2-Methoxynaphthazarin

A saturated solution of crude chloronaphthazarin (1 g) (21) in methanol was added very slowly to 1 of a saturated solution of sodium methoxide in methanol (from sodium) at reflux and under nitrogen. The mixture was refluxed for 2-3 d and then evaporated in vacuo. The residual solid was treated carefully with dil hydrochloric acid and the product was extracted into benzene. Chromatography on an 80 x 5 cm column of acid-treated, deactivated silica gel using benzene as the eluant separated the crude product into a small amount of naphthazarin and recovered chloronaphthazarin (red band), methoxynaphthazarin containing a small amount of naphthopurpurin, and chloromethoxynaphthazarins (orange band) and a small amount of 2,6-dimethoxynaphthazarin and 2,7-dimethoxynaphthazarin (red-orange band). The main orange band gave, after two crystallizations from absolute methanol, an

82% yield of 2-methoxynaphthazarin as black needles, m.p. 195-196°. (Literature (36), m.p. 178°.)

b. 2-Hydroxy-6 (and 7)-methoxynaphthazarins

A solution of 1 g of 2-methoxynaphthazarin in 1 l of benzene was shaken with 2.5 g of lead tetracetate. The yellow solution was filtered and evaporated in vacuo and the residue was treated with 25 ml of acetic anhydride and 2-3 ml of conc sulfuric acid. After standing for an hour the mixture was decomposed in ethanol and hydrochloric acid and the resulting solution was warmed to 50° for 5-10 min. The products were removed by benzene-ether extraction and chromatography on an 80 x 5 cm column of acid-treated deactivated silica gel achieved the following separation: an orange band (small amount of methoxynaphthazarin); an orange yellow band, 205 mg of 2-hydroxy-6-methoxynaphthazarin (20%), small brick-red needles from benzene-chloroform, m.p. 265-267°; and a red band, 600 mg of 2-hydroxy-7-methoxynaphthazarin (55%), small purple needles from chloroform m.p. 240-241°.

c. 1,2,4,5,8-Pentaacetoxy-7-methoxynaphthalene

The leucoacetate was prepared in 72% yield from 2-hydroxy-7-methoxynaphthazarin using method B above for the preparation of 1,4,5,8-tetraacetoxynaphthalene. The product crystallized from acetic acid as white needles m.p. 222-223°.

NMR spectrum in deuteriochloroform:

C_1-, C_2-, C_4-, C_5-, and C_8-$OCOCH_3$, δ 2.27, 2.36 and 2.38 (Singlets, relative intensities 1:3:1); C_3- and C_6-H, 7.00; C_7-OCH_3, 3.86.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 56.2; H, 4.5

Found: C, 56.1; H, 4.2.

Infrared spectra of 2-methoxy-1,4,5,7,8-pentaacetoxy naphthalene showed following maxima 5.67, 6.14 sh, 6.18, 6.6, 6.85, 7.10, 7.32, 7.9, 8.3-8.5 b, 8.65, 8.85 infl, 9.39, 9.81, 10.12, 10.68, 11.1, 11.61 w, 12.7 μ.

d. 2,7-Dihydroxy-3,6-diacetylnaphthazarin

A solution of 36 mg of 1,2,4,5,8-pentaacetyoxy-7-methoxy-naphthalene in 5 ml of acetic acid was treated with boron trifluoride gas until it had turned dark yellow. The solvent was removed in vacuo and the residual solid was treated with cold dil hydrochloric acid or sodium hydroxide solution followed by acidification with ice-cold hydrochloric acid. The product was extracted with chloroform, chromatographed on acid-treated, deactivated silica gel and crystallized from isooctane-chloroform to give 5 mg of 2,7-dihydroxy-3,6-diacetylnaphthazarin (20%) as purple needles m.p. 237-238°.

Anal. Calcd. for $C_{14}H_{10}O_8$: C, 54.9; H, 3.3

Found: C, 55.3; H, 4.1.

UV spectrum of 2,7-dihydroxy-3,6-diacetylnaphthazarin in methanol:

λ max: 542, 450, 308 mu; λ min: 495, 375, 255.5 m .

The IR spectrum showed following bands: 6.15 sh, 6.3, 6.4 infl, 7.02, 7.2, 7.55 w, 7.75 w, 8.2, 8.82 w, 9.2 w, 10.2, 11.12, 12.77.

e. Spinochrome A

A solution of 5 mg of 2,7-dihydroxy-3,6-diacetylnaphthazarin

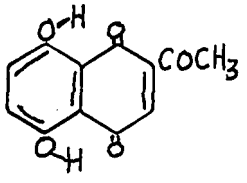
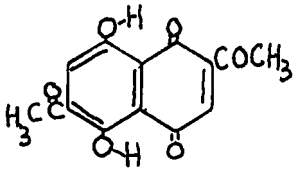
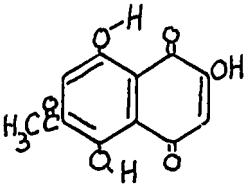
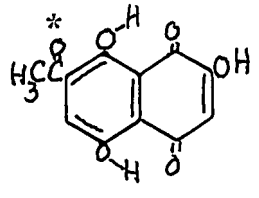
was dissolved in a minimum amount of methanol and about 10 ml of dil hydrochloric acid was added to it. This solution was boiled for a few min, while a portion of the solution was taken out after every two min, extracted with chloroform and spotted on a thin-layer plate. After the reaction had gone for about 10 min the solution was extracted with chloroform and chromatographed on a thin-layer plate of deactivated silica gel. Three products were isolated; the first band was starting material; the second band was that of spinochrome A (50%) as determined by its melting point, ultraviolet and infrared spectra, which were identical with those of the naturally occurring spinochrome A; the third band was confirmed to be 2,7-dihydroxynaphthazarin by its ultraviolet spectrum and R_f-value comparison with an authentic sample of 2,7-dihydroxynaphthazarin.

5. Spinochrome C

To produce spinochrome C, the leucoacetate of D (10 mg), m.p. 212-213° (Literature (13) m.p. 210°), was treated with boron trifluoride in acetic acid (2 ml). Most of the acetic acid was removed under vacuum. To the residue was added hydrochloric acid (1 ml) and dil ethanol (2 ml) and the mixture was heated at 80° for 20 min. The solvent was removed under reduced pressure. After chromatography, spinochrome C (5%), identical with the natural pigment, was obtained.

IR absorption maxima, UV and NMR data of acetylnaphthazarins are given in Tables III, IV and V, respectively.

TABLE III. INFRARED ABSORPTION MAXIMA OF ACETYLNAPHTHAZARINS IN POTASSIUM BROMIDE

Compound	Absorption Maxima, μ.
	3.3, 5.97S, 6.22S, 6.43S, 7.01S, 7.25w, 7.40, 7.50, 7.69w, 7.9sh, 8.02, 8.24S, 8.38S, 8.98, 9.01S, 10.32w, 10.6, 11.53S, 12.02, 12.3, 12.85, 13.25w, 14.75w, 15.18s.
	2.95w, 3.43w, 4.3w, 6.25s, 6.36infl, 6.87, 6.99, 7.1, 7.2sh, 7.6sh, 7.8, 8.12, 8.26, 8.5sh, 8.82, 9.1, 9.93, 10.51, 11.52, 12.3, 13w, 15vw.
	3.09, 3.25vw, 3.45w, 4.25w, 5.99, 6.05sh, 6.15sh, 6.21S, 6.42, 7.12, 7.2, 7.45, 7.6, 7.9, 8.35, 8.5, 9.25, 10.05, 10.49, 10.61, 11.25, 11.9, 12.23w, 13.75.
	3.1, 5.93, 6.05, 6.2, 6.3infl, 6.45vw, 6.82, 7.00 7.15, 7.25infl, 7.45, 7.65w, 7.9w, 8.3, 8.48, 9.2ws, 9.62, 10.1w, 10.75w, 11.2w, 11.12, 11.95w, 12.3w, 12.95w, 13.36w, 14.4w.

*Tentatively assigned structure.

TABLE IV. ULTRAVIOLET SPECTRAL DATA OF ACETYLNAPHTHAZARINS

2-Acetylnaphthazarin

In chloroform, λ max: 555sh mu, 530,508,271

λ min: 380-385,248.

2-Hydroxy-3-acetylnaphthazarin

In chloroform, λ max: 568sh,525sh,490,296,250sh

λ min: 375-400,272.

2-Hydroxy-6-acetylnaphthazarin

In chloroform, λ max: 511,295

λ min: 375.

2-Hydroxy-7-acetylnaphthazarin

In chloroform, λ max: 496,285.

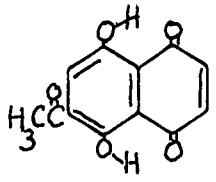
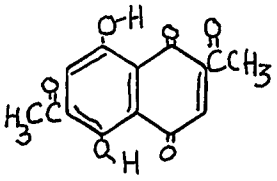
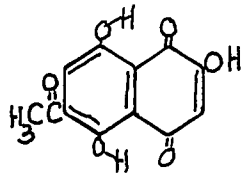
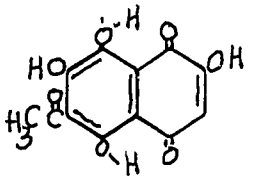
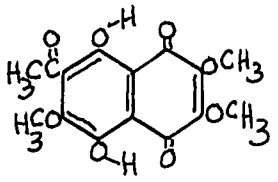
λ min: 362.

Spinochrome A

In chloroform, λ max: 557sh,530,507sh,319,253

λ min: 300,420-430.

TABLE V. NUCLEAR MAGNETIC RESONANCE DATA OF ACETYLNAPHTHAZARINS IN DEUTIRIOCHLOROFORM

Compound	Signals, δ
	C_2-H and C_3-H, 7.08; C_6-$COCH_3$, 2.75; C_5-OH 13.08; C_8-OH 12.15; C_7-H, 7.56.
	C_3-H and C_7-H, 7.14; C_2-$COCH_3$ and C_6-$COCH_3$ 2.62, C_5 and C_8-OH 12.57.
	C_3-H 6.43; C_7-H, 7.63; C_6-$COCH_3$ 2.73 (Hydroxyl signals not determined)
	* C_6-H 6.68; C_3-$COCH_3$ 2.85; (Hydroxyls signals not observed)
	C_2-OMe, 4.13; C_3-OMe 4.07; C_6-OMe 4.08; C_7-$COCH_3$ 2.51S; C_5-OH 12.82 or 12.72; C_8-OH 12.82 or 12.72.

*Determined in DMSO-d_6.

6. Attempted introduction of an acetyl group into naphthazarin

a. Bromoethylnaphthazarin

Ethylnaphthazarin (2 mmol) was placed in a 100 ml round-bottomed flask fitted with condenser and carbon tetrachloride was added until all of the naphthazarin was dissolved. Bromine (6 mmol) dissolved in carbon tetrachloride was added to the solution, followed by heating under reflux in presence of light. Heating was continued for 1 hr; the solvent was then evaporated and a-bromoethylnaphthazarin was separated by TLC on deactivated acid-washed silica gel in a benzene-carbontetrachloride-heptane (1:4:1) system. The product was crystallized from isooctane as dark brown-red platelets (45% yield), m.p. 138.5-139.5°.

NMR spectrum in deuteriochloroform: C_3-H, δ 7.28; C_6- and C_7-H, 7.2; C_2-CH, 5.46 (quartet), C_2-C-CH_3, 1.98 (doublet); C_5-OH, 12.2 or 12.44; C_8-OH, 12.2 or 12.44.

Anal. Calcd. for $C_{12}H_9O_4Br$: C, 48.48; H, 3.03; Br, 26.90

Found: C, 50.13; H, 3.17; Br, 24.64.

Absorption maxima in IR spectrum: 2.9, 6.23, 6.39, 6.90, 6.99, 7.09, 7.19 w, 7.42, 7.92, 8.1, 8.29, 8.45, 8.65, 8.95, 9.2 w, 9.29, 9.85 w, 8.92, 10.31, 10.60, 11.02, 11.22, 11.55, 12.35, 14.4 w, 14.75 w, 15.00 μ.

UV spectrum in chloroform: λ max, 570, 728, 496, 347, 281 mμ.

b. 2-(1-Bromoethyl)-3,6-dimethoxynaphthazarin

The title compound was prepared in 30% yield from 2-ethyl-3, 6-dimethoxynaphthazarin by the method given in section a. It

could not be crystallized. NMR spectrum in deuteriochloroform: C_7-H δ6.28; C_2-CH, 5.75 (quartet); C_2-C-CH_3, 2.08; C_3-OCH_3, 3.95 or 4.21; C_6 -OCH_3, 3.95 or 4.21; C_5-OH, 12.65 or 13.53; C_8-OH, 12.65 or 13.53.

c. 2-(1-Hydroxyethyl)-3,6,7-trimethoxynaphthazarin

2-Ethyl-3,6,7-trimethoxynaphthazarin (6 mg) was dissolved in carbon tetrachloride (25 ml) and refluxed for 15 min in bright light with bromine (2 mol). The solvent was removed under vacuum. To the residue was added 10 ml of a 1N KOH solution and the blue solution was heated on a water bath for 7 min. This solution was acidified with dil hydrochloric acid. This solution was extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was purified on thick-layer plates of deactivated silica gel, using chloroform as the solvent and it was crystallized as beautiful orange needles from isooctane m.p. 90°; yield 3 mg. NMR spectrum in deuteriochloroform: C_2-C-CH_3 δ 1.58 (doublet), C_3-OMe, 4.17; C_6-OMe, 4.2; C_7-OMe, 4.2; C_2-CH= 5.34 (quartet); C_5OH, 13.08 or 13.58; C_8-OH, 13.08 or 13.58.
UV spectrum in chloroform λ max: 532, 502, 479 sh, 320 mu.
IR absorption maxima: 2.9, 3.40, 6.25, 6.9, 7.02 w, 7.152, 7.75, 8.3, 8.65, 9.1, 10.09, 10.45 mμ.

E. POLYHYDROXY AND METHOXYNAPHTHAZARINS

1. 2,3-Dihydroxynaphthazarin

a. Gallacetophenone-3,4-dimethyl ether

To a boiling mixture of gallacetophenone (16.8 g), benzene (400 ml) and anhydrous potassium carbonate (55 g) was added dimethyl sulfate (26 g) in one portion and the mixture was refluxed for 14 hr with occasional shaking. After addition of water (750 ml) and shaking the benzene layer was separated and shaken with 4 x 100 ml portions of 10% sodium hydroxide solution, and the alkaline extracts were acidified with hydrochloric acid. The precipitated gallacetophenone-3,4-dimethyl ether was recrystallized from methyl alcohol to give 16 g of product, m.p. 75-77°. (Literature (12), m.p. 76°.)

b. 1,2-Dihydroxy-3,4-dimethoxybenzene (45)

Gallacetophenone 3,4-dimethylether (9.8 g) in 10% sodium hydroxide solution (40 ml) was oxidized by the addition of a 3% solution of hydrogen peroxide (75 ml) in a nitrogen atmosphere. Considerable rise of temperature occurred and the mixture darkened. After about 0.5 hr the solution was acidified, extracted with ether, the extract dried (sodium sulfate) and distilled; 1,2-dihydroxy-3,4-dimethoxybenzene was obtained as a pale yellow oil (5 g), b.p. 120°C/0.5 mm.

c. 1,2,3,4-Tetramethoxybenzene (46)

To a boiling mixture of 1,2-dihydroxy-3,4-dimethoxybenzene (5 g), benzene (200 ml) and anhydrous potassium carbonate

(20 g) was added dimethyl sulfate (10 g) in one portion, and the solution was refluxed for 10 hr with occasional shaking. After the addition of water (250 ml) and shaking, the benzene layer was separated and extracted with sodium hydroxide solution; evaporation of the dried benzene layer gave 1,2,3,4-tetramethoxybenzene, m.p. 80°.

d. 2,3-Dihydroxynaphthazarin

Method A. To a fused mixture of sodium chloride (20 g) and anhydrous aluminum chloride (100 g) at 180° was added a melted mixture of maleic anhydride (10 g) and 1,2,3,4-tetramethoxybenzene (20 g) with constant stirring. The reaction was conducted in a nitrogen atmosphere and maintained at 180° until the evolution of hydrogen chloride had subsided, when reaction mixture was allowed to cool. It was decomposed with dil hydrochloric acid. The solid material was filtered off, dried in vacuo, and extracted in a Soxhlet apparatus with chloroform. The chloroform extract was introduced onto a column of deactivated silica gel and the 2,3-dihydroxynaphthazaain was eluted with chloroform or, more conveniently, after removal of less polar impurities with chloroform, with 25% ethyl acetatechloroform. The yield was about 30% m.p. 265°.

Method B. 1,2-Dihydroxy-3,4-dimethoxybenzene (1.8 g) was condensed with maleic anhydride (1.0 g) as in the above method at a temperature of 200° to yield 40% of 2,3-dihydroxynaphthazarin.

e. 2,3-Dimethoxynaphthazarin

Careful addition of diazomethane to a solution of 2,3-dihydroxynaphthazarin in methanol gave a 90% yield of 2,3-dimethoxynaphthazarin m.p. 136-137°. (Literature (12) m.p. 133.5°.)

2. Spinochrome D

a. 1,2-Dihydroxy-3,4-dimethoxybenzene and chloromaleic anhydride

A 100 ml 3-necked flask was fitted with a mechanical stirrer, an air condenser having a nitrogen supply and a calcium chloride tube attached to it, and a dropping funnel. The flask was heated in a Wood's metal bath, and introduced into it was a finely powdered mixture of 52 g anhydrous aluminum chloride and 12 g sodium chloride. The bath was heated to 130°C and the stirrer was started. A mixture of 2 g 1,2-dihydroxy-3,4-dimethoxybenzene and 0.8 g chloromaleic anhydride was slowly released from the dropping funnel while the temperature was maintained at 180-190° for about six min until hydrochloric acid gas evolution stopped. The flask was cooled. To it was added 20 ml of ice-cold hydrochloric acid and the decomposed reaction mixture was allowed to stand for six hr. The solid was filtered, dried in vacuo and extracted with chloroform in a Soxhlet apparatus. The crude product on DSG plates showed three distinct spots on TLC plates. The crude mixture, 50% yield, was a 12:1 mixture of 2,3-dihydroxynaphthazarin, 2,3-dihydroxy-6-chloro-naphthazarin, and 2,3-dihydroxy-6,7-dichloronaphthazarin. The

crude mixture, about 25 mg, was dissolved in a minimum amount of methanol and to this was added slowly a diazomethane solution in ether. The reaction was followed on a thin-layer plate of deactivated silica gel until the starting material had completely disappeared. The methanol was evaporated and the residue was dissolved in chloroform and was applied to thick-layer plates of deactivated silica gel with benzene as the developing solvent. Three bands were removed from the plate, washed separately with chloroform, and the three bands were characterized.

Band 1 crystallized from methanol as brownish-yellow needles, m.p. 204-205°, 5 mg NMR spectrum of 2,3-dimethoxy-6,7-dichloronaphthazarin in deuteriochloroform: C_2-OCH_3 and C_3-OCH_3, δ 4.5; C_5-OH and C_8-OH, 13.04.

UV spectrum in chloroform: λ max: 525 sh, 515, 485, 482 sh, 325, 260 mμ.

Anal. Calcd. for $C_{12}H_8O_6Cl$: C, 45.17; H, 2.50;
Found: C, 45.34; H, 2.76.

Band 2 also crystallized from methanol as brownish plates 11 mg, m.p. 134-135°. NMR spectrum of 2,3-dimethoxy-6-chloronaphthazarin in deuteriochloroform: C_2-OCH_3, δ 3.83; C_3-OCH_3, 3.77; C_7-H, 7.17; C_5-OH, 12.43 or 16.05; C_8-OH, 12.43 or 16.05.

UV spectrum in chloroform: λ max: 535 sh, 498, 470, 305 mu.

Anal. Calcd. for $C_{12}H_9O_6Cl$: C, 50.61; H, 3.16.
Found: C, 50.24; H. 3.5.

Band 2 also crystallized from methanol as brownish plates 11 mg, m.p. 134-135°. NMR spectrum of 2,3-dimethoxy-6-chloronaphthazarin in deuteriochloroform: C_2-OCH_3, δ 3.83; C_3-OCH_3, 3.77; C_7-H, 7.17; C_5-OH, 12.43 or 16.05; C_8-OH, 12.43 or 16.05.

UV spectrum in chloroform: λmax: 535 sh, 498, 470, 305 mu.

Anal. Calcd. for $C_{12}H_9O_6Cl$: C, 50.61; H, 3.16.

Found: C, 50.24; H, 3.5.

