NITROGEN DERIVATIVES OF NAPHTHOQUINONE

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NITROGEN DERIVATIVES OF NAPHTHOQUINONE

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. EXPERIMENTAL</td>
<td>4</td>
</tr>
<tr>
<td>III. DISCUSSION</td>
<td>18</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>24</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

Naphthoquinone and its derivatives have held the interest of many investigators since the efforts of Dam\(^1\) and Almquist\(^2\) led to the discovery of the antihemorrhagic K vitamins. Vitamin K\(_1\) proved to be 2-methyl-3-phytyl-1,4-naphthoquinone.\(^3\) Doisy and his associates\(^4\) found that vitamin K\(_2\) was 2-methyl-3-difarnesyl-1,4-naphthoquinone.

Many other derivatives of 1,4-naphthoquinone have also been found to exhibit antihemorrhagic properties. Menadione (2-methyl-1,4-naphthoquinone) is just as potent, molecule for molecule, as vitamin K\(_1\) itself.\(^5\) A yellow pigment from the human tubercle bacillus which has very effective antihemorrhagic


properties was isolated by Anderson and Newman. This compound is called Phthiococ and has the structure 2-methyl-3-hydroxy-1,4-naphthoquinone.

In 1943, Fieser and coworkers found that Hydrolapachol (2-isopentyl-3-hydroxy-1,4-naphthoquinone) and two related naphthoquinones possessed antimalarial activity. This discovery led to the synthesis of new naphthoquinones, some of which proved to be as much as one hundred times as active against avian malaria as Hydrolapachol.

Calandra, Fosdick and Fancher found that 1,4-naphthoquinones are useful inhibitors of acid formation by bacteria in the oral cavity. These workers synthesized four types of amine derivatives of 2-chloro-1,4-naphthoquinone. Amino pyridine, sulfonamide, amino alkane and aminoacid derivatives were tested. All were inhibitors of acid forming bacteria.

Many sulfanilamides have been unsuccessful in destroying the tubercle bacillus. However, it was found by Rich and Follis that sulfanilamide has an inhibitory effect on

the tubercle bacillus in guinea pigs, but the large doses required were fatal to many test animals.

Sjögren\textsuperscript{11} tried to increase the affinity of sulfanilamide for the tubercle bacillus by synthesizing a series of compounds which were substituted at the amido nitrogen with naphthalene derivatives, similar in nature to 1,4-naphthoquinones. These compounds were active against the tubercle bacillus \textit{in vitro}. Buu Hoï\textsuperscript{12} found that certain aryl amine derivatives of 2,3-dichloro-1,4-naphthoquinone are capable of inhibiting the growth of the tubercle bacillus. Lloyd and Middlebrook\textsuperscript{13} tested fifteen naphthoquinone derivatives against the tubercle bacillus \textit{in vitro}. One, 3-sulfanilyl-1,4-naphthoquinone, shows sufficient promise for further investigation. Zetterburg\textsuperscript{14} reported quantitative measurements on naphthoquinone inhibition of the growth of the tubercle bacillus.

A series of nitrogen derivatives of 1,4-naphthoquinone has been prepared by this worker to be tested for antitubercular activity by Parke, Davis and Company. Because of the structural similarities of these compounds to those tested for antitubercular activities by Sjögren and Buu Hoï, it is believed that they may have similar physiological activities.

\textsuperscript{11} Bertil Sjögren, \textit{The Svedburg, Mem. Vol.}, 547-57 (1944); G. A., 39, 1460 (1945).

\textsuperscript{12} Buu Hoï, \textit{Bull. soc. chim.}, 11, 578-84 (1944).

\textsuperscript{13} J. B. Lloyd and G. Middlebrook, \textit{Am. Rev. Tuberc.}, 49, 539-42 (1944); G. A., 38, 5246 (1944).

A 500 ml., three-necked flask equipped with a mechanical stirrer, condenser and dropping funnel was charged with 25 g. of 2,3-dichloro-1,4-naphthoquinone and 300 ml. of 95 per cent ethanol. The mixture was then heated to boiling and 75 ml. of concentrated ammonium hydroxide were added dropwise, with stirring, over a period of twenty minutes. The reaction mixture was refluxed for two hours, allowed to cool, then filtered. Purification was accomplished by treatment with charcoal and recrystallization from glacial acetic acid. A 75 per cent yield of bright orange crystals, melting at 192°C, was obtained.