Band 3 crystallized from methanol as green needles 6 mg, m.p. 136-137°. It was identical in all respects with an authentic sample of 2,3-dimethoxynaphthazarin. Literature, m.p. 133.5° (12).

b. 2,3-Dimethoxy-6-chloronaphthazarin and sodium methoxide

A concentrated solution (300 ml) of sodium methoxide in methanol was prepared from sodium. Nitrogen gas was passed for 2 hr. 2,3-Dimethoxy-6-chloronaphthazarin was dissolved in methanol and dropped slowly into the above solution. This solution was refluxed for 2 d under nitrogen. The color of the solution had changed from dark blue to violet. Methanol was removed under vacuum. The residue was acidified with ice-cold hydrochloric acid and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and chloroform was removed under vacuum. The residue was dissolved in a minimum amount of benzene. It was chromatographed on deactivated acid-washed silica gel and the following fractions were collected

using benzene as the eluant: an orange red band (Fraction 1), red band (Fraction 2) and brick-red band (Fraction 3). Fraction 1 was confirmed to be 2,3-dimethoxy-6-chloronaphthazarin (5 mg). Fraction 2 crystallized from methanol as brown needles, m.p. 161-162°. It was confirmed to be 2,3,6-trimethoxynaphthazarin through mixture melting point, NMR and IR and UV spectra comparison with an authentic sample of the trimethyl ether of spinochrome D. Fraction 3 crystallized from methanol, m.p. UV spectrum of 6-hydroxy-2,3-dimethoxynaphthazarin in chloroform: λ max: 530, 492, 465, 325 mμ. It was converted to spinochrome D trimethyl ether on treatment with diazomethane in methanol.

c. Spinochrome D

2,3,6-Trimethoxynaphthazarin (10 mg) was suspended in 10 ml 48% hydrobromic acid. This was refluxed for 1 hr under nitrogen. The solution was cooled in an ice bath and filtered. The residue (4 mg) was sublimed at 150° under vacuum. The pure product m.p. 280-290° (sublines without melting) was identical in all respects with an authentic sample of spinochrome D.

3. Spinochrome E

a. 2,3-Dihydroxy-3,4-dimethoxybenzene was condensed with dichloromaleic anhydride in aluminum chloride-sodium chloride to give a 75% yield of 2,3-dihydroxy-6,7-dichloronaphthazarin, m.p. 256-257°.

UV spectrum in chloroform: λ max: 515, 485, 462 sh, 325, 260 mμ.

It was converted quantitatively to 2,3-dimethoxy-6,7-dichloronaphthazarin on treatment with diazomethane in methanol.

b. 2,3-Dimethoxy-6,7-dichloronaphthazarin (80 mg) was dissolved in methanol and dropped into a solution of sodium methoxide in methanol. It was refluxed for 2 d under nitrogen. Solvent was removed in vacuo and the residue acidified with ice-cold hydrochloric acid and extracted with chloroform. The chloroform extract was chromatographed on deactivated acid-washed silica gel and the following bands were identified.

Band 1 crystallized from methanol, light brown needles, m.p. 140-141°.

NMR spectrum in deuteriochloroform: δ C_2-OCH_3, 4.11 or 4.15; C_3-OCH_3, 4.11 or 4.15; C_6-OCH_3, 4.18; C_5-OH, 12.58 or 12.84; C_8-OH, 12.58 or 12.84

Anal. Calcd. for $C_{13}H_{11}O_7Cl$: C, 49.60; H, 3.49.

Found: C, 49.33; H, 3.98.

UV spectrum of 2,3,6-trimethoxy-7-chloronaphthazarin in chloroform: λ max: 530 sh, 500, 478, 324, 240 mμ.

Band 2 crystallized from petroleum ether, orange red square needles m.p. 210-211°.

NMR spectrum in deuteriochloroform: C_2-OCH_3, δ 4.10 or 4.16; C_3-OCH_3, 4.10 or 4.16; C_5-OH, 12.16 or 13.2; C_8-OH, 12.16 or 13.20.

UV sprectrum of 2,3-dimethoxy-6-chloro-7-hydroxynaphthazarin in chloroform: λ max: 535, 500, 475, 335, 241 mμ.

Anal. Calcd. for $C_{12}H_9O_7Cl$: C, 48.00; H, 3.00.

Found: C, 47.9; H, 3.03.

The structure of this compound was further confirmed through its conversion to 2,3,6-trimethoxy-7-chloronaphthazarin by treating it with diazomethane in methanol.

Band 3 also crystallized from chloroform as garnet red needles, m.p. 134-135°.

NMR spectrum of 2,3,6-trimethoxy-7-hydroxynaphthazarin in deuteriochloroform: C_2-OCH_3, δ 4.04 or 4.08; C_3-OCH_3, 4.04 or 4.08; C_6-OCH_3, 4.16; C_7-OH, 6.90 (broad) C_5-OH, 12.16 or 13.30; C_8-OH, 12.16 or 13.30.

UV spectrum in chloroform: λ max: 522, 490, 460, 333.

The structure of this compound was further confirmed by the fact that on treatment with diazomethane it was converted to the tetramethyl ether of spinochrome E, m.p. 185-186°. Literature m.p.

c. 2,3,6,7-Tetrahydroxynaphthazarin

2,3,6-Trimethoxy-7-hydroxynaphthazarin (6 mg) was refluxed in 48% aqueous hydrobromic acid for 2 hr. It was cooled and the precipitates were filtered and dried, dark red needles, m.p. 300-320° (subl). Literature m.p.

UV and IR spectra and R_f-value were identical with those of natural spinochrome E.

F. 2,3-DICHLORONAPHTHAZARIN AND SODIUM METHOXIDE

1. 2,3-Dichloronaphthazarin

To a fused mixture of sodium chloride (20 g) and anhydrous aluminum chloride (100 g) at 180° was added a mixture of 2,3-dichloromaleic anhydride (18 g) and hydroquinone (10 g) with constant stirring. The temperature was kept at 180° until evolution of hydrogen chloride had ceased and the melt was then allowed to cool. After decomposition with dil hydrochloric acid the red precipitate of the quinone was collected, dried, and extracted with chloroform in a Soxhlet extractor. Solvent was evaporated and the residue crystallized from petroleum ether or isooctane in scarlet plates, m.p. 199-202°, (65%). Literature m.p. 192° (37).

NMR in deuteriochloroform: C_6-H δ 7.30; C_7-H, 7.30; C_5OH 12.2; C_8-OH, 12.2

UV spectrum in chloroform: λ max: 5.69 mμ (, 49150), 528 (7540), 494 (6270), 359 (680), 294 (8880).

The impurities present in the crude 2,3-dichloronaphthazarin were shown to be monochloronaphthazarin, which traveled more slowly and trichloronaphthazarin which traveled faster than 2,3-dichloronaphthazarin. 2,3-Dichloronaphthazarin (500 mg) was dissolved in acetic anhydride (5 ml) and to this solution were added 2 drops of conc sulfuric acid; the red color changed to yellow; the solution was treated with ice-cold water and the precipitate was filtered with suction and crystallized from acetone, yellow needles, m.p. 236°. Literature m.p. 233° (37).

NMR spectrum of 5,8-diactoxy-2,3-dichloro-1,4-naphthoquinone C_5-OAC, δ 2.46; C_8- OAC, 2.46; C_6-H, 7.38; C_7-H, 7.38.

2. Reaction of 2,3-dichloronaphthazarin with sodium methoxide in methanol.

A 1 l 3-necked flask was fitted with a mechanical stirrer and a reflux condenser fitted with a calcium chloride tube. A conc solution (500 ml) of sodium methoxide was prepared from sodium and methanol. To this solution was added very slowly a solution of crude 2,3-dichloronaphthazarin (50 mg) in methanol over a period of 3 hr, while the solution was refluxed under nitrogen and continually stirred.

After the addition of 2,3-dichloronaphthazarin solution the whole solution was refluxed under nitrogen for 2 to 3 d. The methanol was evaporated under reduced pressure and the remaining bluish solid was neutralized with ice-cold conc hydrochloric acid. The solution was extracted with chloroform and dried over anhydrous sodium sulfate. The chloroform was stripped and the residue was dissolved in a minimum amount of a chloroform-benzene mixture. This solution was placed on top of a column of DSG and eluted with benzene. Various bands separated, which in turn were further separated by thick-layer chromatography. Sixteen bands were observed and they are listed in order of elution in Table VI.

Bands 1 and 2 were confirmed to be 2,3-dichloronaphthazarin and 2-chloronaphthazarin, respectively.

Band 3 crystallized from isooctane, yellow needles, m.p. 155-157°.

NMR spectrum of 2-methoxy-3-chloronaphthazarin in deuteriochloroform:

TABLE VI. PRODUCTS OF NUCLEOPHILIC REACTION OF METHOXIDE WITH CRUDE 2,3-DICHLORONAPHTHAZARIN IN ORDER OF SEPARATION

Compound	Relative R_f	Approx. yield, %
2,3-Dichloronaphthazarin (starting material)		8
2-Chloronaphthazarin		3
2-Methoxy -3-chloronaphthazarin		8
Naphthazarin	1.000	3
2,3-Dimethoxynaphthazarin	0.577	5
2-Methoxynaphthazarin	0.415	8
Naphthopurpurin	0.412	3
2-Hydroxy-3-methoxynaphthazarin	0.308	5
2,3,6-Trimethoxynaphthazarin	0.228	7
2,6-Dimethoxynaphthazarin	0.217	10
2,7-Dimethoxynaphthazarin	0.257	10
2-Hydroxy-3-chloro-6-methoxynaphthazarin		7
2-Hydroxy-3-chloro 7-methoxynaphthazarin		3
2-Hydroxy-3,6-dimethoxynaphthazarin		4
2-Hydroxy-3,7-dimethoxynaphthazarin		6
Unknown		2

C_6- and C_7-H, δ 7.26 (singlet); C_2-OCH_3, 4.32; C_5-OH, 12.10 or 12.28; C_8-OH, 12.10 or 12.28.

Anal. Calcd. for $C_{11}H_7O_5Cl$: C, 51.97; H, 2.83; Cl, 14.17.

Found: C, 51.91; H, 2.91; Cl, 15.35.

UV spectrum of 2-methoxy-3-chloronaphthazarin in chloroform:

λ max: 553, 512, 479, 297, 240 mu.

λ min: 540, 490, 340, 265.

Principal IR bands: 6.2, 6.34, 6.91, 7.1, 7.38 w, 7.53, 7.95, 8.13, 8.4, 8.6 w, 8.78, 9.2, 10.2, 10.95, 11.92, 12.27, 12.85, 14.1 μ.

Band 7 also crystallized from isooctane as red needled, m.p. 222-224°, subl with decomposition. Literature m.p. 183-187° (27). (Subl with decomposition.)

NMR in deuteriochloroform: C_6-H, and C_7-H, δ 7.18 (Singlet); C_5-OH, 11.32 or 12.32, C_8-OH, 11.32 or 12.32.

UV spectrum of 2-hydroxy-3-chloronaphthazarin: λ max: 542, 530 sh, 505, 474, 298, 237 mμ. λ min: 536, 480, 333, 263 mu.

Absorption maxima in IR spectrum: 3.15 w, 6.23, 6.37 infl, 6.91, 7.1 w, 7.26, 7.45, 7.8, 8.1, 8.47, 8.72, 9.27, 10.4, 11.8, 12.05, 12.05, 12.32 w, 13.05, 12.7 b, 14.13 μ.

Band 9 was crystallized from methanol as dark red micro crystals, m.p. 181-182° (dec).

UV spectrum of 2-hydroxy-3-methoxynaphthazarin in chloroform:

λ max: 522, 490, 462, 293, 245 mμ. min: 519, 466, 350, 275 mμ.

Absorption maxima in IR spectrum: 3.1 w, 6.23, 6.35 sh, 6.9, 7.75, 8.00, 8.65, 9.2, 9.35 w, 10.29, 10.54 w, 11.35, 11.9, 12.95,

14.05 u.

Band 13 crystallized from chloroform as orange plates.

UV spectrum in chloroform: λ max: 532, 494, 464, 328 mμ. λ min: 512, 472, 370, 272 mμ.

Absorption maxima in IR spectrum of 2-hydroxy-3-chloro-6-methoxy-naphthazarin: 3.09, 6.30, 6.87, 6.98, 7.10 w, 7.50 w, 7.8, 8.27, 8.5 infl, 9.42, 10.19, 10.7 u, 11.55, 12.80, 14.90 w, μ.

2-Hydroxy-3-chloro-6-methoxynaphthazarin was converted to 2-acetoxy-3-chloro-6-methoxynaphthazarin by reaction with ketene in benzene and it crystallized from isooctane, orange needles, m.p. 160-161°.

NMR spectrum of 2-acetoxy-3-chloro-6-methoxynaphthazarin in deuteriochloroform: C_2-$OCOCH_3$, δ 2.47; C_6-OCH_3, 4.02; C_7-H, 6.35; C_5-OH, 12.64; C_8-OH, 12.6

UV spectrum in chloroform: λ max: 560 sh, 515 sh, 490, 303 mμ; λ min: 350, 265 mμ.

Band 14 crystallized from isooctane, red needles, m.p. 217-221° (dec).

NMR spectrum of 2-hydroxy-3-chloro-7-methoxynaphthazarin in deuteriochloroform: C_6-OCH_3, δ 4.03.

UV spectrum in chloroform: λ max: 550, 512, 491 sh, 485 sh, 318 mμ. λ min: 541, 362 mu.

2-Hydroxy-3-chloro-7-methoxynaphthazarin was converted to 2-acetoxy-3-chloro-7-methoxynaphthazarin by reaction with ketene in benzene. It crystallized from isooctane as orange micro crystals.

NMR spectrum in deuteriochloroform: C_2-$OCOCH_3$, 2.47; C_7-OCH_3, 4.03.

UV spectrum in chloroform: λ max: 570 sh, 525 sh, 486, 300 mu.

Band 15 was crystallized from isooctane as orange needles, m.p. 182-183°.

UV spectrum in chloroform: λ max: 522, 488, 463, 320 mu. λ min: 510, 465, 365 mu.

Band 16 also crystallized from isooctane as orange needles, m.p. 204-205° (dec).

UV spectrum of 2-hydroxy-3,7-dimethoxynaphthazarin in chloroform: λ max: 525, 488, 468, 320 mu. λ min: 515, 470, 370 mu.

Bands 15 and 16 were converted to 2,3,6-trimethoxynaphthazarin on treatment with diazomethane in methanol.

NMR data of methoxynaphthazarins and hydroxy-methoxynaphthazarins are given in Tables VII and VIII.

G. GENERAL METHOD FOR THE PREPARATION, ISOLATION AND PURIFICATION OF ACETOXY NAPHTHAZARINS.

Hydroxy naphthazarin (20-25 mg) was taken up in benzene (15 ml) and the solution was heated if necessary. Into this solution was passed ketene for about 5 min. This solution was spotted during this period on a plate of deactivated silica gel in a chloroform system. The solution was allowed to stand until no starting material was left. The color of the compounds was usually red to orange on the plate, but in all cases studied there was always noticed a yellow spot immediately after the main product, which could be hydrolyzed to the starting material.

TABLE VII. NUCLEAR MAGNETIC RESONANCE DATA OF METHOXYNAPHTHAZARINS IN DEUTERIOCHLOROFORM

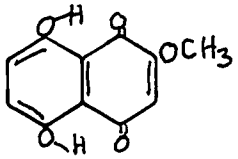
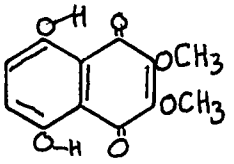
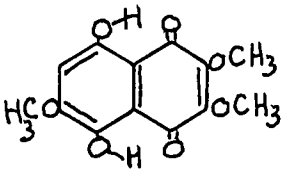
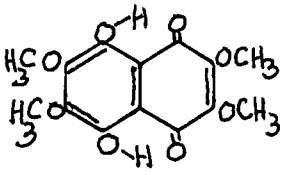
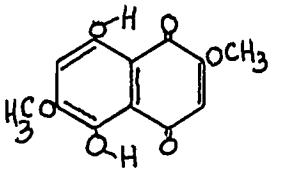
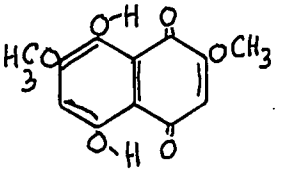
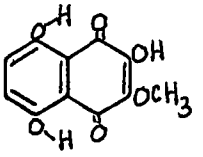
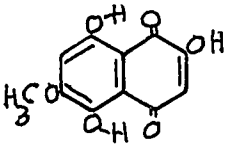
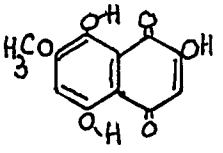
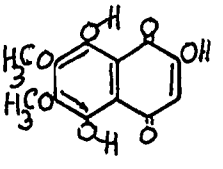
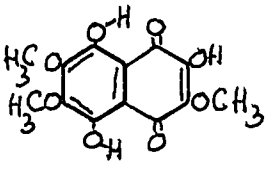
Compound	Signals, δ
	C_3-H, 6.17; C_6-H and C_7-H, 7.23s C_2-OMe, 3.92; C_5-OH, 12.63; C_8-OH, 12.17.
	C_2-OMe and C_3-OMe 4.12s; C_6-H and C_7-H 7.18; C_5-OH, 12.32; C_8-OH, 2.32.
	C_7-H, 6.41; C_2-OMe, 4.14; C_3- OMe; 4.06; C_6-OMe, 3.93; C_5-OH, 12.95 or 13.00; C_8-OH, 12.95 or 13.00.
	C_2-OMe, C_3-OMe, C_6-OMe and C_7-OMe 4.10; C_5-OH and C_8-OH 12.68.
	C_3-Hand C_7-H 6.36 s; C_2-OMe and C_6-OMe 3.93 s C_5-OH and C_8-OH, 13.07 s.
	C_3-H and C_6-H 6.40; C_2-OMe and C_7-OMe 3.94; C_5-OH 13.12; C_8-OH 12.70.

TABLE VIII. NUCLEAR MAGNETIC RESONANCE DATA OF HYDROXY-METHOXYNAPHTHAZARINS IN DEUTERIOCHLOROFORM

Compound	Signals, δ
	C_6-H 7.24d; C_7-H 7.20d; C_3-OMe 4.22; C_5-OH 12.40; C_8-OH 11.55.
	C_3-H 6.42; C_7-H 6.48; C_6-OMe 3.98; C_5-OH 13.29; C_8-OH 12.33.
	C_3-H 6.47; C_6-H 6.53; C_7-OMe 3.97; C_5-OH 13.13; C_8-OH 12.07.
	C_7-H, 6.50; C -OMe 4.16; C_6-OMe 3.93; C_5-OH 12.95 or 13.00; C_8-OH 12.95 or 13.00.
	C_2-OH 6.90 (broad); C_3-OMe 4.16; C_6-OMe 4.08; C_7-OMe 4.04; C_5-OH 13.30; C_8-OH 12.16.

Benzene was evaporated and the product was purified on thick-layer plates, using benzene or chloroform. However, a first purification in benzene and a second in chloroform was found to be very satisfactory. For all these compounds, isooctane was found to be the most satisfactory solvent for crystallization. The data for these compounds are given in Tables IX, X, and XI.

TABLE IX. MELTING POINTS AND INFRARED ABSORPTION MAXIMA OF ACETOXYNAPHTHAZARINS IN POTASSIUM BROMIDE

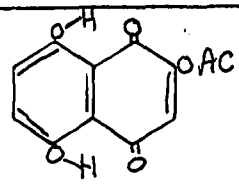
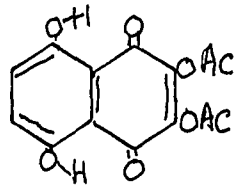
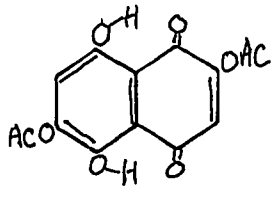
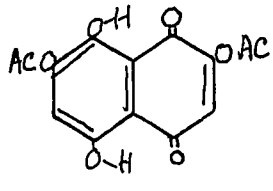
Compound	Melting Point	Absorption Maxima, μ
	132-133°	5.65, 6.02sh, 6.2, 6.35sh, 6.92, 7.09, 7.3, 7.5, 7.85, 8.18, 8.45, 8.65sh, 9.0, 9.54, 9.90, 10.55w, 10.8w, 10.9w, 11.68, 12.33, 14.7, 15.5w.
	158-160°	3.00w, 5.61, 6.04sh, 6.15, 6.35, 6.49sh, 6.9, 7.28, 7.5, 7.85, 8.12w, 8.4, 8.65, 9.25, 9.36, 9.5sh, 9.9, 10.28, 10.42w, 11.02, 11.41w, 11.6w, 12.35, 13.00, 14.0.
	161-162°	5.63, 6.05sh, 6.18, 6.32w, 6.52, 7.08w, 7.3, 7.82, 8.5b, 9.15w, 9.31, 9.55w, 9.88, 10.55, 10.93, 11.30w, 12.352, 12.852.
	166-167°	5.62, 6.19, 6.32w, 6.4 infl, 6.95, 7.08, 7.3, 7.49, 7.82, 8.6b, 9.32, 9.55, 9.88, 10.55, 10.95, 11.3, 12.35, 12.8, 13.2b, 15.1w, 15.55w.

TABLE X. MELTING POINTS AND INFRARED ABSORPTION MAXIMA OF THE ACETOXYNAPHTHAZARINS IN POTASSIUM BROMIDE

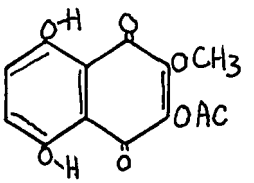
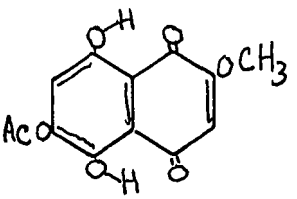
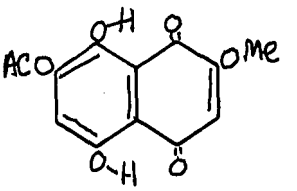
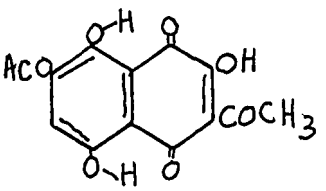
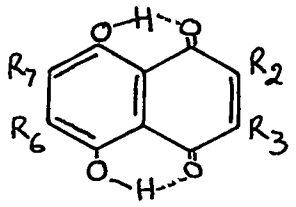
Compound	Melting Points	Absorption Maxima, μ
		3.42, 3.51, 5.65, 6.25, 6.35sh, 6.82sh, 6.9, 7.1, 7.25, 7.82, 8.00, 8.40, 7.62, 9.1, 9.32, 10.28, 10.62, 11.25, 11.75w, 11.98w, 12.60, 14.00, 14.60.
	178-179°	3.3w, 3.42w, 5.60, 5.70, 6.29, 6.92, 7.09, 7.3, 7.6, 7.85, 8.3b, 8.72, 9.62, 9.87, 10.37w, 10.8w, 10.95w, 11.3, 11.52, 12.2, 12.65, 13.65, 14.00, 14.6, 15.85w.
		5.65, 6.05sh, 6.2, 6.35sh, 6.91, 7.08, 7.3, 7.45w, 7.85, 8.03w, 8.36, 8.60, 9.02, 9.22, 9.52, 9.89, 10.51, 10.9, 11.4w, 11.7, 12.28w, 12.8, 13.54, 14.18
	185-189° (dec)	3.42, 3.50, 5.68, 6.18, 6.35w, 6.9, 7.1, 7.3, 7.52, 7.82, 8.35, 8.6, 8.9, 9.12, 9.82, 10.37, 10.85, 11.6w, 11.85w, 12.25, 12.85, 13.1w, 13.4, 13.99, 14.66w.

TABLE XI. NMR DATA OF ACETOXYNAPHTHAZARINS IN DEUTERIOCHLOROFORM



Substituents				Chemical shift of Ri				
R_2	R_3	R_6	R_7	H	O CH_3	$OCOCH_3$	C_5-OH	C_8-OH
O AC	H	H	H	6.85(3)[a] 7.25s(6.7)		2.39	12.33	12.07
O AC	O AC	H	H	7.28		2.42	12.08	12.08
O AC	H	O AH	H	6.93		2.38	12.28	12.28
O AC	H	H	O AC	6.98		2.40	13.12	12.70
O Me	H	O AC	H	6.17(3) 7.02(7)	3.96	2.37	12.82	12.25
O Me	H	H	O AC	6.22(3) 7.09(6)	3.94	2.37	12.65	12.23

[a]Number in parenthesis refers to position i of substituent Ri.

CHAPTER III

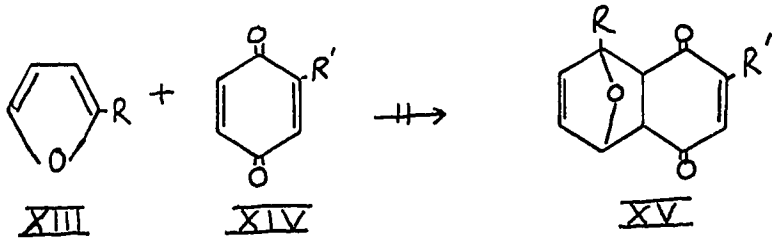
RESULTS AND DISCUSSION

A. Synthetic Approaches

The twofold aim of this research, to explore new approaches to the synthesis of naphthazarin derivatives and to prove the structure of naturally occuring naphthazarins by synthesis, received unequal emphasis. While the exploratory work was proceeding, degradative work in our laboratory on several naturally occuring spinochromes was culminating in the elucidation of a number of structure which had long been controversial. It seemed therefore most fruitful to place major emphasis on the synthetic structure proof of these spinochromes.

1. Diene Syntheses

Construction of a naphthazarin skeleton by a Diels-Alder synthesis appears attractive at first sight, but becomes less so in the light of previous failures (29) to effect a reaction between benzoquinone and furan. We confirmed this lack of reactivity, but felt that further investigation was warranted. In particular, we believed that the probability of reaction might be enhanced if the diene-furan-was rendered more electron-rich and the dienophile-p-benzoquinone-more electron-poor. However, under a variety of conditions, 2-acetoxyfuran and 2-acetyl-p-benzoquinone failed to react. These reactions are shown in Scheme I.

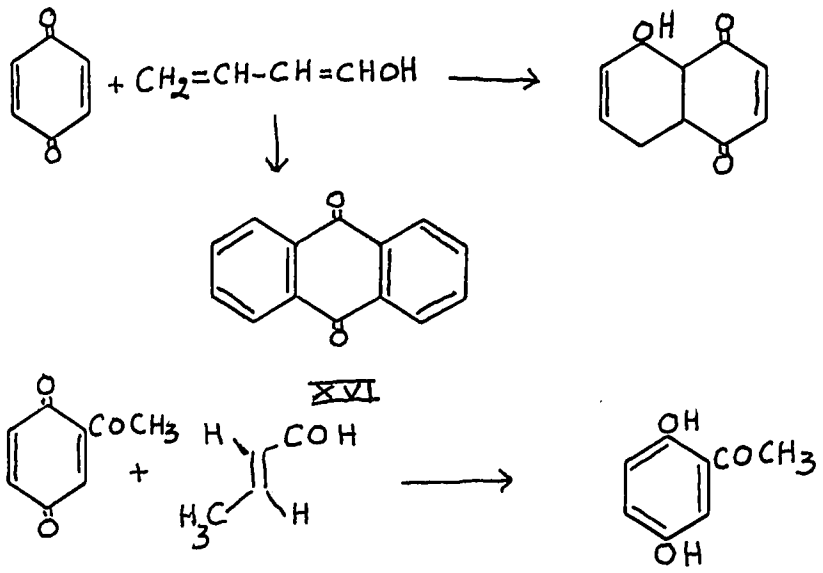


XIII XIV XV

R = $OCOCH_3$	R = H
R = H	R = H
R = $OCOCH_3$	R = $COCH_3$

SCHEME I

In another variation, the reaction of p-benzoquinone and crotonaldehyde in the presence of piperidine led only to anthraquinone XVI, while crotonaldehyde and 2-acetyl-p-benzoquinone yielded 2,5-dihydroxyacetophenone. The reactions are summarized in Scheme II.

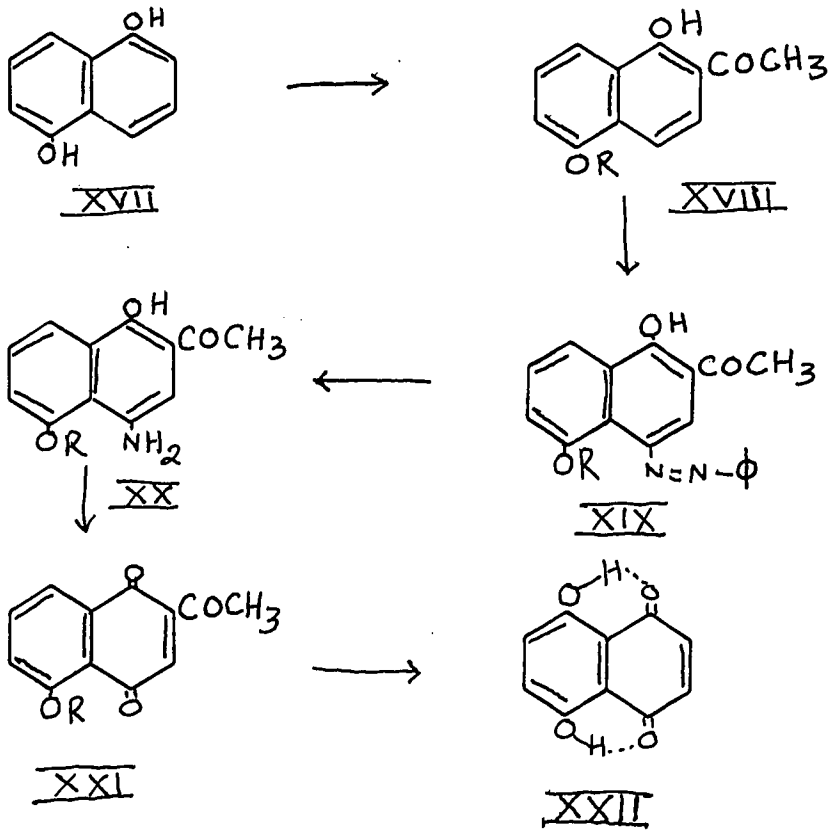


XVI

SCHEME II

2. Introduction of an acetyl side chain in naphthalene derivatives

Research progress in our laboratory at that time had demonstrated that spinochrome A(V) and C(VII) have an acetyl side chain. This feature would pose a particularly difficult synthetic problem. It was therefore held desirable to study this problem on some model systems. The first pathway under consideration involved a synthesis of an actyljuglone derivative, which then might be oxidized to the corresponding naphthazarin. This sequence is outlined in Scheme III.



SCHEME III

This scheme required the preparation of 1,5-dihydroxy-2-acetylnaphthalene (XVIII, R = H) as the starting material from 1,5-dihydroxynaphthalene by the method of Spruit (34). We found that boron trifluoride and acetic anhydride gave higher yields of this compound. Furthermore, this method was found to be of general applicability for the syntheses of dihydroxyacetylnaphthalenes; e.g. the reaction of 1,2-dihydroxynaphthalene with boron trifluoride and acetic anhydride gave a percent yield of 1,7-dihydroxy-2-acetylnaphthalene. Similar reaction with 1,8-dihydroxynaphthalenesulfonic acid yielded 1,8-dihydroxy-2-acetyl-naphthalene. The acetyl group is probably introduced through a Fries rearrangement, since 1,4-diacetoxybenzene was isolated when boron trifluoride was passed through a suspension of 1,4-dihydroxy-benzene and the solution heated for only five min.

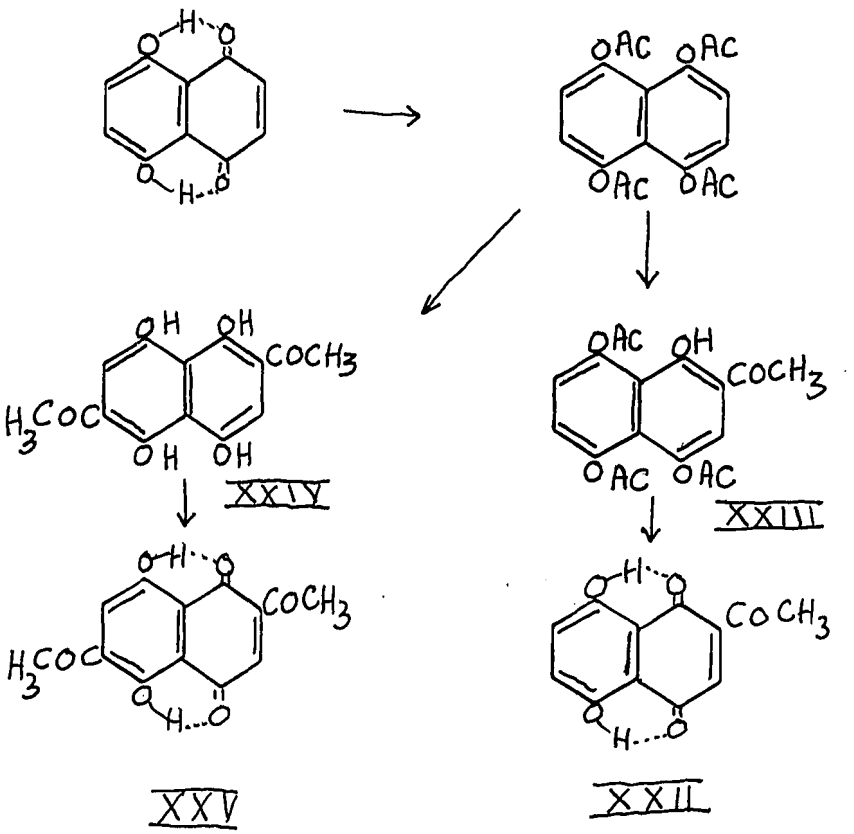
3. Coupling of 1-naphthols with diazotized aniline

The next step in Scheme III was the introduction of an amino group in position 4 of naphthalene to make it easily oxidizable to naphthoquinone. 1,5-Dihydroxy-2-acetylnaphthalene in sodium hydroxide solution reacted with diazotized aniline to yield a 40 percent yield of 1,5-dihydroxy-2-acetyl-4-diazoanilinonaphthalene (XIX, R = H) m.p. 207-208°. Similarly, 1-hydroxy-5-methoxy-2-acetylnaphthalene yielded 1-hydroxy-5-methoxy-2-acetyl-4-diazoanilinonaphthalene (XIX, R = CH_3). This scheme was not pursued further beyond the formation of diazo compounds because another scheme outlined below in section 4 was successful for the

synthesis of acetylnaphthazarin.

4. Introduction of an acetyl group into naphthazarins

With a good method available for introducing an acetyl side chain into dihydroxynaphthalenes it was considered desirable to extend this method to 1,4,5,8-tetrahydroxynaphthalene. But the instability of this compound and the lack of reactivity of naphthazarin towards boron trifluorideacetic anhydride led to the formulation of another successful sequence of reactions for the synthesis of acetylnaphthazarin, which is outlined in Scheme IV.



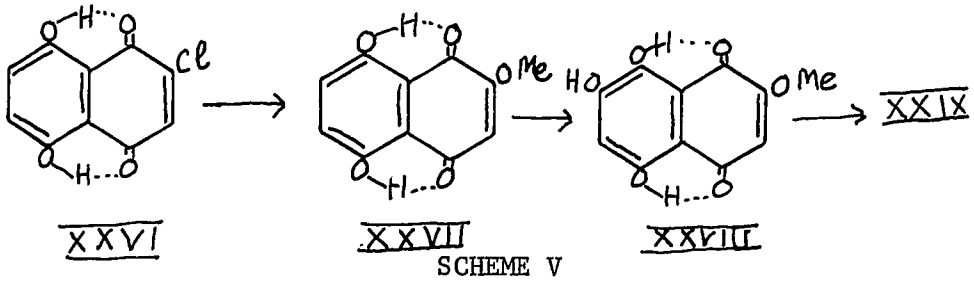
SCHEME IV

Naphthazarin was reductively acetylated to 1,4,5,8-tetraacetoxy-naphthalene in very good yields using five percent Palladium-on-charcoal, hydrogen and acetic anhydride. This reaction is cleaner and proceeds in higher yield than the classical method using a zinc, acetic anhydride, and acetic acid mixture. 1,4,5,8-Tetra-acetoxynaphthalene on treatment with boron trifluoride in acetic anhydride yielded 1-hydroxy-2-acetyl-4,5-8-triacetoxynaphthalene (XXIII) and a small amount of 2,6-diacetyl-1,4,5,8-tetrahydroxy-naphthalene; the diacetyl compound can be obtained exclusively by treatment with boron trifluoride in acetic acid. 1-Hydroxy-2-acetyl-4,5,8-triacetoxynaphthalene (XXIII) could be hydrolyzed with sodium hydroxide to 2-acetyl-1,4,5,8-tetrahydroxynaphthalene which is not isolated but is immediately oxidized to 2-acetyl-naphthazarin (XXII) in the presence of air. On the other hand, 1,4,5,8-tetrahydroxy-2,6-diacetylnaphthalene (XXIV) was oxidized to 2,6-diacetylnaphthazarin (XXV) with lead tetraacetate. Similarly, naphthopurpurin (2-hydroxynaphthazarin) was reductivity acetylated and subsequent treatment with boron trifluoride-acetic anhydride yielded three isomers as expected. The first, fast moving isomer was confirmed to be 2-hydroxy-3-acetylnaphthazarin by comparison with an authentic sample of the naturally occuring pigment (19). The structure of that pigment had been elucidated through the comparison of its ultraviolet spectrum with that of 7-acetoxy-2-hydroxy-3-acetylnaphthazarin, the monoacetate of spinochrome A. Since hydroxy and acetyl groups are vicinal in this compound, they are hydrogen-bonded, and the compound tends to travel fast on

a column or a plate. The ultraviolet spectra are illustrated in Figure 1. The second compound was shown to be 2-hydroxy-6-acetylnaphthazarin. Structure proof rests on the fact that the compound is identical with a product of the sodium borohydride reduction of spinochrome A. Since spinochrome A is 2,7-dihydroxy-3-acetylnaphthazarin, only the 2-hydroxy or the 7-hydroxy group would be eliminated by borohydride. Furthermore, since the first product was already confirmed to be 2-hydroxy-3-acetyl, the second isomer must be 2-hydroxy-6-acetylnaphthazarin. The structure of the third isomer is, without rigorous proof, assumed to be 2-hydroxy-7-acetylnaphthazarin. This assignment is based on the fact that its acetyl signal in the n.m.r. spectrum is at δ 2.75; that its ultraviolet spectrum shows a hypsochromic shift as compared to the spectrum of 2-hydroxy-6-acetylnaphthazarin; and that the 2,6-isomer travels faster on chromatographic adsorbents than the 2,7-isomer, which we have generally observed to be the case.

5. Spinochrome A

The above described method of introducing the acetyl group into naphthazarin was successfully applied to the synthesis of spinochromes A and C according to Schemes V and VI.



SCHEME V

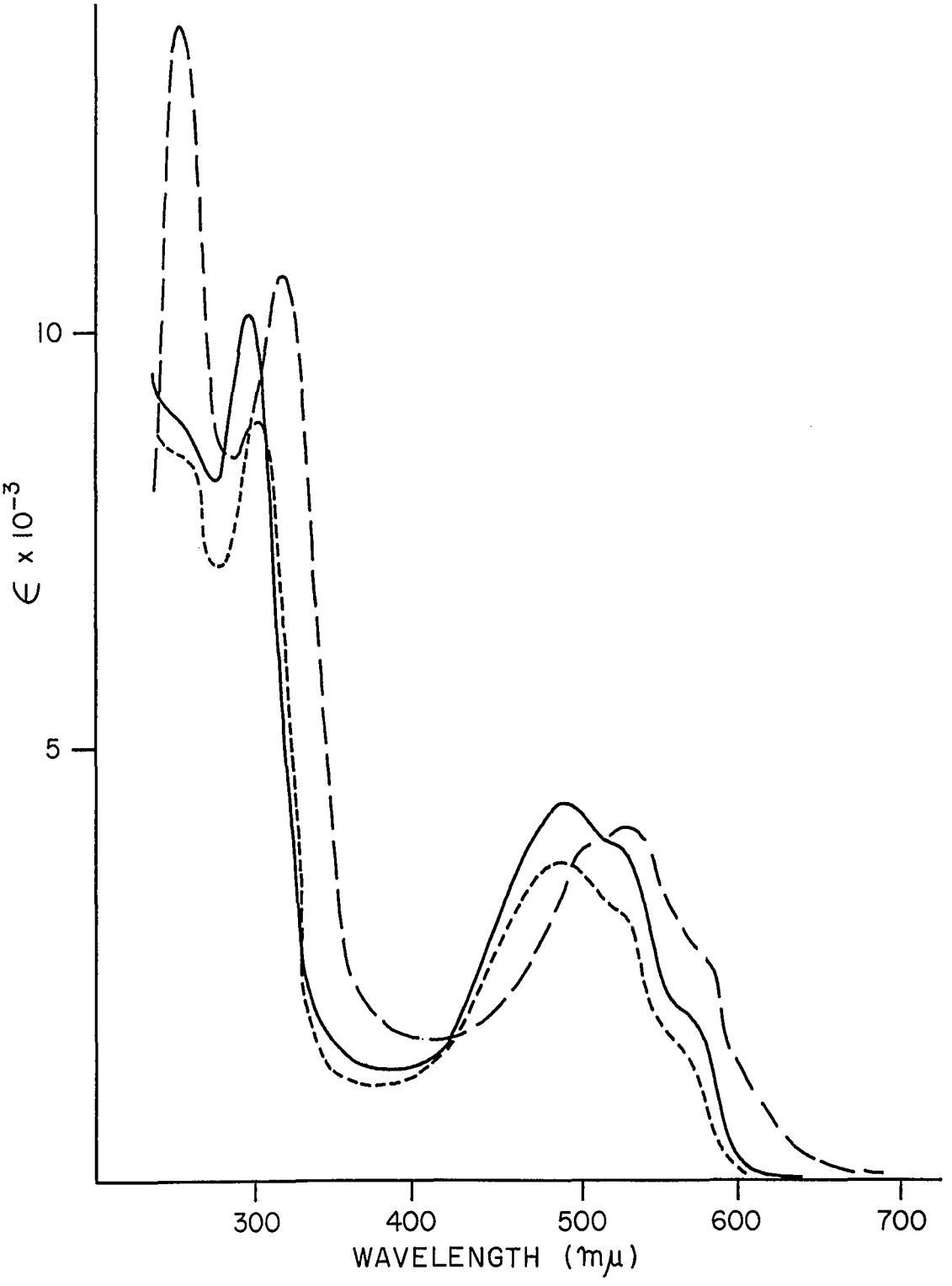
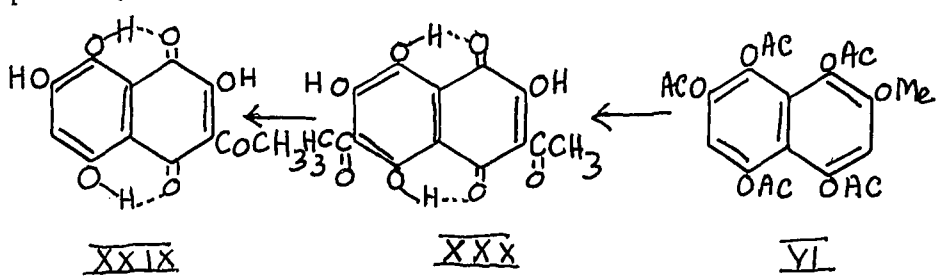


Figure 1 Electronic Absorption Spectra of 2-Hydroxy-3-Acetylnaphthazarin (—), Spinochrome A (— —), and Spinochrome A monoacetate (----) in Chloroform.