(1) K. Fries and P. Ochwat, Ber., 56B, 1291-304 (1923).
2-Acetamido-3-chloro-1,4-naphthoquinone²

A 150 ml. beaker was charged with 7.2 g. of 2-amino-3-chloro-1,4-naphthoquinone and 10 ml. of acetic anhydride. Four drops of concentrated sulfuric acid were added. The mixture was rubbed and pressed with a glass stirring rod until the color had changed from bright orange to a dull yellow. The mass was then diluted with 100 ml. of water, filtered, and washed several times with 100 ml. portions of water. The residue was then removed from the filter and dissolved in 350 ml. of boiling 95 per cent ethyl alcohol. This solution was treated with 2 g. of charcoal, filtered while hot and allowed to cool. Bright yellow, needle-like crystals separated upon the addition of a few milliliters of water. Five grams of crystals melting at 219°C. were obtained.

(2) Ibid.
2-Propamido-3-chloro-1,4-naphthoquinone

A 150 ml. beaker was charged with 5 g. of 2-amino-3-chloro-1,4-naphthoquinone and 10 ml. of propionic anhydride. Four drops of concentrated sulfuric acid were added. The mixture was rubbed and pressed with a glass stirring rod until the color changed from bright orange to a dull yellow. The mass was then diluted with 100 ml. of water, filtered, and washed several times with 100 ml. portions of water. The residue was then removed from the filter and dissolved in 350 ml. of boiling 95 per cent ethyl alcohol. This solution was treated with 2 g. of charcoal, filtered while hot and allowed to cool. Bright yellow, needle-like crystals separated upon the addition of a few milliliters of water. A yield of 4.9 g. of crystals melting at 190°C. were obtained.

Anal. Calcd. for C_{13}H_{10}O_2Cl: N, 5.31; Cl, 13.47. Found: N, 5.31; Cl, 13.54.
A 150 ml. beaker was charged with 5 g. of 2-amino-3-chloro-1,4-naphthoquinone and 10 ml. of butyric anhydride. Four drops of concentrated sulfuric acid were added. The mixture was rubbed and pressed with a glass stirring rod until the color changed from bright orange to a dull yellow. The mass was then diluted with 100 ml. of water, filtered, and washed several times with 100 ml. portions of water. The residue was then removed from the filter and dissolved in 500 ml. of boiling 95 per cent ethyl alcohol. This solution was treated with 2 g. of charcoal, filtered while hot and allowed to cool. Bright yellow, needle-like crystals separated upon the addition of a few milliliters of water. Five grams of crystals melting at 164-165°C. were obtained.

Anal. Caled. for C_{14}H_{12}O_{3}NCl: N, 5.05; Cl, 12.80.
Found: N, 5.35; Cl, 12.86.
A 150 ml. beaker was charged with 1 g. of 2-acetamido-3-chloro-1,4-naphthoquinone and 75 ml. of 95 per cent ethanol. This solution was heated to boiling and 4 ml. of aniline were added dropwise, with stirring, over a period of ten minutes. After another ten minutes of boiling, 5 ml. of water were added and the mixture was allowed to cool. Dark crystals, which resembled crystals of KMnO₄, separated after a few hours. These crystals were collected on a filter and dissolved in 40 ml. of boiling 95 per cent ethanol. The resulting solution was treated with .5 g. of charcoal, filtered while hot and allowed to cool. A yield of .9 g. of dark, blood-red crystals melting with decomposition at 202°C. was obtained.

(3) Ibid.
2-Propamido-3-anilino-1,4-naphthoquinone

[Chemical structure image]

A 150 ml. beaker was charged with 2 g. of 2-propamido-3-chloro-1,4-naphthoquinone and 100 ml. of 95 per cent ethanol. This solution was heated to boiling and 5 ml. of aniline were added dropwise, with stirring, over a period of ten minutes. After another ten minutes of boiling, 5 ml. of water were added and the mixture was allowed to cool. Dark, purplish-red crystals separated after a few hours. This product had the appearance of \( \text{KMnO}_4 \), both in crystalline form and in solution. The crystals were collected on a filter and dissolved in 75 ml. of boiling 95 per cent ethanol. The resulting solution was treated with .5 g. of charcoal, filtered while hot and allowed to cool. A yield of 1.9 g. of dark, blood-red crystals melting with decomposition at 132°C. was obtained.