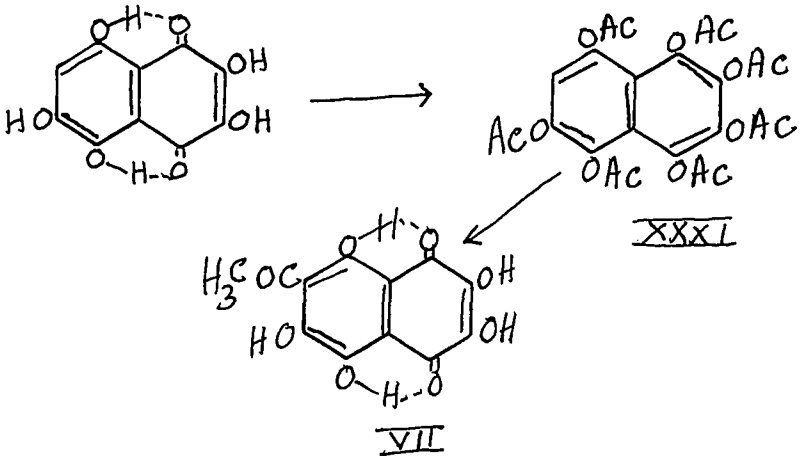
2-Hydroxy-7-methoxynaphthazarin (XXVIII), which was prepared from methoxynaphthazarin (XXVII), was utilized for the synthesis of spinochrome A (V). It was reductively acetylated to 2-methoxy-1,4,5,7,8-pentaacetoxynaphthalene (XXIX) in 72 percent yield. This compound on treatment with boron trifluoride in acetic acid, followed by hydrolysis with hydrochloric acid, yielded a compound, m.p. 237-238°. Its n.m.r. spectrum in deuteriochloroform showed only one signal at δ 2.85, which could be ascribed to an acetyl group. The mass spectrum of the compound indicated a molecular weight of 306, which corresponds to a formula of $C_{14}H_{10}O_8$. Since no methoxyl group was present and since, when spotted on a thin-layer plate along with spinochrome A and its monomethyl ether, the compound traveled along with monomethoxyspinochrome A but exhibited a different color, it could only be 2,7-dihydroxy-3,6-diactyl-naphthazarin (XXX). Since in the elucidation of the structure of spinochrome A the deacetylation of spinochrome A to 2,7-dihydroxy-naphthazarin was the key step (17), 2,7-dihydroxy-3-diacetyl-naphthazarin (XXX) was subjected to mild hydrolysis with dilute hydrochloric acid. This reaction indeed yielded spinochrome A in 50 percent yield, identical in all respects with the natural product.



XXIX XXX VI

SCHEME V

6. Spinochrome C



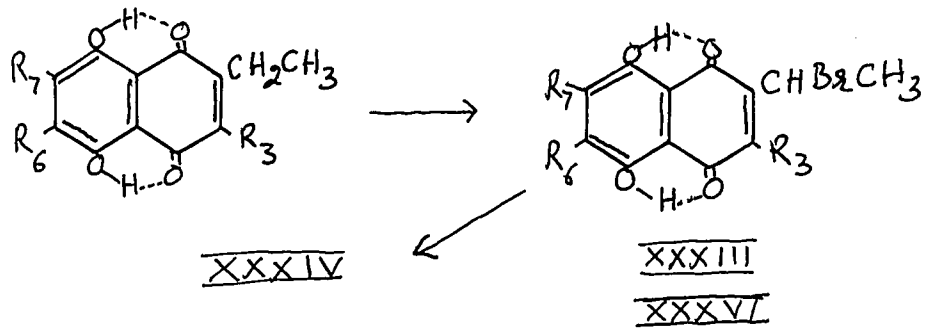
XXXI

VII

SCHEME VI

Spinochrome D was reductively acetylated to its leucoheptaacetate (XXXI) (13) with a mixture of acetic anhydride and zinc. On further treatment with boron trifluoride-acetic acid and mild acidic hydrolysis in the presence of air, there was obtained spinochrome C (VII), which was identical with the natural product by comparison of R_f-values, ultraviolet spectrum, and melting point.

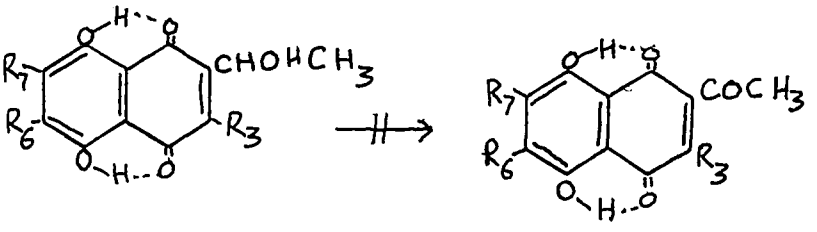
Since the yield of spinochrome C was very poor, it was desired to find a more favorable reaction for the introduction of the acetyl group. The reaction sequence is shown in the Scheme VII.



XXXIV

XXXIII

XXXVI



XXXIV

R_3, R_6, R_7 oMe

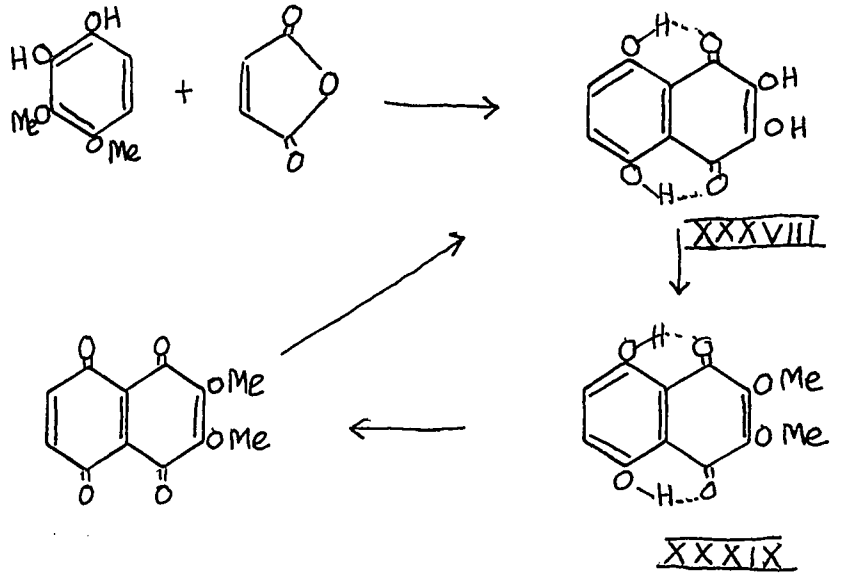
SCHEME VII

In a simple model experiment ethylnaphthazarin was brominated in the presence of light. The product, -bromoethylnaphthazarin (XXXVI), was very difficult to separate from starting material. Separation was accomplished by repeated chromatography on deactivated silica gel plates, which had been dried for several hours. The solvent system was benzenecarbontetrachloride-heptane (1:4:1). The product had an R_f-value of 1.12 as compared with that of the starting material of 1.10. Its n.m.r. spectrum with signals at δ 5.46(1H, quartet) and δ 1.98 (eH, doublet) confirmed that the product was the expected α-bromoethyl compound. Since a proton alpha to a quinone is a benzyl proton and since ethyl groups on a quinone ring are known to be reactive, α-bromoethyl-naphthazarin was kept in warm dilute sodium hydroxide solution for only a few minutes in order to achieve a clean replacement of bromine by hydroxyl. Yet on isolation of the product after acidification with cold dilute hydrochloric acid, followed by extraction with chloroform, thin-layer spotting revealed no fewer

than six spots. Because of lack of material these were not further investigated. Similarly, 2-(1-bromoethyl)-3,6-dimethoxy- and 2(1-bromoethyl)-3,6,7-trimethoxynaphthazarin (XXXIII) were prepared from their respective ethyl compounds. The latter compound on treatment with aqueous potassium hydroxide yielded 2-(1-hydroxyethyl)-3,6,7-trimethoxynaphthazarin (XXXIV). Its n.m.r. signals at δ 1.58 (3H, doublet) for the side chain methyl group and at δ 5.34 (1H, quartet) for the proton at C-1 as well as its molecular weight of 324 determined by mass spectrum confirmed its assigned structure. Surprisingly, attempted oxidation of the compound with acidic chromium trioxide was unsuccessful. Starting material was recovered. Lack of material prevented further study of this reaction.

7. 2,3-Dihydroxynaphthazarin

While this work was in progress Thomson (24) published a synthesis of spinochrome D by the lengthy tetralone route. As spinochrome D differs from 2,3-dihydroxynaphthazarin only by one additional hydroxyl group, it was thought desirable to introduce this group directly, and then extend this method to introduce a sixth hydroxyl group to produce spinochrome E. To this end, 2,3-dimethoxynaphthazarin was prepared from 1,2-dihydroxy-3,4-dimethoxy benzene (XXXVII), by condensation with maleic anhydride in an aluminum-sodium chloride melt, followed by conversion to the dimethyl ether by treatment with diazomethane. When XXXIX was oxidized to the diquinone with lead tetraacetate, and it in

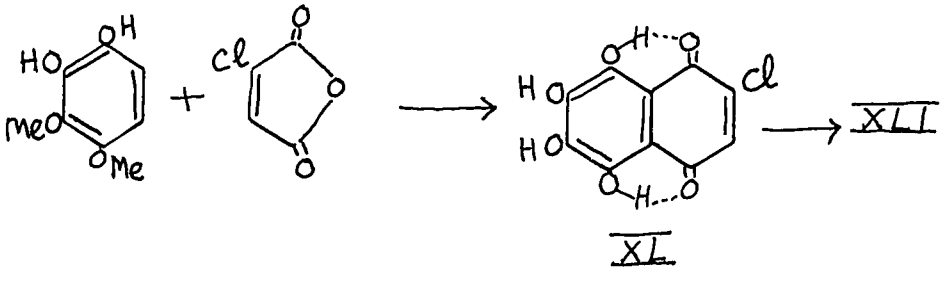


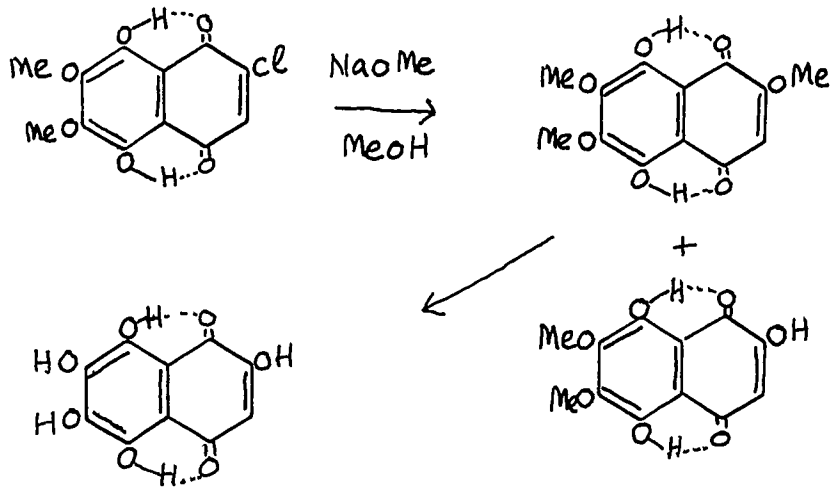
SCHEME VIII

turn was treated with acetic anhydride in presence of sulfuric acid as catalyst, the resulting compound was hydrolyzed to give back only 2,3-dihydroxynaphthazarin (XXXVIII). These reactions are summarized in Scheme VIII.

8. Spinochrome D

The above described failure to introduce further hydroxyl groups and the knowledge that the chloro group in naphthoquinone is susceptible to nucleophillic displacement, the following Scheme IX was pursued for the synthesis of spinochrome D.





SCHEME IX

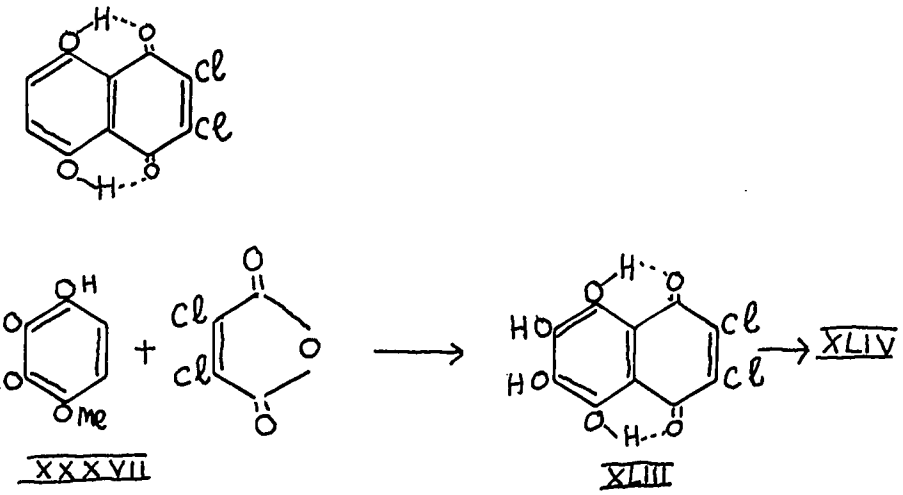
Condensation of 1,2-dihydroxy-3,4-dimethoxynaphthazarin with chloromaleic anhydride in an aluminum-sodium chloride melt yielded an unexpected mixture of three compounds. This mixture could be separated by thick-layer chromatography on deactivated silica gel, which was found to be more efficient than a corresponding column. Band I was analyzed and pointed to a formula of $C_{10}H_4O_6Cl_2$. On treatment with diazomethane it gave a compound of melting point 210-211°, with signals in its n.m.r. spectrum at δ 3.85 (6H) and δ 13.04 (2H). These data and its elemental analysis confirmed its structure to be 2,3-dimethoxy-6,7-dichloronaphthazarin (XLIV). Therefore, Band I was 2,3-dihydroxy-6,7-dichloronaphthazarin. Band III was confirmed to be 2,3-dihydroxynaphthazarin by its comparison with an authentic sample. As we had noted earlier that introduction of a chloro group into the naphthazarin molecule enhances its movement on chromatographic adsorbents, we suspected

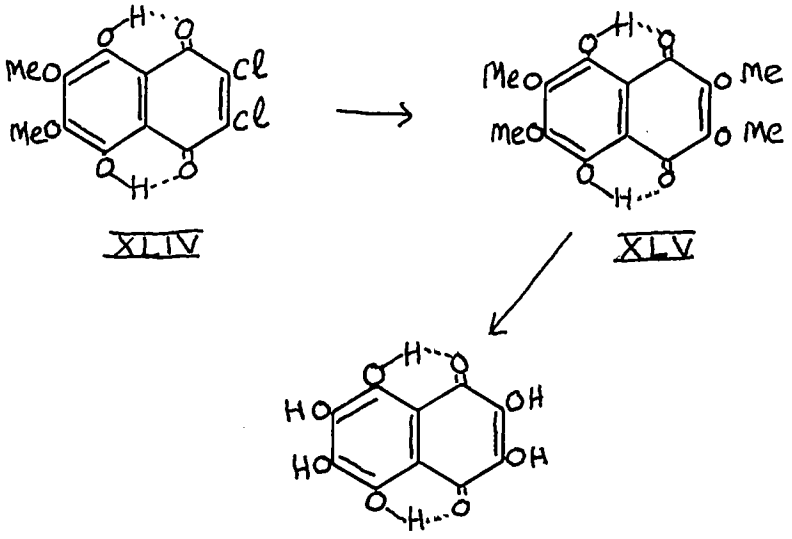
band II to be 2,3-dihydroxy-6-chloronaphthazarin since it traveled more slowly than 2,3-dihydroxy-6,7-dichloro and faster than 2,3 dihydroxynaphthazarin. Band II also reacted with diazomethane to yield a compound of melting point 134-135°. Its signals in the n.m.r. spectrum at δ 3.83 (3H), δ 3.77 (3H), δ 7.17 (1H), δ 13.05 (1H) and δ 12.43 (1H) and its elemental analysis confirmed its structure to be 2,3-dimethoxy-6-chloronaphthazarin (XLI), which in turn confirmed the assigned structure of band II. Since an excess of aluminum chloride is used in the condensation, it is conceivable that the monochloro compound which is formed first becomes disproportionated into dichloro-and 2,3-dihydroxy-naphthazarins through aluminum chloride catalysis. In the above reaction, demethylation also takes place in addition to condensation. This is not unexpected since aluminum chloride is a standard reagent for the cleavage of aromatic ethers (47). However, the mechanism of the disproportionation is not clear.

2,3-Dimethoxy-6-chloronaphthazarin was converted to the trimethyl ether of spinochrome D by refluxing for two days with sodium methoxide in methanol. A second product was 2,3-dimethoxy-6-hydroxynaphthazarin, which most probably resulted from nucleophilic displacement of chloride by hydroxide ion, which is present in a saturated solution of sodium methoxide in methanol. An analogous case in the anthraquinone series is recorded in the literature (38). Compound XLII was demethylated to spinochrome D by refluxing with 48 percent aqueous hydrobromic acid.

9. Spinochrome E

1,2-Dihydroxy-3,4-dimethoxybenzene was condensed with 2,3-dichloromaleic anhydride in the usual way and yielded a single product, 2,3-dihydroxy-6,7-dichloronaphthazarin, in contrast to the reaction of chloromaleic anhydride. The compound was quantitatively converted to 2,3-dimethoxy-6,7-dichloronaphthazarin, which in turn was transformed to 2,3,6,7-tetramethoxynaphthazarin (XLV) by nucleophilic displacement of chloride with methoxide in methanol. In addition, 2-hydroxy-3,6,7-trimethoxy-2-chloro-3,6,7-trimethoxy-and 2-chloro-3-hydroxy-6,7-dimethoxynaphthazarin were isolated from the same reaction and identified through their n.m.r. and ultraviolet spectra and elemental analyses. The desired product, the tetramethyl ether of spinochrome E, was demethylated with aqueous hydrobromic acid to yield spinochrome E (IX) almost quantitatively and identical in all respects with natural spinochrome E. The reaction sequence is shown in Scheme X.





SCHEME X

10. 2,3-Dichloronaphthazarin and sodium methoxide

It was desired to synthesize 2,3-dichloronaphthazarin in order to prepare from it 2,3-dihydroxynaphthazarin in reasonable quantity. Thomson (37) had prepared 2,3-dichloronaphthazarin in 37 percent yield by the condensation of 2,3-dichlorohydroquinone and maleic anhydride in an aluminum-sodium chloride melt. We prepared the same compound in yields of by using 2,3-dichloromaleic anhydride and hydroquinone. In our case, however, traces of monochloro and trichloronaphthazarin were also formed. This might indicate that at least one pathway of disproportionation proceeds directly from maleic anhydride. When 2,3-dichloronaphthazarin was allowed to react with methoxide ion in methanol in a

nitrogen atmosphere, there were isolated and identified some seveteen products. These compounds are listed in Table VI. The formation of 2,3-disubstituted products is expected from a nucleophilic displacement reaction. Since the starting material contained monochloronaphthazarin and trichloronaphthazatin, formation of trace amounts of monohydroxy, monomethoxy, as well as corresponding tri-substituted products can be explained. But the percentage of these products is too high to account for all of them. Even more puzzling is the formation of 2,6- and 2,7-disubstituted products and the presence of naphthazarin in the reaction mixture, since the starting material was free from naphthazarin. A possible rationalization might involve the presence of traces of sodium and/or hydrogen in a saturated solution of sodium methoxide in methanol. When crude 2,3-dichloronaphthazarin is added to this mixture, dissolved hydrogen is responsible for eliminating chloro groups thus leading to chloronaphthazarin and to naphthazarin, which in turn give rise to monohydroxy and monomethoxynaphthazarin. To check on these possibilities the reaction was carried out with pure 2,3-dichloro-naphthazarin and commercial sodium methoxide. From this reaction only 2,3-dimethoxy,hydroxymethoxynaphthazarin, and spinochrome D trimethyl ether were isolated. No monomethoxy, no monohydroxy nor naphthazarin itself were present. However, formation of spinochrome D trimethyl ether (2,3,6-trimethoxynaphthazarin in the presence of alkali and air leads to 2-hydroxynaphthazarin, an attempt was made to check on the formation of the trimethoxy

compound. Naphthazarin was refluxed in a sodium methoxide-methanol mixture, from which naphthopurpurin and its methyl ether, in 50 percent yield each, were isolated, as well as traces of other products, which were not further investigated. Furthermore, when methoxynaphthazarin was refluxed in methoxide-methanol, 2,6- and 2,7-dimethoxynaphthazarin were isolated in almost 50 percent yield each. These experiments indicate that methoxide ion can displace hydride ion in the naphthazarin molecule. Further work is needed to study the scope of this reaction.

B. Electronic Spectra

Since the electronic spectra of substituted naphthoquinones are important factors in the elucidation of their structures and since very few such data have been recorded in the literature (39), it was considered desirable to study the electronic spectra of these compounds. This study has been confined to emperical correlations but even with this limitation it was the most important basic tool to predict the structure of these compounds. First, the spectra of naphthoquinones were studied; this was extended to explain the spectra of juglone derivatives, and finally the spectra of naphthazarins were studied in detail.

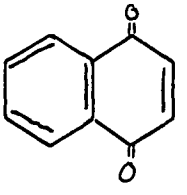
1. 1,4-Naphthoquinones

The multibanded spectra of 1,4-naphthoquinones can be divided roughly into three regions. First, the absorption at longest wavelength (400-600 mu) is attributed to the $n \rightarrow \pi^*$ transition of the quinone carbonyls. 1,4-Naphthoquinone (XLVI)

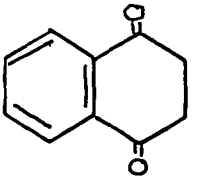
exhibits this excitation as a broad band at 425 mu of low intensity (32) when observed in hexane and as a shoulder on the much larger 335 mu band when observed in chloroform.

Secondly, the bands of medium intensity in the region 330-400 mu are overlapping benzenoid and E.T. (electron transfer) bands. Also included in this is the quinoid local $\pi \rightarrow \pi^*$ excitation. The last band is due to intense E.T. absorption (250-300 mu).

The band at 335 mu (3040) in the spectrum of XLVI (Fig. 2) is assigned to the benzenoid local excitation (L.E.) and its



XLVI



XLVII

position appears to be relatively independent of substitution on the quinoid ring (Table XII). Although the quinone carbonyls insulate the aromatic portion from the quinoidal double bond and its substituents, the presence of the double bond does facilitate the benzenoid $\pi \rightarrow \pi^*$ excitation as its position is bathochronically displaced by 40 mu from the transition for 2,3-dihydro-1,4-naphthoquinone (XLVII) (295 mu, 2100 in ethanol (39) . The quinoid L.E. $\pi \rightarrow \pi^*$ transition is not discernable in the spectra of 1,4-naphthoquinone and its 2-substituted derivatives, e.g., the spectrum of 2-methoxy-1,4-naphthoquinone (XLVIII) (Fig. 3). This is probably due to low intensity and masking by the larger

benzenoid $\pi \rightarrow \pi^*$ transition.

The spectrum of 2-hydroxy-3-methoxy-1,4-naphthoquinone (XLIX) (Fig. 3), on the other hand, displays an additional medium intensity band at 418 mμ (1320). Probably this transition is due to an excitation of a non-bonding p-orbital electron ($p \rightarrow \pi^*$) of the hydroxyl group and we shall refer to it as a quinoid L.E. transition. The position of this band is dependent on the nature of the substituents (Table XII). The quinoid L.E. band is also observed in the spectrum of 2-methoxy-3-ethyl-1,4-naphthoquinone, but the band is clearly not as intense. This transition appears absent in the spectra of 2-acetoxy-3-methyl-1,4-naphthoquinone and 2,3-dichloro-1,4-naphthoquinone.

Finally the intense bands in the 235-290 mμ region are assigned to benzenoid and quinoid electron transfer p or $\pi \rightarrow \pi^*$ transitions. The peaks at 245 (22,100) and 251 mu (23,450) in the spectrum of XLVI (Fig. 2) are due to benzenoid E.T. processes and shift only slightly with substitution on the quinoid ring (Table XII). The quinoid E.T. transitions shoulder at 257 mμ (13,100) are quite sensitive to substitution on the quinoid ring (Table XII). Note that the benzenoid E.T. bands of 2-hydroxy-1,4-naphthoquinone appear at essentially the same positions as in XLVI, but that the quinoid E.T. bands have shifted bathochromically to 277 mμ (15,900) and 283 mμ (15,960).

In conclusion, the diagnostic features of the 1,4-naphthoquinone electronic spectrum are intense E.T. bands in the 235-290 mμ region (15,000-25,000) and a L.E. band at about 335 mμ

(2500-3000).

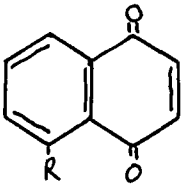
2. Juglones and 1,4-Naphthoquinones Substituted on the Benzenoid Ring.

When the 5-position of 1,4-naphthoquinone is substituted with an electron-releasing group, the benzenoid L.E. band is bathochromically displaced (Table XIII). Juglone (Fig. 2) exhibits this band in the visible region at 429 mμ with a somewhat higher intensity (3800) whereas 5-methoxy-1,4-naphthoquinone (L1) displays the benzenoid L.E. transition at 296 mμ (3320). Even the benzenoid L.E. band for 5-acetoxy-1,4-naphthoquinone (L11) has shifted by the expected 10 mu as compared with 1,4-naphthoquinone.

L, R = OH

L1, R = OCH_3

L11, R = $OCOCH_3$



The bathochromic shift of the benzenoid L.E. transition is not as pronounced upon substitution at C-6 when one compares the position of the band for 6-hydroxy-1,4-naphthoquinone (388 mμ) with that of L .

The quinoid L.E. transition can be seen in the spectrum of juglone (L, Fig. 2) as a medium intensity band at 337 mμ (1210) and also in the spectra of L1 sh 324 mμ (1270) and L111 sh 344 mμ (1760) . The position of this transition is affected by further substitution on the benzenoid ring of juglone as

evidenced in the spectrum of 7-hydroxyjuglone (LIV), where an appreciable bathochromic shift to 371 mμ is observed. Substitution of the quinoid ring with an electron-releasing group causes the expected bathochromic shift. The red shift is large enough for 2-hydroxyjuglone (LV, Fig. 4) and 3-hydroxyjuglone (Fig. 5) that it is masked by the larger benzenoid L.E. band.

The intense bands in the region 240-320 mu mark the benzenoid and quinoid E.T. transitions. The benzenoid and quinoid E.T. bands for compounds L, L1, and L11 overlap and appear at essentially the same position as 1,4-naphthoquinone. Surprisingly, the substitution at C-5 produces no appreciable shift of either transition. On the other hand, C-6 substitution does have an effect on the benzenoid E.T. band as evidenced by the ca. 10 mu red shift of the band in the spectrum of 6-hydroxy-1,4-naphthoquinone (Table XIII) while the position of the quinoid E.T. band is essentially the same. Substitution on the quinoid ring with an electron-releasing substituent causes a pronounced shift of the quinoid E.T. band see the spectra of LV, Fig. 4 and LVI, (Fig. 5) , while the position of the benzenoid E.T. band remains essentially unaltered. Both the benzenoid and quinoid E.T. bands shift bathochromically as expected in the spectra of 3,7-dimethoxyjuglone (LVII, Fig. 6) and of spinochrome B, 2,3,7-trihydroxyjuglone (LVIII, Table XIV).

3. Naphthazarins

The benzenoid L.E. band for naphthazarin (Fig. 2) has

shifted entirely into the visible region and is centered at ca. 525 mμ. Its multibanded structure is a diagnostic feature of all naphthazarin compounds and is one of the two most important criteria for ascertaining the nature of the substituents and their positions on the naphthazarin system (see for example the spectra of the three isomers of dihydroxynaphthazarin, Fig. 9). The center of the benzenoid L.E. band is generally near 500 mμ with 6000-9000 for a substituted naphthazarin.

The small band in the valley between the benzenoid L.E. band and the intense E.T. band is assigned to the quinoid L.E. band. It can always been seen in the spectra of unsubstituted and of monosubstituted naphthazarins (Table XVI), but further substitution of electron-releasing groups usually causes a large enough bathochromic shift that it becomes hidden by the much larger benzenoid L.E. band.

The second most important feature of the naphthazarin spectrum is the medium intense E.T. band in the near ultraviolet region, usually between 270-350 mμ with 5000-10,000. The position of this band is very important for elucidating the nature of β-substituents on the naphthazarin system. For example, it is readily apparent from Table XVI that each addition of a β-hydroxy group to the naphthazarin ring shifts the E.T. band ca. 20 mμ to the red. Methoxy groups also shift the E.T. band dramatically to the red, although for adjacent methoxyls the effect is less pronounced, probably because of steric crowding which causes less overlap of the naphthazarin π- system. As long as no alkoxyl

groups are present the position of the E.T. band immediately reveals the number of β -hydroxyls on the naphthazarin nucleus (Figs. 7 and 9).

When a β -hydroxyl group is acetylated, the spectra of the β -acetoxy compound no longer resemble that of the parent compound but instead they resemble -alkyl derivatives of naphthazarin. This phenomenon can be seen by comparing the spectra of 2-acetoxy-naphthazarin (Table XVII), 2-hydroxynaphthazarin, naphthazarin, and ethylnaphthazarin (Table XVI). However, inaccessibility of disubstituted alkylnaphthazarins has prevented the direct comparison of the spectra of other compounds. The acetoxy-naphthazarins were prepared by the reaction of ketene on hydroxy-naphthazarins. Ketene first reacts only with non-hydrogen-bonded hydroxyl groups and then if present in excess it will react with hydrogen-bonded hydroxyl groups. But only the acetoxyl compounds were isolated by TLC and identified. Other physical data for these compounds are given in Tables IX, X, and XI.

All spectra of hydroxynaphthoquinones (39) show a bathochromic shift of their visible bands when they are observed in methanol to which base was added. Juglone shows the bathochromic shift of the 422 mμ band by 92 mμ concomitant with an increased extinction. Among monohydroxyjuglones listed in Table XIII 3-hydroxyjuglone shows the smallest base shift of only 20 mμ. Methoxy substituents do not seem to affect the base shift greatly. This is seen in the bathochromic shift of the 423 mμ band of 3,7-dimethoxyjuglone by 86 mμ. In case of naphthazarin this shift is about 78 mμ but with almost double the extinction; 2,3-dimethoxy naphthazarin shows similar behavior. However, 2,6-dimethoxynaphthazarin shows a shift of 52 mμ whereas the 2,7-isomer shows only 24 mμ. The spectra in base were determined immediately after the addition of base, since on standing side reactions take place. The results are shown in Table XVIII and Figures 4, 5, 6 and 8.

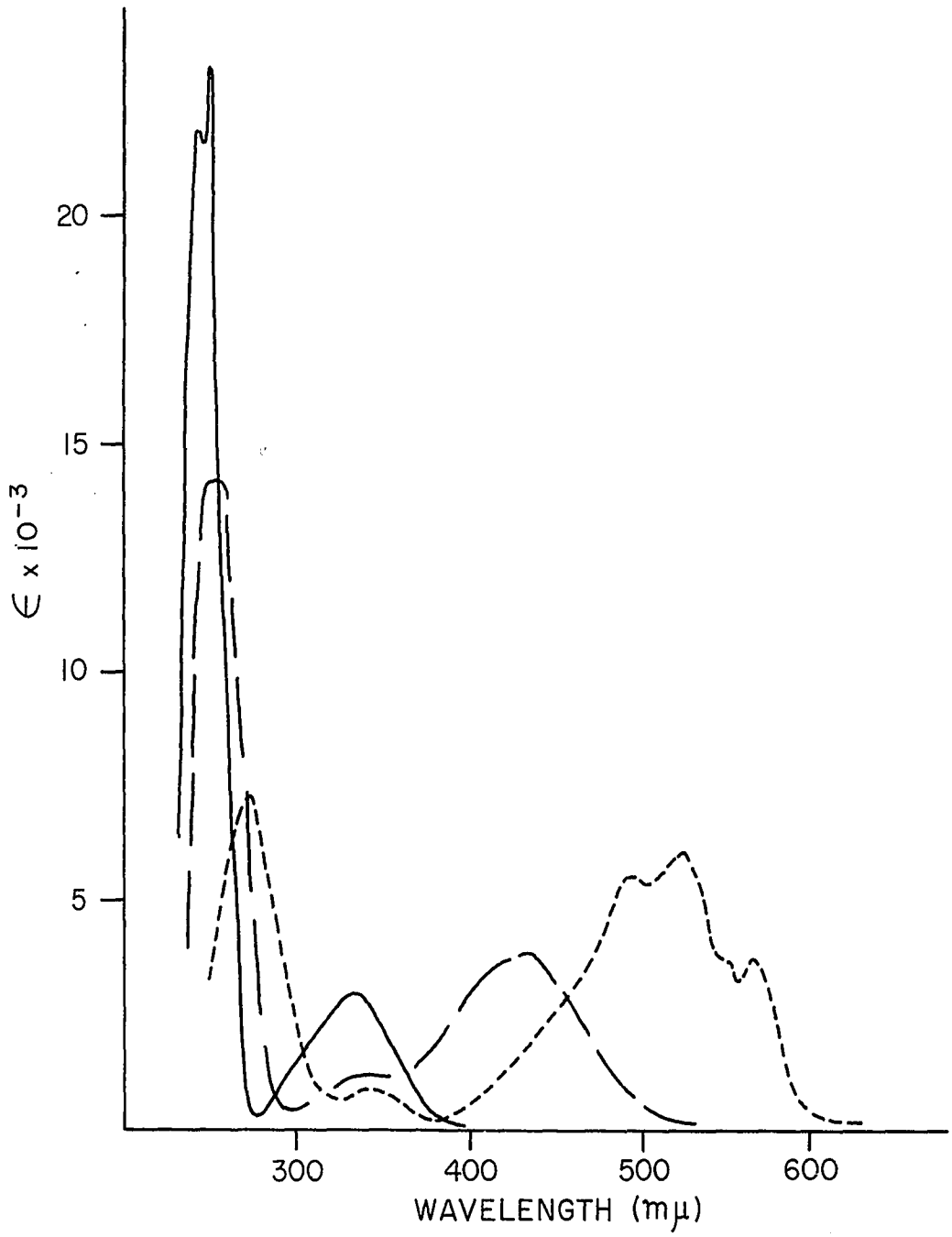


Figure 2 Electronic Absorption Spectra of 1,4-Naphthoquinone (—), Juglone (— —), and Naphthazarin (----) in Chloroform.

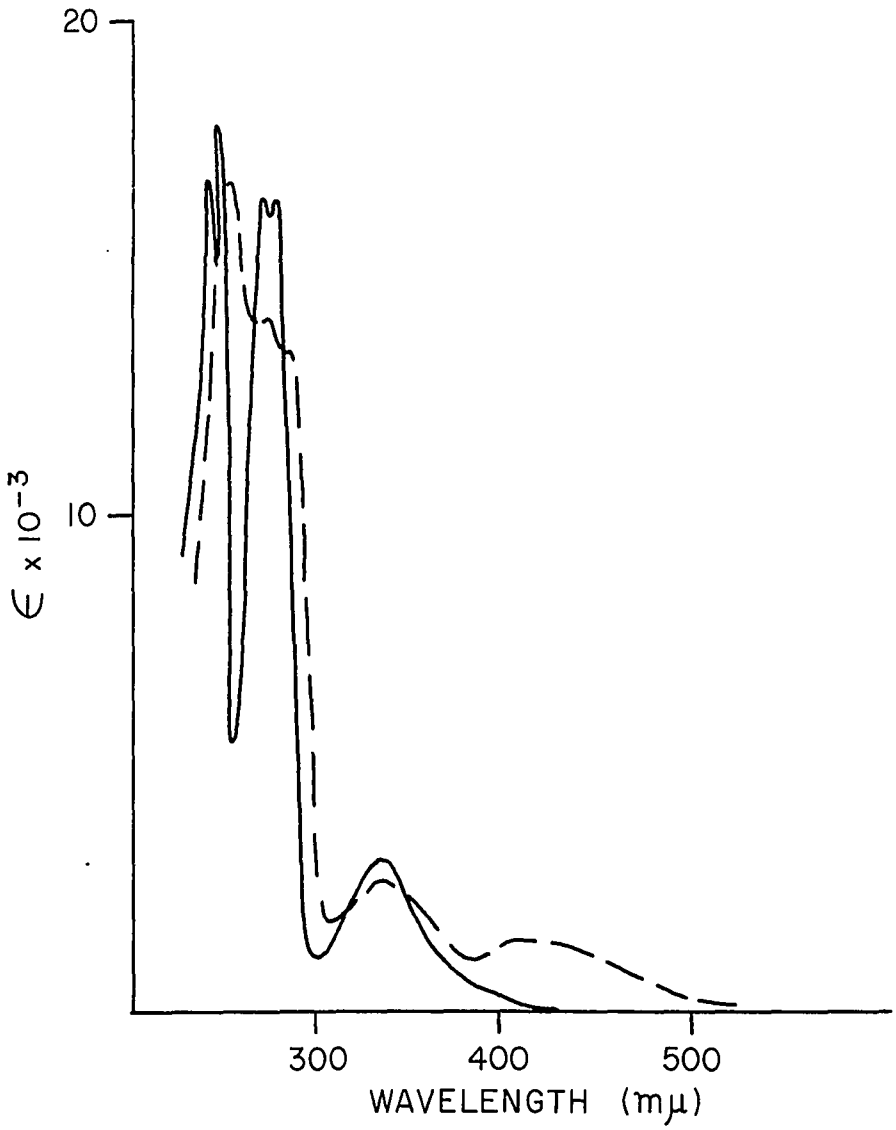


Figure 3 Electronic Absorption Spectra of 2-Methoxy-1, 4-Naphthoquinone (—) and 2-Hydroxy-3-Methoxy-1, 4-Naphthoquinone (— —) in Chloroform.

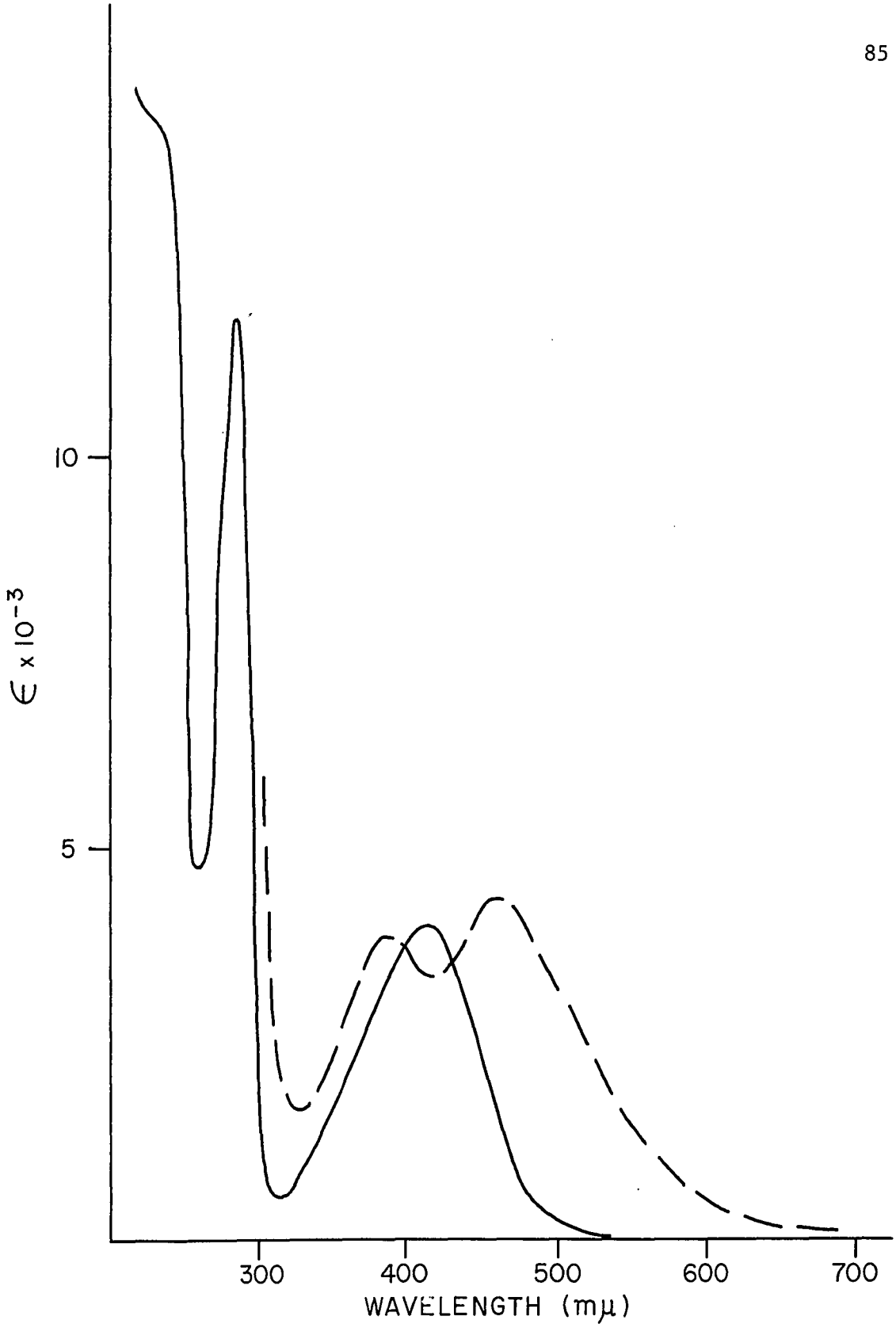


Figure 4 Electronic Absorption Spectra of 2-Hydroxyjuglone (—) in Methanol and in Methanolic KOH (– – –).