Anal. Calcd. for \( \text{C}_{15}\text{H}_{17}\text{O}_3\text{N}_2 \): N, 8.75. Found: N, 8.67.
2-Butyramido-3-anilino-1,4-naphthoquinone

A 150 ml. beaker was charged with 1 g. of 2-butyramido-3-chloro-1,4-naphthoquinone and 75 ml. of 95 per cent ethanol. This solution was heated to boiling and 4 ml. of aniline were added dropwise, with stirring, over a period of ten minutes. After another ten minutes of boiling, 5 ml. of water were added and the mixture was allowed to cool. After a few hours crystals formed which were similar in appearance to those of 2-acetamido-3-anilino-1,4-naphthoquinone and 2-propamido-3-anilino-1,4-naphthoquinone. These crystals were collected on a filter and dissolved in 50 ml. of boiling 95 per cent ethanol. The resulting solution was treated with .5 g. of charcoal, filtered while hot and allowed to cool. A yield of .8 g. of dark, blood-red crystals melting with decomposition at 207°C. was obtained.

Anal. Calcd. for C_{20}H_{19}O_{3}N_{3}: N, 8.38. Found: N, 8.29.
2-Amido-3-n-tetradecylamino-1,4-naphthoquinones

![Chemical Structure](image)

**2-Acetamido-3-n-tetradecylamino-1,4-naphthoquinone (I).**—A mixture of 2.5 g. (.01 mole) of 2-acetamido-3-chloro-1,4-naphthoquinone, 2.13 g. (.01 mole) of n-tetradecyl amine, and 75 ml. of 95 per cent ethanol was refluxed for two hours. The reaction mixture was then diluted to 200 ml. with more ethanol, boiled for two minutes with 2 g. of charcoal, and filtered while hot. The filtrate, upon cooling, yielded 3.15 g. of red-orange crystals. Recrystallization from ethanol gave crystals melting at 141°.

**Anal.** Calcd. for C_{29}H_{33}O_2N_2: N, 6.57. Found: N, 6.78.

**2-Propamido-3-n-tetradecylamino-1,4-naphthoquinone (II).**—2-Propamido-3-chloro-1,4-naphthoquinone (2.4 g., .01 mole) was reacted with n-tetradecyl amine (2.13 g., .01 mole) in the same manner as above to give a 2.9 g. yield of red-orange crystals melting at 124°.

**Anal.** Calcd. for C_{27}H_{40}O_2N_2: N, 6.36. Found: N, 6.38.
2-Butyramido-3-n-tetradecylamino-1,4-naphthoquinone (III).

A mixture of 2.22 g. (.01 mole) of 2-butyramido-3-chloro-
1,4-naphthoquinone, 2.13 g. (.01 mole) of n-tetradecyl amine,
and 75 ml. of 95 per cent ethanol was refluxed for two hours.
The reaction mixture was then diluted to 200 ml. with more
ethanol, boiled for two minutes with 2 g. of charcoal, and
filtered while hot. The filtrate, upon cooling, yielded 2.8 g.
of red-orange crystals. Recrystallization from ethanol gave
crystals melting at 112°.

Anal. Calcd. for C_{38}H_{43}O_{2}N_{2}: N, 6.23. Found: N, 6.16.
3-Hydroxy-1,4-naphthoquinone-2-(4-n-octylpyridinium) Anhydride

To a solution of 23 g. of 2,3-dichloro-1,4-naphthoquinone in 100 ml. of dioxane was added 20 ml. of 4-n-octylpyridine in 50 ml. of dioxane. The solution was warmed to 80° and held at that temperature until it had turned green and no further evidence of reaction was noted. The mixture was allowed to stand five days, then diluted to 475 ml. with water. A brown precipitate formed with the evolution of heat. The precipitate was filtered, washed with water and ether, then boiled with acetic acid and water. This final solution, upon cooling, yielded an orange powder which melted at 215° with decomposition.

2-Propanido-3-p-tolylamino-1,4-naphthoquinone

A solution of 2.5 g. of 2-propanido-3-chloro-1,4-naphthoquinone and 1.2 g. of p-toluidine in 50 ml. of 95 per cent ethanol was refluxed for two hours. At the end of this time, water was added, dropwise, to the cloudpoint and the solution was allowed to cool. The resulting precipitate was collected on a filter, dried, then redissolved in 50 ml. of hot ethanol. This solution was treated with 1 g. of charcoal and filtered while hot. The filtrate, upon cooling, yielded 2.3 g. of bright, purplish-red crystals melting at 165° with decomposition. In determining the melting point it was found that the crystals darkened momentarily at 94°. However, when the temperature was raised above this point the dark color disappeared and the crystals regained their original appearance.