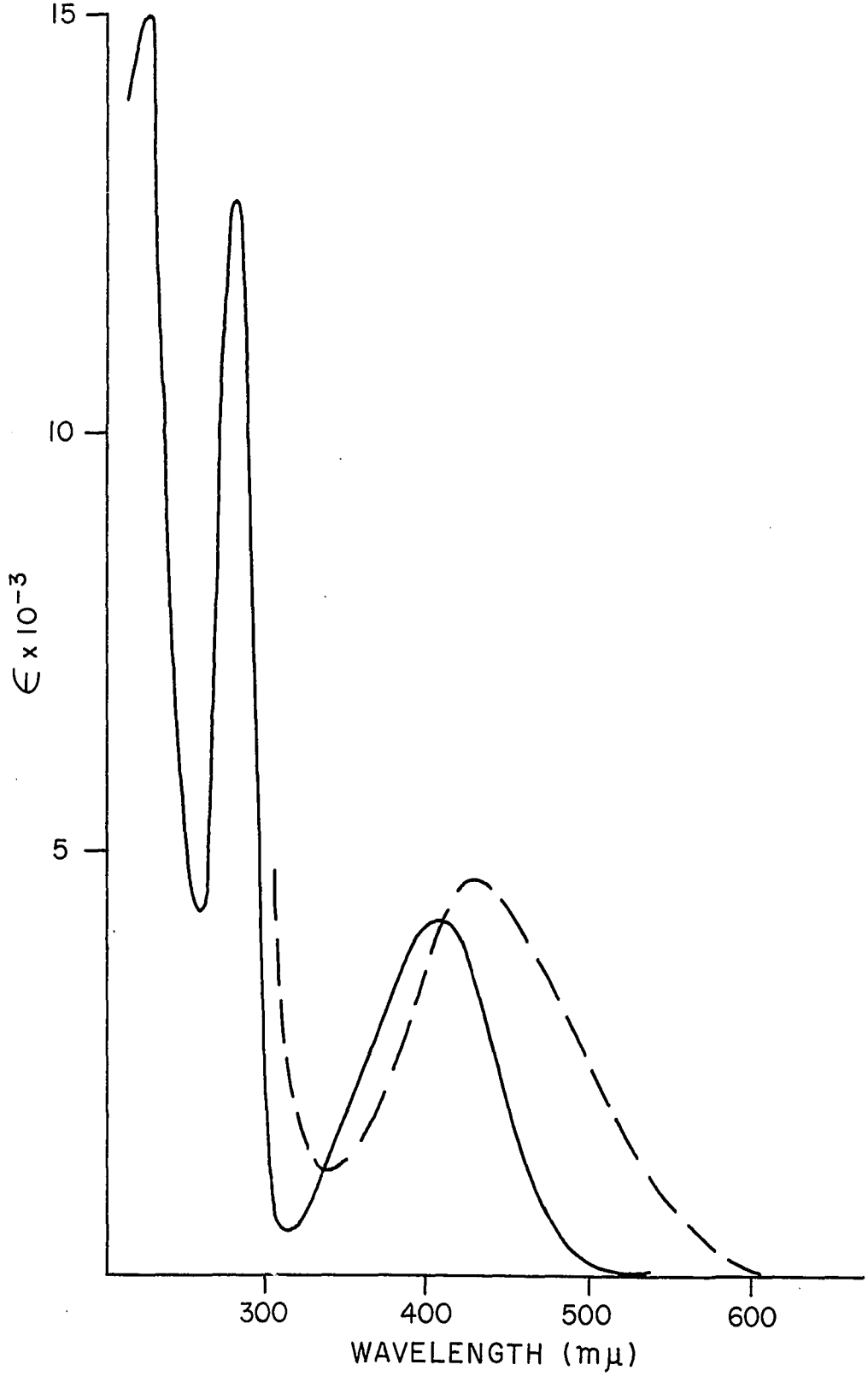


Figure 5 Electronic Absorption Spectra of 3-Hydroxyjuglone (—) in Methanol and in Methanolic KOH (--).

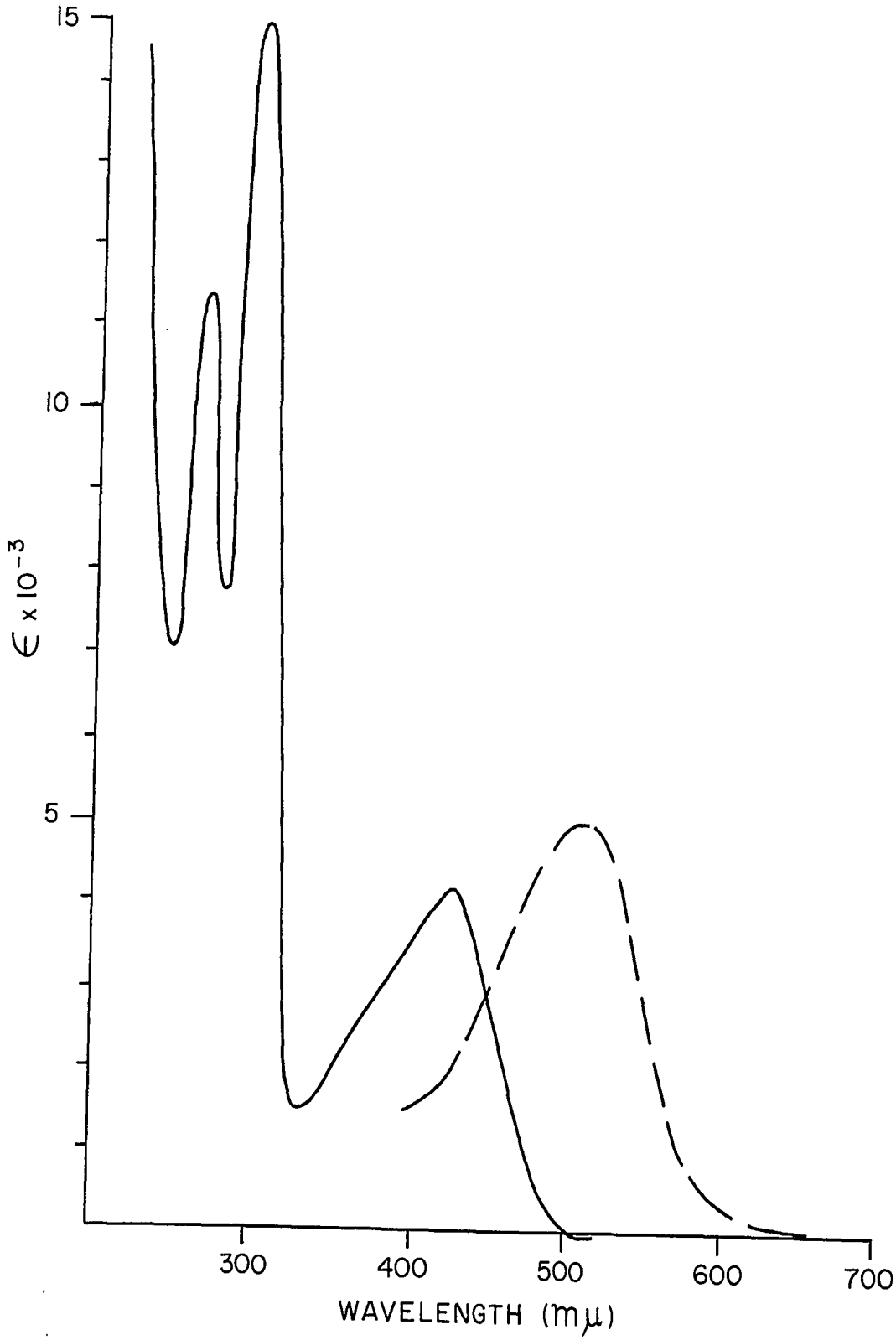


Figure 6 Electronic absorption spectra of 3,7-Dimethoxyjuglone in Methanol (—) and in Methanolic KOH (– –).

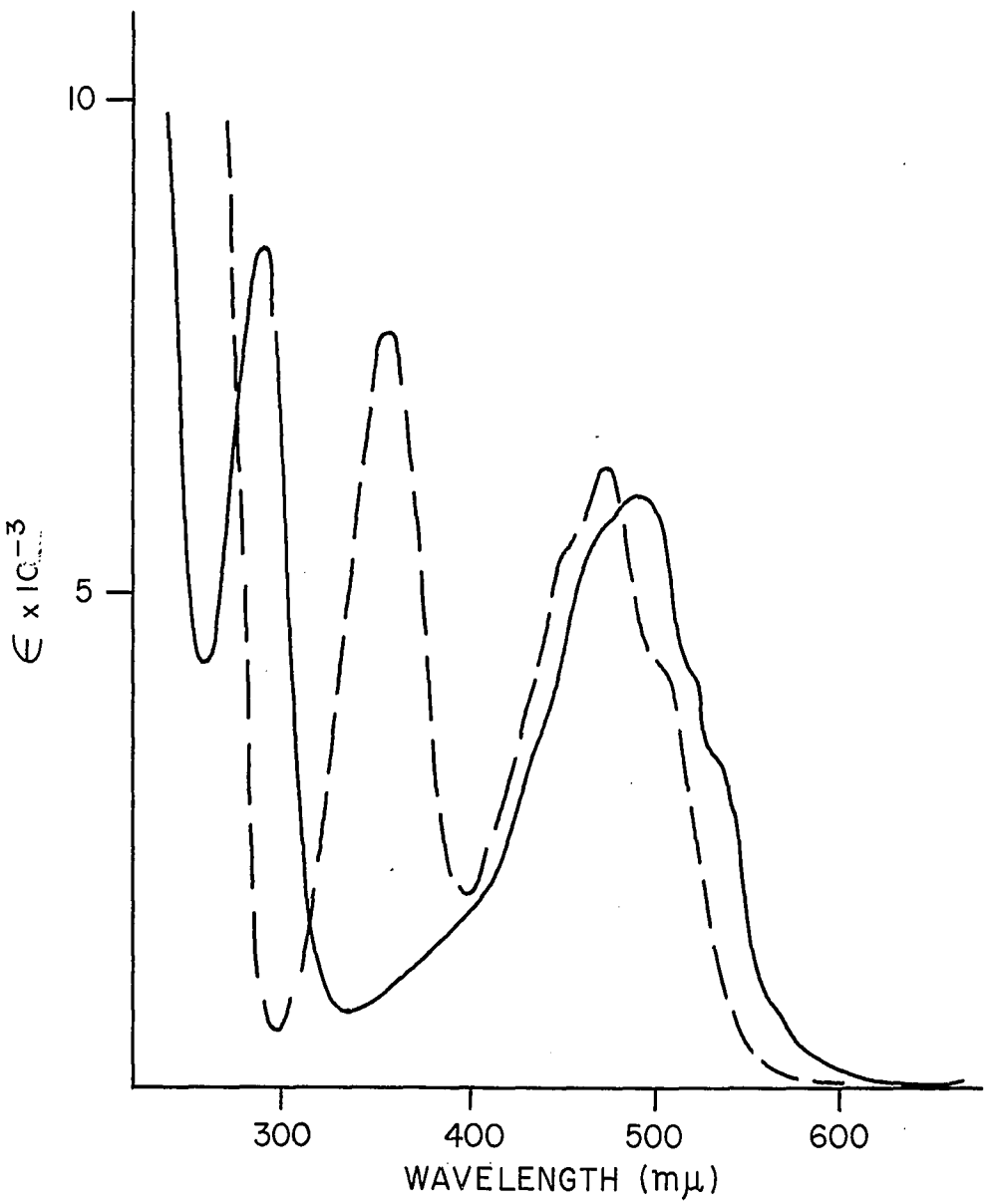


Figure 7 Electronic Absorption Spectra of Naphthopurpurin (—) and Spinochrome E (– –) in Methanol.

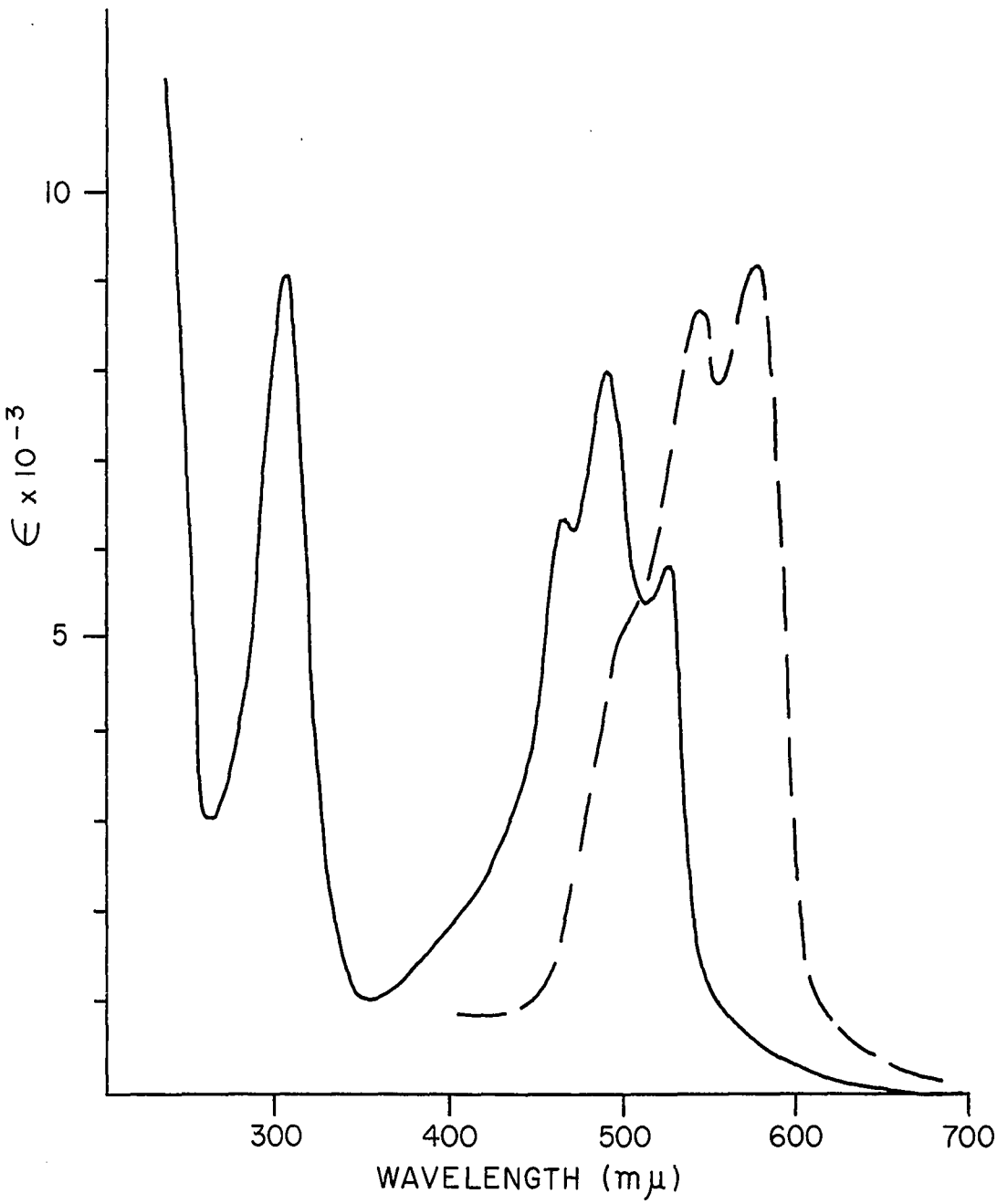


Figure 8 Electronic Absorption Spectra of 2,6-Dimethoxynaphthazarin in Methanol (—) and Methanolic KOH (--).

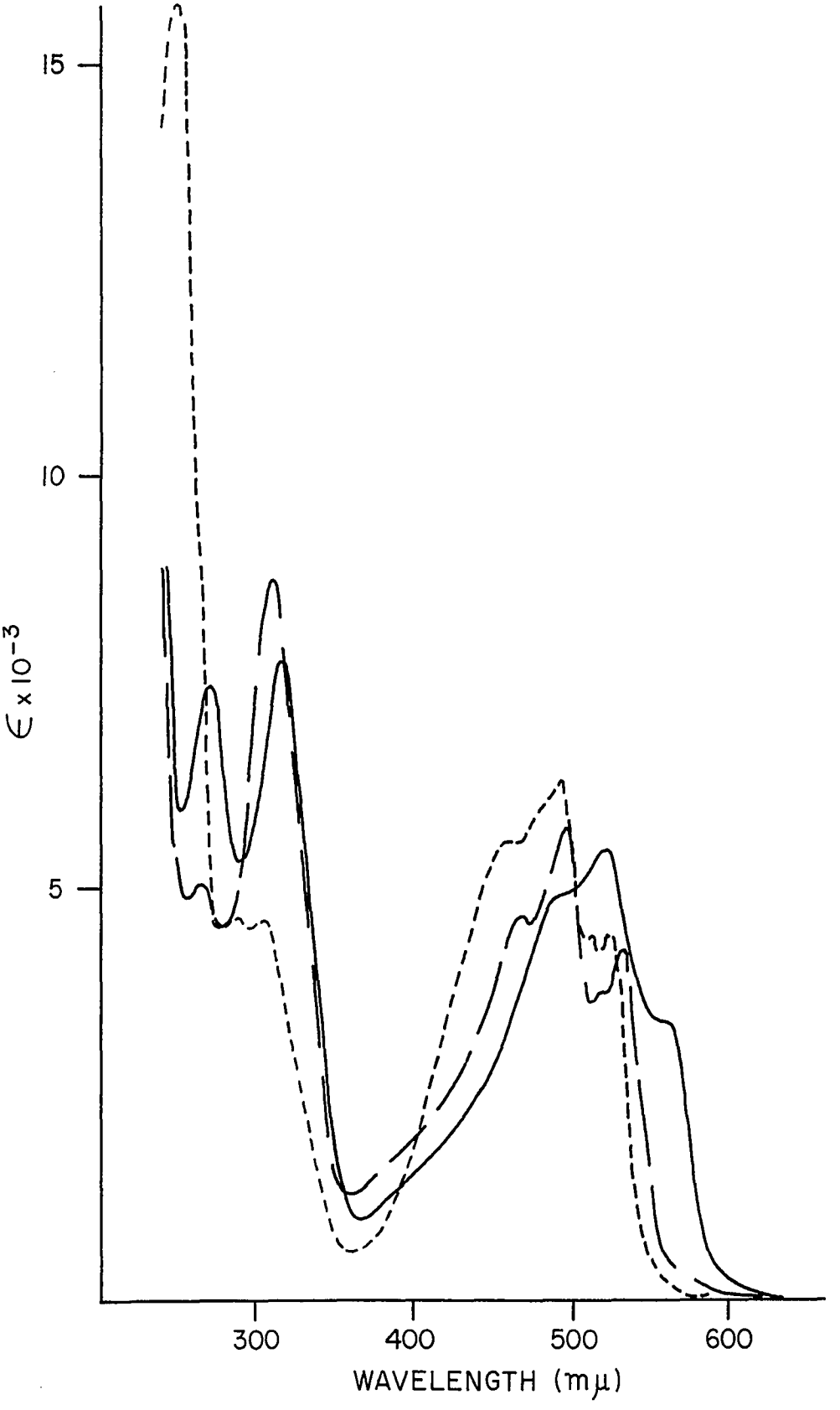


Figure 9 Electronic Absorption Spectra of 2,3—,2,6—, and 2,7— Dihydroxynaphthazarin.

TABLE XII. ELECTRONIC ABSORPTION SPECTRA OF 2(3)-SUBSTITUTED 1,4-NAPHTHOQUINONE IN CHLOROFORM

	λ max (ε) of E.T. bands benzenoid		quinoid		λ max (ε) of L.E. bands benzenoid	quinoid[a]
XLVI	245(22,100);	251(23,450)	sh257(13,100)		335(3040)	
$_1CH_3$	245(18,230);	251(19,910)	259(17,860);	268(16,430)	335(2680)	
1	244(15,580);	250(18,150)	277(15,900);	283(15,960)	337(3020)	
2 XLVIII	242(16,680);	248(17,950)	274(16,260);	280(16,300)	333(2950)	
$:H_3$	252(17,800)		252(17,800)		341(3190)	
OCH_3 3	244,250		264,269		337	
	252(21,700)		sh281(13,900);	286(15,100)	340(2950)	sh380(1270)
H_3 XLIX	253(16,790)		273(13,900);	sh283(13,280)	337(2620)	418(1320)
$:H_3$ $_2CH_3$	sh245(20,700);	252(22,000)	sh280(17,700);	sh288(15,800)	338(2990)	sh388(860)

TABLE XII. ELECTRONIC ABSORPTION SPECTRA OF 2(3)-SUBSTITUTED 1,4-NAPHTHOQUINONE IN CHLOROFORM

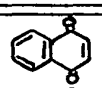
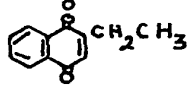
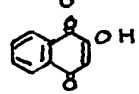
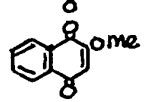
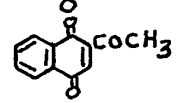
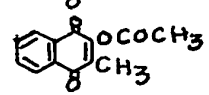
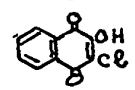
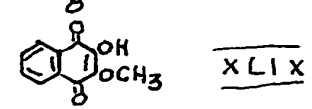
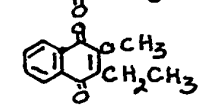
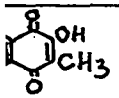
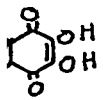
Compound	λ max (ε) of E.T. bands benzenoid		quinoid		λ max (ε) of benzenoid
 XLVI	245(22,100);	251(23,450)	sh257(13,100)		335(3040)
	245(18,230);	251(19,910)	259(17,860);	268(16,430)	335(2680)
	244(15,580);	250(18,150)	277(15,900);	283(15,960)	337(3020)
 XLVIII	242(16,680);	248(17,950)	274(16,260);	280(16,300)	333(2950)
	252(17,800)		252(17,800)		341(3190)
	244,250		264,269		337
	252(21,700)		sh281(13,900);	286(15,100)	340(2950)
 XLIX	253(16,790)		273(13,900);	sh283(13,280)	337(2620)
	sh245(20,700);	252(22,000)	sh280(17,700);	sh288(15,800)	338(2990)

TABLE XII. (Continued) ELECTRONIC ABSORPTION SPECTRA OF 2(3)-SUBSTITUTED 1,4-NAPHTHOQUINONE IN CHLOROFORM

ound	λ max (ε) of E.T. bands			λ max (ε) of L.E. bands	
	benzenoid	quinoid		benzenoid	quinoid[a]
	sh247,252	281,sh287		337	sh385
	262(17,650)[d]	sh274(17,000);	sh288(14,350)	335(2275)	439(1470)

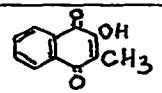
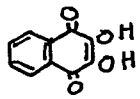
inoid L.E. band for the monosubstituted 1,4-naphthoquinones is probably of low intensity (100) and masked by d L.E. band.

ectrum was determined immediately as an anomalous reaction with chloroform occurs on standing.

extinction coefficients were not determined.

hough no distinct inflections are discernible on the low wavelength side of this band, there is enough absorption u to indicate the presence of the more familiar 245 and 250 mu bands. The 262 mu may be a new transition.

TABLE XII. (Continued) ELECTRONIC ABSORPTION SPECTRA OF 2(3)-SUBSTITUTED 1,4-NAPHTHOQUINONE IN CHLOROFORM

Compound	λ max (ε) of E.T. bands benzenoid	quinoid		λ max (ε) of benzenoid
	sh247,252	281,sh287		337
	262(17,650)[d]	sh274(17,000);	sh288(14,350)	335(2275)

[a]The quinoid L.E. band for the monosubstituted 1,4-naphthoquinones is probably of low intensity (100) and n the benzenoid L.E. band.

[b]The spectrum was determined immediately as an anomalous reaction with chloroform occurs on standing.

[c]Molar extinction coefficients were not determined.

[d]Even though no distinct inflections are discernible on the low wavelength side of this band, there is enough ab at 245-255 mu to indicate the presence of the more familiar 245 and 250 mu bands. The 262 mu may be a new transition

TABLE XIII. ELECTRONIC ABSORPTION SPECTRA OF JUGLONES AND 1,4-NAPHTHOQUINONES SUBSTITUTED ON THE BENZENOID RING

		Solvent	λ max, mu (ε) of E.T. bands benzenoid		quinoid	λ max, mu (ε) of L.E. bands benzenoid		quinoid
	L	$CHCl_3$	251(14,320)		251(14,320)	429(3800);	sh415(3640)	337(1210)
		MeOH	248(12,880)		248(12,880)	422(3630);	sh407(3550)	sh340(1130)
	LI	$CHCl_3$	247(17,590)		247(17,590)	396(3320)		sh324(1270)
	LII	$CHCl_3$	244(17,700);	250(18,100)	sh259(11,650)	345(2850)		----[a]
		$CHCl_3$	261(19,520)		254(19,900)	388(2410)		sh344(1760)
		MeOH	259(17,930)		254(17,700)	374(2880)		340(2320)
OH	LV	$CHCl_3$	240(9980)		286(12,600)	430(3660);	418(3610)	----[b]
		MeOH			282(11,800)	409(3920)		
OH	LVI	$CHCl_3$	240(7810)		283(14,000)	419(4410)		----[b]
		MeOH			282(12,760)	410(4220)		
	LIV	$CHCl_3$	263(12,720)		sh249(10,920)	436(3680)		sh371(2230)

TABLE XIII. ELECTRONIC ABSORPTION SPECTRA OF JUGLONES AND 1,4-NAPHTHOQUINONES SUBSTITUTED ON THE BENZENOID RING

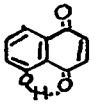
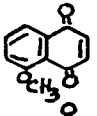
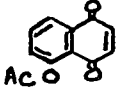
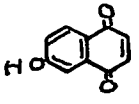
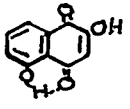
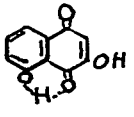
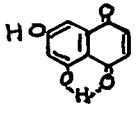
Compound		Solvent	λ max, mu (ε) of E.T. bands benzenoid		quinoid	λ max, mu (ε) of L.E. ba benzenoid		q
	L	$CHCl_3$	251(14,320)		251(14,320)	429(3800);	sh415(3640)	
		MeOH	248(12,880)		248(12,880)	422(3630);	sh407(3550)	sh
	LI	$CHCl_3$	247(17,590)		247(17,590)	396(3320)		sh
	LII	$CHCl_3$	244(17,700);	250(18,100)	sh259(11,650)	345(2850)		---
		$CHCl_3$	261(19,520)		254(19,900)	388(2410)		sh
		MeOH	259(17,930)		254(17,700)	374(2880)		
	LV	$CHCl_3$	240(9980)		286(12,600)	430(3660);	418(3610)	---
		MeOH			282(11,800)	409(3920)		
	LVI	$CHCl_3$	240(7810)		283(14,000)	419(4410)		---
		MeOH			282(12,760)	410(4220)		
	LIV	$CHCl_3$	263(12,720)		sh249(10,920)	436(3680)		sh

TABLE XIV. ELECTRONIC ABSORPTION SPECTRA OF JUGLONES AND 1,4-NAPHTHOQUINONES SUBSTITUTED ON THE BENZENOID RING

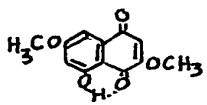
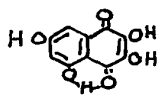
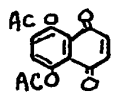
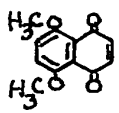
		Solvent	λ max, mu (ε) of E.T. bands benzenoid	quinoid	λ max, mu (ε) of L.E. bands benzenoid	quinoid
OCH_3	LVII	$CHCl_3$	268(10,900)	300(15,380)	430(4430)	----[b]
		MeOH	266(11,230)	299(14,520)	423(4180)	
OH OH	LVIII	MeOH	270(15,940)	320(7790)	387(2800)	----[b]
					470(1440)	
		$CHCl_3$	244(16,540); 250(16,620)	sh259(10,980)	355(3190)	----[a]
		$CHCl_3$	255(13,780)	255(13,780)	445(4340)	313(764)
		MeOH	254(11,100)	254(11,100)	448(4170)	324(946)

---

of low intensity (compare with 1,4-naphthoquinone) and masked by the larger benzenoid L.E. transition.

t observed. Substituents on quinoid ring effects a bathochromic shift of the transition and the larger
. band masks its position.

TABLE XIV. ELECTRONIC ABSORPTION SPECTRA OF JUGLONES AND 1,4-NAPHTHOQUINONES SUBSTITUTED ON THE BENZENOID RING

Compound		Solvent	λ max, mu (ε) of E.T. bands benzenoid		quinoid	λ max, mu (ε) of L.E. ban benzenoid	qu
	LVII	$CHCl_3$	268(10,900)		300(15,380)	430(4430)	----
		MeOH	266(11,230)		299(14,520)	423(4180)	
	LVIII	MeOH	270(15,940)		320(7790)	387(2800)	----
						470(1440)	
		$CHCl_3$	244(16,540);	250(16,620)	sh259(10,980)	355(3190)	----
		$CHCl_3$	255(13,780)		255(13,780)	445(4340)	313
		MeOH	254(11,100)		254(11,100)	448(4170)	324

[a]Band is of low intensity (compare with 1,4-naphthoquinone) and masked by the larger benzenoid L.E. transition.

[b]Band not observed. Substituents on quinoid ring effects a bathochromic shift of the transition and the larger benzenoid L.E. band masks its position.

TABLE XV. ELECTRONIC ABSORPTION SPECTRA OF NAPHTHAZARINS

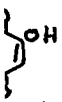
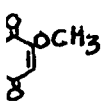
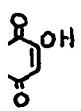
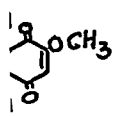
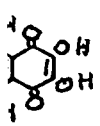
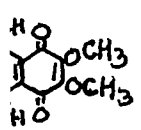
	Solvent	λ max, mu (ε) of L.E. bands benzenoid	quinoid	λ max, mu (ε) of E.T. band
	$CHCl_3$	532(4180),sh520(3660),494(5670),465(4550)		310(8920) 267(4780)
	MeOH	527(3920),514(3580),489(5450),462(4560)		310(8640) 260(5220)
	$CHCl_3$	529(6470),sh518(5500),492(8790),464(6710)		307(10,520)
	MeOH	523(5880),488(8070),461(6380)		303(9150)
	$CHCl_3$	558(3290),520(5380),490(4800)		316(7630) 269(7320)
	MeOH	552(3350),515(5520),488(5150)		315(7460) 267(7820)
	$CHCl_3$	550(5140),532(4850),511(8040),499(6950),479(6720)		309(8580) 286(7920)
	MeOH	543(4570),506(7330),sh491(6700),478(6540)		307(7370) 280(7420)
	$CHCl_3$	559sh(1430),519sh(3570),494(4170),469(3950)		323(5610) 259sh(9030) 249(9040)
	MeOH	512sh(3590),488(4090),468(4020)		321(5170) 259sh(9220) 250(9420)
	$CHCl_3$	529(5400),496(7710),sh474(6580)		314(8710)
	MeOH	sh518(5420),492(7460),sh478(6750)		312(7910)

TABLE XV. ELECTRONIC ABSORPTION SPECTRA OF NAPHTHAZARINS

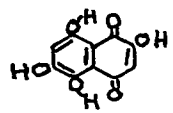
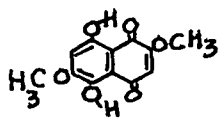
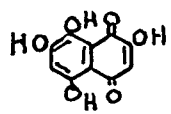
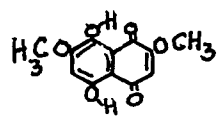
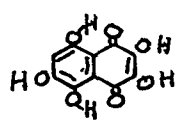
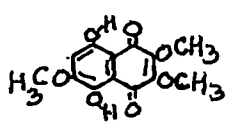
Compound	Solvent	λ max, mu (ε) of L.E. bands benzenoid quinoid
	$CHCl_3$	532(4180),sh520(3660),494(5670),465(4550)
	MeOH	527(3920),514(3580),489(5450),462(4560)
	$CHCl_3$	529(6470),sh518(5500),492(8790),464(6710)
	MeOH	523(5880),488(8070),461(6380)
	$CHCl_3$	558(3290),520(5380),490(4800)
	MeOH	552(3350),515(5520),488(5150)
	$CHCl_3$	550(5140),532(4850),511(8040),499(6950),479(6720)
	MeOH	543(4570),506(7330),sh491(6700),478(6540)
	$CHCl_3$	559sh(1430),519sh(3570),494(4170),469(3950)
	MeOH	512sh(3590),488(4090),468(4020)
	$CHCl_3$	529(5400),496(7710),sh474(6580)
	MeOH	sh518(5420),492(7460),sh478(6750)

TABLE XVI. ELECTRONIC ABSORPTION SPECTRA OF NAPHTHAZARINS

	Solvent	λmax, mu (ε) of L.E. bands benzenoid	quinoid	max, mu ( ) of E.T. band
›H	MeOH	498sh(4350),475(6060),459sh(5350)		357(7660)
›H	$CHCl_3$	531(5450),496(7940),469(6680)		328(8500)
›CH_3	MeOH	524(5260),493(7440),468(6580)		325(7440)
›CH_3				235(21,400)
	$CHCl_3$	564(3720),547(3630),524(6050),490(5420)	338(990)	269(7460)
›CH_2CH_3	$CHCl_3$	553(3840),516(6450),502(5920),486(5820)	343(570)	278(9260)
›Cl	$CHCl_3$	569(4140),526(6760),494(5980)	340(700)	284(8560)
›Cl	$CHCl_3$	569(4950),528(7540),494(6270)	359(680)	294(8880)
›Cl				
›H	$CHCl_3$	543(4000),528(4640),506(6280),sh496(6160),481(5520)	sh390( )	292(8130)
›CH_3	$CHCl_3$	540(4150),522(4710),503(6980),sh494(6760),475(6300)	sh380( )	291(10,790)
›H	$CHCl_3$	524(4420),511(4490),488(6280),sh478(5960),463(5560)		302(4570)
›H				288(4630)
				249(15,680)
›CH_3	$CHCl_3$	533(4370),499(7070),473(6410)		295(7920)
›CH_3	MeOH	sh525(4050),494(6680),472(6360)		292(7350)

TABLE XVI. ELECTRONIC ABSORPTION SPECTRA OF NAPHTHAZARINS

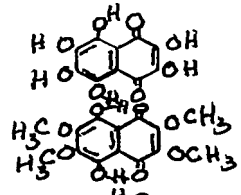
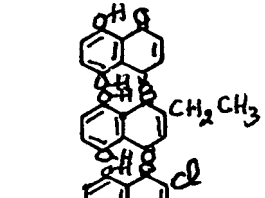
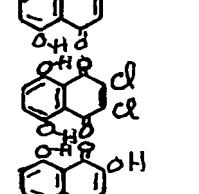
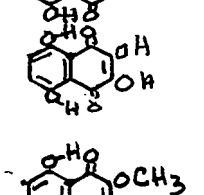
Compound	Solvent	λmax, mu (ε) of L.E. bands benzenoid	quinoid	max of
	MeOH	498sh(4350),475(6060),459sh(5350)		35
	$CHCl_3$	531(5450),496(7940),469(6680)		32
	MeOH	524(5260),493(7440),468(6580)		32 23
	$CHCl_3$	564(3720),547(3630),524(6050),490(5420)	338(990)	26
	$CHCl_3$	553(3840),516(6450),502(5920),486(5820)	343(570)	27
	$CHCl_3$	569(4140),526(6760),494(5980)	340(700)	28
	$CHCl_3$	569(4950),528(7540),494(6270)	359(680)	29
	$CHCl_3$	543(4000),528(4640),506(6280),sh496(6160),481(5520)	sh390( )	29
	$CHCl_3$	540(4150),522(4710),503(6980),sh494(6760),475(6300)	sh380( )	29
	$CHCl_3$	524(4420),511(4490),488(6280),sh478(5960),463(5560)		30 28 24
	$CHCl_3$	533(4370),499(7070),473(6410)		29
	MeOH	sh525(4050),494(6680),472(6360)		29

TABLE XVII. ELECTRONIC ABSORPTION SPECTRA OF ACETOXY NAPHTHAZARINS IN CHLOROFORM

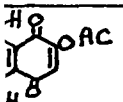
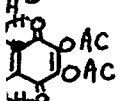
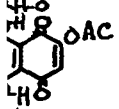
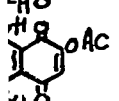
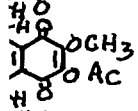
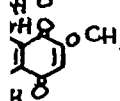
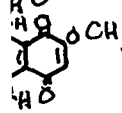
Compound	λ max, mμ (ε) of L.E. bands		λ max, mμ (ε)
	benzenoid	quinoid	of E.T. band
	555,519,485	338	280
	553(3590),592sh(3380),513(5370)481(4890)		288(9560)
	552(2735),513(4690),483(4490)		280(8210)
	565,522,484	352	280
	537(3950),501(6090),486sh(5600),479(5490)		293(7970)
	528sh(4040),515sh(4900),493(6940),485sh(6870)		295(11,000)
	535sh(3710),521sh(4680),501(6580),492(6610),475(6270)		295(10,150)

TABLE XVII. ELECTRONIC ABSORPTION SPECTRA OF ACETOXY NAPHTHAZARINS IN CHLOROFORM

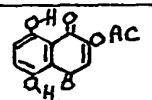
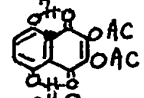
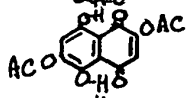
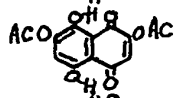
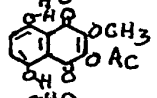
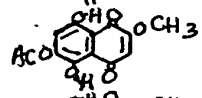
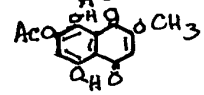
Compound	λ max, mμ (ε) of L.E. bands benzenoid	quinoid	λ max, mμ (ε) of E.T. band
	555,519,485	338	280
	553(3590),592sh(3380),513(5370)481(4890)		288(9560)
	552(2735),513(4690),483(4490)		280(8210)
	565,522,484	352	280
	537(3950),501(6090),486sh(5600),479(5490)		293(7970)
	528sh(4040),515sh(4900),493(6940),485sh(6870)		295(11,000)
	535sh(3710),521sh(4680),501(6580),492(6610),475(6270)		295(10,150)

TABLE XVIII. ELECTRONIC ABSORPTION SPECTRA OF SUBSTITUTED JUGLONES AND NAPHTHAZARINS IN 0.05N METHANOLIC KOH (350mu to 700mu)

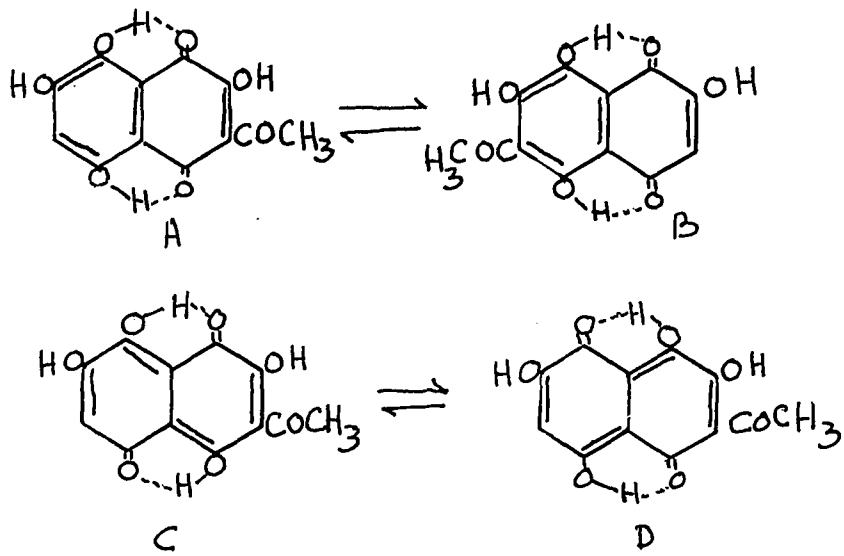
Compound	λ max, mu (ϵ) of L.E. bands		
Juglone	514(3840)		
2-Hydroxyjuglone	468(4890)	383(4540)	
3-Hydroxyjuglone	432		
7-Hydroxyjuglone	509(5010)		
Naphthazarin	621(7400)	585(8120)	558sh(6370)
2-Chloronaphthazarin	588(8440)	553(8000)	523sh(5260)
2-Ethylnaphthazarin	616(6600)	579(7500)	551sh(5770)
2-Methoxynaphthazarin	589(9220)	550(9600)	523sh(6450)
2-Hydroxynaphthazarin	569(4700)	531(6550)	505(5900)
2,3-Dimethoxynaphthazarin	593(8200)	563(8600)	
2,6-Dimethoxynaphthazarin	575(9300)	538(8700)	505sh(5400)
2,7-Dimethoxynaphthazarin	567(10,400)	532(10,350)	505sh(6600)
2,3,6-Trimethoxynaphthazarin	569(10,900)	536(10,300)	
2,3,6,7-Tetramethoxynaphthazarin	579(9050)	546(9300)	513sh(7000)

CHAPTER IV

CRYSTAL STRUCTURE STUDIES OF NAPHTHOQUINONES*

A. Spinochrome A

Because of the occurrence of tautomerism in the naphthazarin series, the only physical method which reveals the complete molecular structure of such a molecule in the solid phase is X-ray crystallography. This method will provide two important pieces of information, the crystal structure and the molecular structure of a given crystalline substance. Spinochrome A can exist in a number of quinoid forms, A, B, C, and D. To determine which of these is the major contributing structure in solution NMR and UV spectra have been investigated thoroughly. But in order to find out what is the structure of spinochrome A in the solid state a crystal structure analysis using X-rays was undertaken.



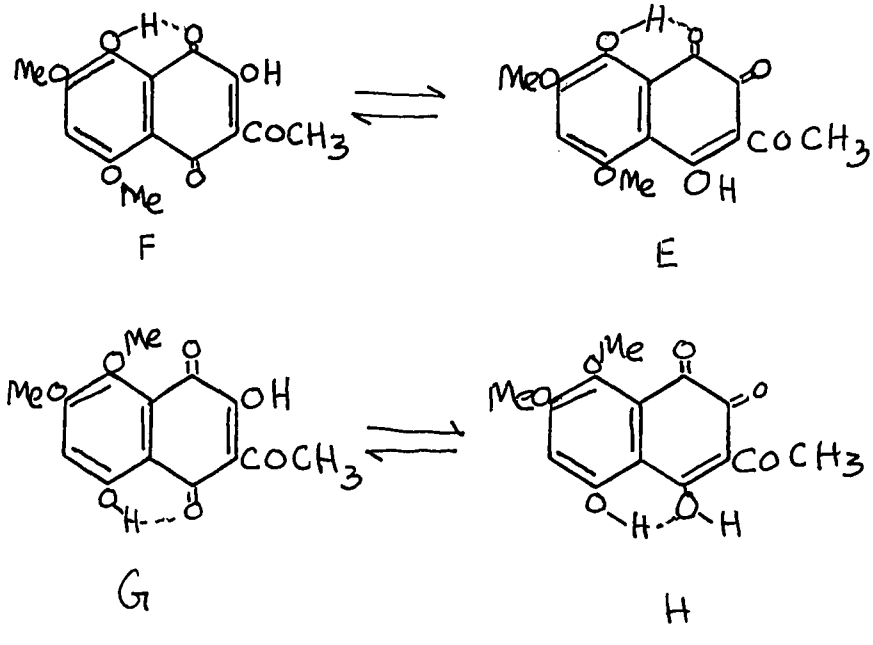
*Since this chapter is not an intrinsic part of this dissertation, the experimental details will be presented along with the discussion.