Anal. Calcd. for C_{29}H_{18}O_{2}N_{2}: N, 8.35. Found: N, 8.56.
A solution of 2 g. of 2-propamido-3-chloro-1,4-naphthoquinone and 1 g. of o-toluidine in 50 ml. of 95 per cent ethanol was refluxed for two hours. At the end of this time, water was added, dropwise, to the cloudpoint and the solution was allowed to cool. The resulting precipitate was collected on a filter, dried, then dissolved in 50 ml. of hot ethanol. This solution was treated with 1 g. of charcoal and filtered while hot. The filtrate, upon cooling, yielded 2.1 g. of brown crystals melting at 165° with decomposition.

Anal. Caled. for C_{30}H_{18}O_{3}N_{3}: N, 8.38. Found: N, 8.39.
A mixture of 2.5 g. of 2-propamido-3-chloro-1,4-naphthoquinone and 2 g. of p-tolidene in 75 ml. of 95 per cent ethanol was refluxed for six hours. Water (5 ml.) was added and the solution was placed in the refrigerator and allowed to stand overnight. The dark solid which settled out was collected on a filter, washed with water, then dissolved in 75 ml. of hot ethanol. This solution was treated with 1 g. of charcoal and filtered while hot. The filtrate, upon cooling, yielded 2.3 g. of fine, dark purple crystals which decomposed at 160°.

2-Propamido-3-(2-butylamino)-1,4-naphthoquinone

A mixture of 1.8 g. of 2-propamido-3-chloro-1,4-naphthoquinone and 3 ml. of secondary butyl amine in 50 ml. of 95 per cent. ethanol was refluxed for two hours. Water was added, dropwise, to the cloudpoint and the solution was allowed to cool. The solid material which formed was filtered from the cold mixture and dissolved in 50 ml. of hot ethanol. This solution was treated with 1 g. of charcoal and filtered while hot. The filtrate, upon cooling, yielded 1.7 g. of fine powder, which was orange in color, melting at 111-113°.

Anal. Calcd. for C_{17}H_{25}O_{2}N_{2}: N, 9.33. Found: N, 9.30.
It was desired in this investigation to prepare derivatives of 1,4-naphthoquinone substituted at the 2- and 3-positions with various amino groups. 2,3-Dichloro-1,4-naphthoquinone was selected as the starting material for the subsequent attempts because of the possibility of replacing both chloro groups in direct substitution reactions with various amines or amides.

It was known that in certain reactions it was possible to replace both chloro groups with other structures. For instance, in the reaction of 2,3-dichloro-1,4-naphthoquinone with potassium phenolate, both chlorines are replaced, giving 2,3-diphenoxy-1,4-naphthoquinone.\(^1\) Pyridine replaces both chlorines, giving 3-hydroxy-1,4-naphthoquinone-2-pyridinium anhydride.\(^2\) However, in most reactions, particularly with primary and secondary amines, only one chlorine is replaced from the 2,3-dichloro-1,4-naphthoquinone. Even with boiling alcoholic potassium hydroxide, only one chlorine is replaced.

Fries and Oehwät prepared 2-amino-3-chloro-1,4-naphthoquinone and discovered that acylation of the amino group in

that compound rendered the chlorine atom reactive toward ammonia, amines, and alcohohates. This reactivity suggested the general procedure for the preparation of the compounds in this series.

2-Amino-3-chloro-1,4-naphthoquinone was prepared from 2,3-dichloro-1,4-naphthoquinone. The amino compound was then treated with acetic, propionic, or butyric anhydride to give, respectively, acetamido, propamido, or butyramido grouping in the 2- position. The 2-amido-3-chloro-1,4-naphthoquinone thus obtained was reacted with various amines to give the desired 1,4-naphthoquinones which were substituted at both the 2- and 3- positions with amide and amine groupings.

Attempts to replace only one chlorine by direct reaction with amides were unsuccessful. In the first of these attempts, urea, sodium acetate, and 2,3-dichloro-1,4-naphthoquinone, in equimolar proportions, were refluxed in ethanol. The product was found to be 2-chloro-3-ethoxy-1,4-naphthoquinone. Thiourea, sodium acetate, and the dichloro compound, in equimolar quantities, were refluxed in ethanol to give a dark purple powder. This product, containing both chlorine and sulfur, failed to melt below 290° and charred when placed in an open flame, leaving a black residue. The substance was very slightly soluble in hot dioxane and completely insoluble in other organic solvents. Attempts to recrystallize were unsuccessful.