Spinochrome A crystallizes beautifully as dark purple needles from methanol (43). A crystal of spinochrome A was chosen and cut into a rectangular shape. It was mounted on a goniometer head along its obvious long axis. The crystal so mounted had stayed out of contact with the solvent for about two hours and its rotation picture was taken using the supercamera. The X-ray tube was operating at 40 kv and 26 ma and the exposure time for the above picture was one hour. The films were developed and it was observed that, in addition to the oscillation spots, there were extra spots other than layer line spots. This immediately indicated that in addition to the single crystal of spinochrome A there were some small crystallites deposited on the surface of the crystal. The crystal was viewed under a microscope, but the crystallite structure of spinochrome A could not be detected. Spinochrome A was again crystallized from methanol and the crystals were allowed to remain in the air for about eight hours. A crystal of suitable size was chosen, mounted, and a rotation photograph was again taken. This picture showed clearly the absence of any layer lines. This proved that the crystal had been transformed completely into the crystallite state, that is, it had gone from the crystalline to the amorphous state. This may be rationalized as follows. Spinochrome A freshly crystallized from methanol, contains one mole of methanol as solvent of crystallization. But on standing the solvent molecules are lost and the crystal structure is ruptured. To overcome this difficulty a crystal structure analysis can be carried out either by working at low temperature or by sealing the crystal in a capillary. The first method could not be used because of lack of low temperature

equipment. In attempting the second method, to seal the crystal in a capillary tube in the presence of solvent, it was found that methanol would evaporate within one day through the silicone oil which is used to seal the tube. Because of the practical difficulties, therefore, the crystal structure studies of spinochrome A were discontinued.

B. The Structure of Dimethylspinochrome A

When spinochrome A (17) is treated with dimethyl sulfate it yields, in addition to other products, a dimethyl derivative, m.p. 181-182°, of composition $C_{14}H_{12}O_7$. Structure E was assigned to this compound, hereafter designated as DMSPIN, on the basis of NMR and UV spectra. However, no rigorous proof was advanced for DMSPIN to have



structure E or the equally plausible structures F, G, or H. In solution E and F are readily interconvertible tautomers, as are G and

H. But in the crystalline state the structure of DMSPIN could be any of the four. In order to prove the position of the doubtful methoxyl group as well as to pinpoint the correct tautomer in the crystalline state, an X-ray crystallographic study was undertaken.

The crystal structure determination method consists of two parts, the determination of the cell parameters and of the cell structure.

1. The cell parameters. Measurement of the cell parameters entails the determination of the cell constants a, b, c and α, β, γ, where a, b, and c are the lengths of the unit cell in Angstrom units, where α is the angle between sides b and c, β the angle between a and c, and γ the angle between a and b.

The main requirements for a good crystal for collecting X-ray diffraction data are the crystal purity at the molecular, ionic, and atomic levels; the absence in the crystal of twinning, bending or fracturing. A good-sized crystal was obtained as follows: DMSPIN was dissolved in chloroform and the solution was just warmed to enhance solubility. It was placed in a vessel containing methanol, was covered, and allowed to stand for about two days. The crystal was filtered and treated with carbon tetrachloride solution to clear it and to remove some small crystallites adhering to the main crystal. The crystal was viewed under a polarizing microscope to make sure that it was a single good crystal. This seemed to be the case since on rotation of the polarizer the crystal appeared uniformly dark once every 90^{o}.

Since the intensities of the rays diffracted from a given crystal are proportional to the amount of material present in the specimen,

there is an advantage in selecting as large a crystal as possible. Because of energy absorption, on the other hand, there is an optimum thickness; for diffracted rays will show decrease in intensity after having passed through an excessively thick crystal. The optimum thickness for organic compounds is 0.2 mm.

Density measurements were made using the flotation technique with n-heptane and carbon tetrachloride. A centrifuge was used in order to be able to determine the flotation point accurately. The results were as follows:

Weight of picnometer 5.132 g.

Weight of picnometer + wt of solution 6.526, 6.521, 6.524 g.

Weight of solution (A_2) 1.390 g.

Weight of picnometer + water 6.0130 g.

Weight of water 0.881 g.

Volume of picnometer $\frac{0.881000}{0.996783}$ 0.8838 ml.

Also measured was the volume of the picnometer, 0.8806 ml.

$$\text{Density} = \frac{1.3900 \text{ g}}{0.8821 \text{ ml}} = 1.5727 \ \frac{\text{g}}{\text{ml}}$$

The crystal so chosen was then mounted on a brass pin. The brass pin having a uniform hole was heated on a hot plate and the hole was filled with dental wax. A glass fiber, 10 mm in length was inserted into the soft wax. On cooling the length of glass fiber protruding from the brass pin was reduced to 3 mm. This brass pin was then mounted on a pin holder attached to the crystal mounting device. To the end of the fiber some epoxy glue was applied. Then the mounter

was brought close to the crystal; the two were just allowed to touch, which caused the glue to spread and to adhere to the crystal. The set-up was allowed to stand for several hours until the glue dried. The crystal and the pin holding it were then mounted on a goniometer head and aligned optically. Rotation and Weissenberg photographs of the crystal gave the following cell dimensions:

$CuK_\alpha = 1.5418$ Å

$b = 4.08$ Å

$a = 19.714$ Å

$c = 7.7$ Å

$\beta = 91.46^\circ$

From rotation and Weissenberg photographs, no mirror plane symmetry nor systematic absences could be observed. This indicated that the crystal belonged to the triclinic space group, since $V^* = 5.824 \times 10^{-3}$, $V = \frac{3}{V^*} = 629.3$ Å^3, and $M = 595$.

Since the molecular weight of $C_{14}H_{12}O_7$ is 292, the unit cell contains two molecules. On this basis the space group was chosen to be $P\bar{1}$.

The integrated Weissenberg photographs were taken in Prof. Jensen's Laboratory at the University of Washington. Since the crystal was rotated about the b-axis, the indexing was done as

Zero level photographs were taken using CuK_α -radiation (λ 1.5418Å). Exposures lasted one hundred hours for the zero level with the X-ray tube run at 40 kv and 20 ma. Four sheets of Ilford Industrial G film were used for each exposure. This level showed reflections to the edge of the film. The negatives were scanned with

a microphotometer in a direction at right angles to the direction of camera integration. The output of the microphotometer was amplified by a Leeds and Northrop amplifier and then converted to optical density by a Leeds and Northrop chart recorder equipped with a logarithmic slide wire. The curves obtained from the chart recorder were planimetered to give the relative integrated intensitives. These intensitives were converted in the normal manner to F rel data on the IBM 7094 Computer using the X-ray system program at the University of Washington, Seattle. The total number of reflections was 302, of which 222 were observed. A Wilson temperature plot was made showing the temperature factor B to be 3. (This plot was very bad, but the best value selected was 3.) A Computer 650 was used for this purpose. After data reduction a "datafix" program was tried, which gave output as reflections and their F rel and E values. A vector map was obtained using the program from system tape. Patterson synthesis did not yield much information.

Because of the uncertainty about the structure of DMSPIN it was felt that a direct solution would be most desirable if it could be achieved. The basis of the method is usually considered to be a 1952 paper by Sayre (40). For reflections of a suitable large normalized structure factor E in centrosymmetric space groups,

$$E_{HKL} \times E_{HKL} = S_{H+H,\ K+K,\ L+L}$$

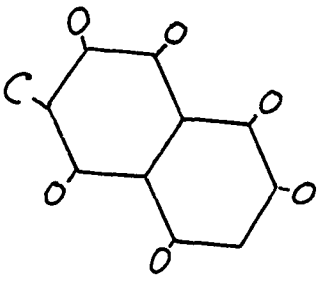
$$= \text{a quantity (1) or}$$

$$S_{HKL} \times S_{HKL} = S_{H+H,\ K+K,\ L+L} \quad (2)$$

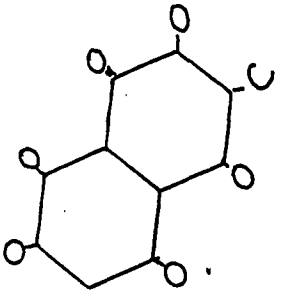
where S_{HKL} is the sign of E_{HKL}. For convenience, equations (1) and (2) have been referred to as "Phase Determining Equations" (PDE).

The principle of the method is a bootstrapping operation in which one starts with a very limited number of phases and uses these in connection with equation (2) to pyramid to a number large enough to give a recognizable Fourier representation. The first problem to be met in practice is that of obtaining some initial phases to work with. This was pointed out by Harker and Kasper (41) and discussed at some length by Karle and Hauptman (42) and by Lonsdale and Grenville (43). With certain restrictions two signs may be chosen for zonal and three for general data. These arbitrarily assigned phases usually constitute the initial set.

To do the first, 1600 was assumed to be + and all reflections for which E was above 1.7 also 1700 to be plus; it was possible to obtain signs of various reflections either determined absolutely or in terms of A, B, C, D (25 reflections). At this stage Fourier syntheses were calculated for the following combinations of signs: ++++, ---+, ----, ++-, --+-, --++, ++-+, ++--, -+++, -++-, -+-+, +-+-, -+--, +--+. All of these Fouriers were bad, except the +-++ combination Fourier which looked good. Therefore, the following two models were used for F_C and F_O calculations, that is from the above Fourier the positions of atoms were assumed so as to fit the molecular packing and other considerations. This led to the following two models, I and J.



I



J

F_c and F_o calculations were then submitted for Model I.
$R(R = \frac{\Sigma||F_o| - |F_c||}{\Sigma|F_o|})$ was 64.4 percent whereas for J, R was 70 percent. This was not pursued further since R was too high.

At this stage it was thought that the sign assigned to 1600 might be wrong and another set of signs assuming 807 as + was used. However, at this time a program written by Professor Stout was used and all reflections having E values above 1.0 were used. Initially 26 signed and 27 unsigned reflections were submitted. They underwent several cycles of combinations and they gave 37 signed reflections. There were indications that ABC = + or AB = + B = +. The Fouriers were bad as compared with the Fourier from Phases +-++ assuming 1600 as + and so on. Because of the failure to obtain concrete information about the structure of DMSPIN using Sayre's method, it was decided to use a mechanized trial and error method based on a model translation program.

The following model (k) was taken as starting point.

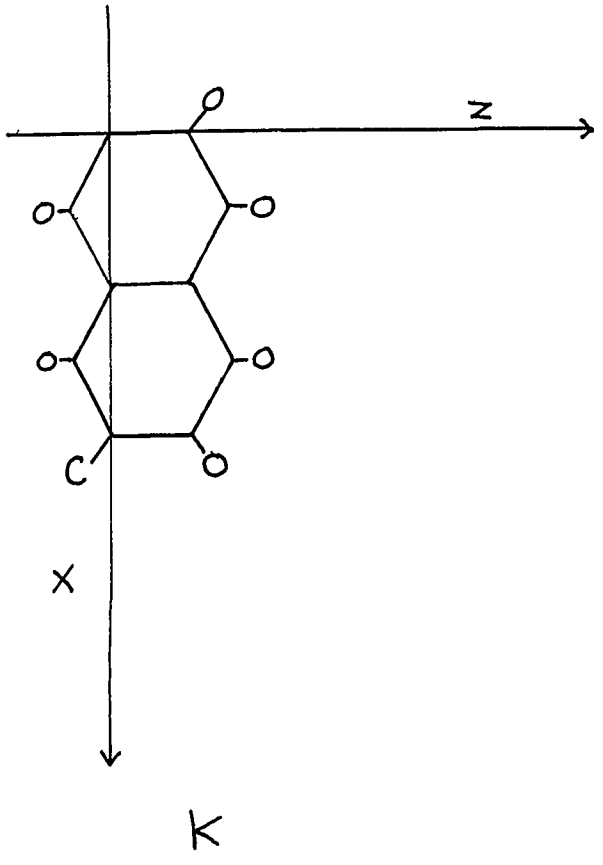

O
N
O
O
O
O
C
O
X
K

TABLE XIX. COORDINATES OF THE ATOMS FOR THE TRIAL AND ERROR MODEL

		X	Z
C_1	=	1.18	-0.68
C_2	=	0	0
C_3	=	0	1.232
C_4	=	1.18	1.912
C_5	=	3.55	1.912
C_6	=	4.74	1.232
C_7	=	4.74	0
C_8	=	3.55	-0.68
C_9	=	2.37	0
C_{10}	=	2.37	1.232
C_{11}	=	4.74	-0.68
O_1	=	1.18	-1.90
O_2	=	-1.18	+1.90
O_3	=	1.18	3.1
O_4	=	3.55	3.1
O_5	=	5.92	1.90
O_6	=	3.55	-1.90

The structure factors for the model were first calculated with respect to some arbitrary origin. In this case the arbitrary local origin 0, 0 (X,Z) was first placed at the origin of the unit cell; structure factors were calculated and from these the R values were calculated. Then this model was moved around the unique part of the unit cell and at each new position of the local origin F_c and R values were calculated. The whole unique space is reached both by translation and orientation. The calculations of the structure in space group PT has two equivalent positions with atoms in pairs at X, Y, Z and $\bar{X}$, $\bar{Y}$, $\bar{Z}$. The structure factor may be written in the form

$$F_{hkl} = \sum_j f_j e^{2\pi i[h(x_j+x_a)+l(z_j+z_a)]} + \sum_j f_j e^{2\pi i[h(-x_j-x_a)+l(-z_j-z_a)]}$$

where Xa, Ya, Za are the coordinates of some arbitrary origin with respect to the unit cell origin and xj, yj, zj are the coordinates with respect to this local origin. Since we were dealing with projections, the Y terms are zero. Factoring the common terms within each summation leads to

$$F_{hkl} = e^{2\pi i(hx_a+lz_a)} \sum_j f_j e^{2\pi i(hx_j+lz_j)} + e^{-2\pi i(hx_a+lz_a)} \sum_j f_j e^{-2\pi(hx_j+lz_j)}$$

Substituting, $a = \cos 2\pi(hx_a+lz_a)$

$b = \sin 2\pi(hx_a+lz_a)$

$A = \sum_j f_j \cos 2\pi(hx_j+lz_j)$; $B = \sum_j f_j \sin 2\pi(hx_j+lz_j)$

$F_{hkl} = (a+b)(A+B)+(a-b)(A-B)$.

$= 2(aA+bB)$

If the model is translated, only Xa, Ya, Za, change, while Xj, yj, zj do not. Thus only the terms a and b change and just these two terms need be evaluated for each reflection for each new position. Using the program written by Professor Stout, this model was rotated to 0^{o}, 60^{o}, 120^{o} and 180^{o} orientations and it was found that a position 24, 40 (x, z) the value of R was a minimum of 57 percent in the 0^{o} orientation position. At this stage only 200 reflections could be used, since that is all the program could handle. At this stage carbon atoms No. 12, 13, and 14 and O_7 were placed in positions according to Structure F. Structure factors (F_c) were calculated for this model and signs from these structure factors were used to calculate the Fourier synthesis. This dropped R to 54 percent. Three cycles of least square refinement on the position of atoms brought down the value of R to 48 percent and this would not refine further. At this state Δ F Fourier synthesis was carried out. The Δ F map strongly indicating that methoxyl on carbon 5 in structure F is in the wrong position. When the position of methoxyl was changed from carbon 5 to carbon 8 as in structure G, above calculations were repeated and gave an R-value of 34 percent. However, this was with only 200 reflections and with all the reflections R was 38 percent. However, it is obvious that with putting in the various hydrogen atoms R could drop to 30 or below and with more refinement R could go down to about 20 percent. On this basis the most probable structure of DMSPIN is G. But looking at the Weissenberg pictures carefully it can be seen that the spots are split into two and in many cases into three. This is indicative of the twinning in the crystals of DMSPIN, which is the

most likely reason why R remained large.

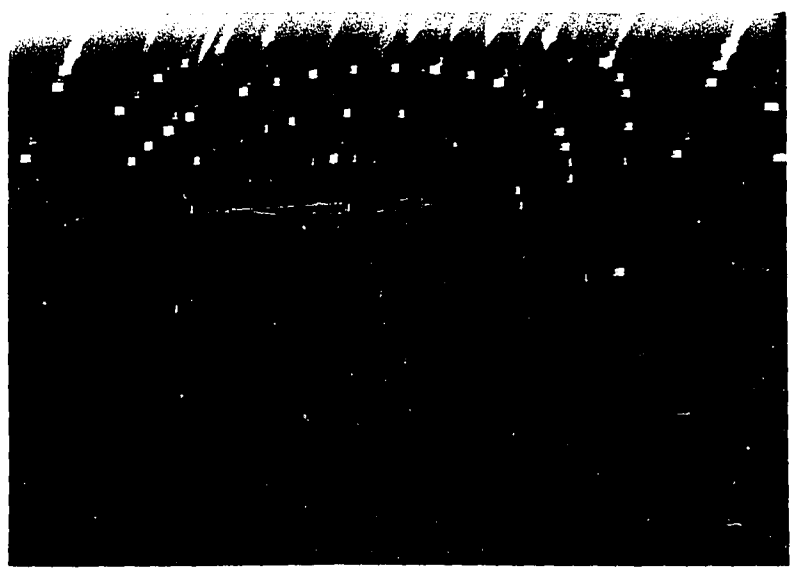


Figure 10 Integrated Weissenberg Photograph of DMSPIN.

REFERENCES

1. R. H. Thomson, "Naturally Occuring Quinones," Butterworth's Scientific Publications, London, 1957.

2. C. A. MacMunn, Quart. J. Microscop. Sci., 25, 469 (1885).

3. R. Kuhn and K. Wallenfels, Ber., 72, 1407 (1939).

4. K. Wallenfels and A. Gauhe, ibid., 76, 325 (1943).

5. E. Lederer and R. Glaser, Compt. Rend., 207, 454 (1938).

6. T. W. Goodwin, E. Lederer, and L. Musajo, Experientia, 7, 375 (1951).

7. K. Nishibori, Nature, 184, 1234 (1959).

8. K. Nishibori, ibid., 192, 1293 (1961).

9. L. Musajo and M. DiFonzo, Boll. Sci. Fac. Chim. Ind. Bologna, 29, 231 (1940).

10. R. Glaser and E. Lederer, Compt. Rend., 208, 1939 (1939).

11. R. Kuhn and K. Wallenfels, Ber., 74, 1594 (1941).

12. C. Kuroda and H. Ohshima, Proc. Imp. Acad. (Tokyo), 16, 214 (1940).

13. C. Kuroda and H. Iwakura, ibid., 18, 74 (1942).

14. C. Kuroda and K. Koyasu, ibid., 20, 23 (1944).

15. J. Smith and R. H. Thomson, J. Chem. Soc., 1008 (1961).

16. J. Gough and M. D. Sutherland, Tetrahedron Letters, No. 6, pp. 269, (1964).

17. C. W. J. Chang, R. E. Moore, and P. J. Scheuer, J. Am. Chem. Soc., 86, 2959 (1964).

18. C. W. J. Chang, R. E. Moore, and P. J. Scheuer, Tetrahedron Letters, 3557 (1964).

19. R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, unpublished results.

20. K. Zahn and P. Ochwat, Ann., 462, 72 (1928).

21. C. W. J. Chang, unpublished results.

22. D. B. Bruce and R. H. Thomson, J. Chem. Soc., 1089 (1955).

23. C. Charrier and G. Tocco, Chem. Zentr., 94, 1159 (1923), Gazz. Chim. Ital., 53, 431 (1923).

24. H. A. Anderson, J. Smith, and R. H. Thomson, J. Chem. Soc., 2141 (1965).

25. R. H. Thomson, J. Org. Chem., 16, 1082 (1951).

26. O. Dimorth and H. Roos, Ann., 456, 191 (1927).

27. J. F. Garden and R. H. Thomson, J. Chem. Soc., 2483 (1957).

28. R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, unpublished results.

29. C. A., 59, 9920 (1963), (F. Farina, M. Lora-Famgo, and C. Suarez, Anales Real Soc. Espan. Fis. Quim. (Madrid) Ser. B, 59 (3), 167 (1963).

30. F. Weygand, K. Vogelbach, and K. Zimmermann, Ber., 80, 391 (1947).

31. B. Eistert and G. Bock, Angew. Chem., 70, 575 (1958).

32. M. C. Kloetzel, R. P. Dayton, and B. Y. Abadir, J. Org. Chem., 20, 38 (1955).

33. N. Clauson-Kaas, S. Li, and N. Elming, Acta Chem. Scand., 4, 1233 (1950).

34. C. J. P. Spruit, Rec. Trav. Chim., 66, 655 (1947).

35. M. Pailer, K. Jentzsch, W. Kump, Monatsh., 89, 540 (1958).

36. T. Kuroda, Proc. Imp. Acad. (Tokyo), 15, 226 (1939).

37. P. B. Bruce and R. H. Thomson, J. Chem. Soc., 1089 (1955).

38. T. F. Low, R. J. Park, M. D. Sutherland, and I. Vessey, Australian J. Chem., 18, 182 (1965).

39. R. A. Morton and W. T. Earlam, J. Chem. Soc., 159 (1941).

40. D. Sayre, Acta Cryst., 5, 60 (1952).

41. D. Harker and J. S. Kasper, J. Chem. Phys., 15, 882 (1947).

42. H. Hauptman and J. Karle, Phys. Rev., 80, 244 (1950).

43. C. W. J. Chang, Ph.D. Thesis, University of Hawaii, Honolulu (1964).

44. C. A. Naylor, Jr., with J. H. Gardner, J. Am. Chem. Soc., 53, 4109 (1931).

45. W. Baker and H. A. Smith, J. Chem. Soc., 2542 (1931).

46. W. Baker and R. I. Savage, ibid., 1602 (1938).

47. G. A. Olah, "Friedel-Crafts and Related Reactions," InterScience Publishers, Inc., New York, N. Y., 1964.