Several diamines, and also compounds containing an amino group and a hydroxy group attached to adjacent carbon atoms, were refluxed with 2,3-dichloro-1,4-naphthoquinone in various
solvents in attempts to form a closed ring structure. It was expected that the amine group would attach at the 2- position, with the elimination of HCl, and that a second molecule of HCl could be removed with some base, allowing the formation of a six-membered ring, incorporating two hetero atoms bonded to the 2- and 3- carbons of the naphthoquinone nucleus.

In the reactions with diamines, such as ethylene diamine and o-phenylene diamine, benzene was used as the solvent and sodium acetate was added as the base. The products were similar in nature to the substance obtained in the reaction of the 2,3-dichloro-1,4-naphthoquinone with thiourea. Each product was a dark powder, contained chlorine, was insoluble in most organic solvents, failed to melt below 290°, and burned with charring, leaving a black residue.

The reactions of the compounds which contained adjacent amino and hydroxy groups, such as ethanolamine and (+)-threo-2-amino-1-p-nitrophenyl-1,3-propanediol, with 2,3-dichloro-1,4-naphthoquinone were carried out in methanol, using sodium acetate again as the base. In these reactions, the products were dark red, oily gums. Each was fairly soluble in acetone, methanol, dioxane, and ethyl ether. All attempted recrystalizations yielded the same gummy, oily substance.

In the reactions involving the replacement of the chlorine atom with various amine groups in the 2-amido-3-chloro-1,4-naphthoquinones, unexpected difficulties arose in the attempt to introduce an amino group with concentrated ammonium hydroxide. The reactions were carried out in boiling alcohol, and in each case
a mixture of products was obtained. Fractional recrystallizations failed to give a pure product. When p-aminobenzene-sulfonylamide was refluxed with the different 2-amido-3-chloro-1,4-naphthoquinones, the reactions seemed to go satisfactorily; however, the amount of product in each case was very low (less than 10 per cent yield). The substances obtained were very slightly soluble in alcohol and dioxane, and purification was not accomplished.

In general, the conditions were the same for the different reactions of the 2-amido-3-chloro-1,4-naphthoquinones with the various amines. Ethanol was used as the solvent in each of these reactions, since both the 2-amido-3-chloro-1,4-naphthoquinones and the amines were fairly soluble in this medium. Recrystallization, with the exceptions already noted, was easily accomplished using this same solvent. Most of the products from these reactions were highly colored, ranging from bright orange to red to dark purple. As a general rule, the melting points decreased with an increase in the number of carbons in the amido chain, and increased with an increase in the number of carbons in the amino grouping. Reaction time varied somewhat for the different amines; two hours being sufficient for most, though some of the heavier amines required up to six hours refluxing for maximum yields. The compounds, with the exception of those already mentioned, were all soluble in hot alcohol, dioxane, and acetone, and were easily precipitated from these solvents with water. No study was made of the effect of acids and bases on these compounds.
Mechanism for the Reaction of 4-n-Octylpyridine with 2,3-Dichloro-1,4-naphthoquinone

(4) Ullman and Ettisch, op. cit., 54B, 259-72. These workers proposed a mechanism for the reaction of pyridine with 2,3-dichloro-1,4-naphthoquinone which is analogous to the mechanism for the reaction of 4-n-octylpyridine with that same compound.
The formation of 3-hydroxy-1,4-naphthoquinone-2-(4-n-octylpyridinium) anhydride prepared in this investigation by the reaction of 2,3-dichloro-1,4-naphthoquinone with 4-n-octylpyridine, has been assumed to follow a route analogous to the reaction of pyridine with 2,3-dichloro-1,4-naphthoquinone. The 4-n-octylpyridine first adds to form compound I. Under the influence of water or alcohol, the excess 4-n-octylpyridine behaves as a base, acting upon compound I to form either II or III. The intermediate, II or III whichever the case may be, loses HCl to give the final compound IV, which is 3-hydroxy-1,4-naphthoquinone-2-(4-n-octylpyridinium) anhydride.

All melting points reported in this investigation were taken on a Fischer-Johns melting point block and are uncorrected. The nitrogen analyses were by the micro Dumas method.
BIBLIOGRAPHY

Articles

Buu Hol, Bull. soc. chim., 11, 578-84 (1944).

Fries, K., and Ochwat, P., Ber., 56, 1291-304 (1923).


Ullman, F., and Ettisch, M., Ber., 54B, 259-72 (1921).