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Reactions of phenoxazine and some of its derivatives

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REACTIONS OF PHENOXAZINE AND SOME OF ITS DERIVATIVES

by

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Dean of Graduate College

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INTRODUCTION

Phenoxazine and its derivatives have not received a great deal of attention either theoretically or commercially. The only derivatives of commercial importance are a few dyes, called the oxazine dyes.[1,2] The research on this compound has been closely related to these dyes and no general systematic study of substitution reactions has been made. This may be due in part to the ready oxidation of phenoxazine to phenoxazone.

The ready oxidation to the oxazone has led to a series of reactions in which the 3-position is attacked by amines and by carbonyl compounds. A little work has been done on the nitration of 10-acetylphenoxazine and related compounds, and a few N-substituted compounds have been prepared.

There has never been a general review of the properties of phenoxazine and its derivatives, though short discussions have been included in several books.[3,4]

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[1]K. Venkataraman, "The Chemistry of Synthetic Dyes," Vol. II, Academic Press, Inc., New York, N. Y., 1952, pp. 780-791.

[2]H. A. Lubs, ed., "The Chemistry of Synthetic Dyes and Pigments," Reinhold Publishing Corp., New York, N. Y., 1955, pp. 259-261.

[3]D. E. Pearson in R. C. Elderfield, "Heterocyclic Compounds," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1957, pp. 684-705.

[4]A. A. Morton, "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1946, pp. 478-480.

This study has been undertaken to review the known chemistry of phenoxazine and to study the relative directive influence of the nitrogen and the oxygen atoms of this compound, and the effect on the directive influence of the nitrogen caused by substituting the nitrogen with various groups such as ethyl, phenyl, and acetyl.

Since some polynuclear heterocycles also are known to act as scintillators in radiation counters,[5] a series of N-aryl phenoxazine compounds has been prepared which are being tested as solutes for these counters.

Some of the oxazine dyes, notably the Nile Blue dyes, are known to stain brain cancer tissue preferentially, and some decrease the rate of growth of tumors. An interest in this use of these compounds is closely related to the use of boron as a source of *in vivo* radiation, since it must be principally in the carcinogenic tissue to be most useful. The methods of preparing boronic acids was studied in an effort to find a method of preparing a boron derivative of phenoxazine, or ideally a Nile Blue dye, which might be useful in cancer chemotherapy.

Because of the similarity of this compound to other heterocycles whose chemistry has been investigated much more thoroughly, frequent recourse has been made to the literature

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[5] F. Newton Hayes, *International Journal of Applied Radiation and Isotopes*, 1, 46 (1956).

concerning these heterocycles. These include carbazole, dibenzofuran, phenothiazine and phenoxathiin.

HISTORICAL

The historical section of this dissertation includes a survey of the methods of preparation and the properties of phenoxazine and its known derivatives. The literature is covered completely through 1955, and the period between January 1956 and June 1957 is covered as completely as possible by survey of the more important and more accessible journals and by a scrutiny of the heterocyclic section of Current Chemical Papers. This publication contains a classified world list of the new papers in pure chemistry.

The chemistry of the boronic acids is considered only since the appearance of the review article by Lappert.[6]

Nomenclature

Phenoxazine has not been known by a large number of different names, phenazoxine being the only common synonym. This is probably due to the fact that most of the early work was done by a single group of workers.

Several systems of numbering this compound have been used. The system which is employed in this dissertation is illustrated below (I) and is that preferred by Chemical Abstracts since 1940. Before that time the system illustrated

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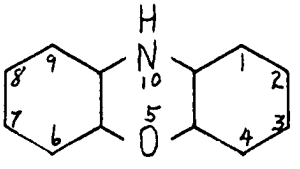
[6] M. F. Lappert, Chem. Revs., 56, 959 (1956).

by III was used. Patterson and Capell[7] have given several ways of numbering but prefer I. In the recent volume by Elderfield[3] the method illustrated by II was used since it was the one used in Beilstein.[8]

All names used in this dissertation are changed to correspond to the numbering system I.



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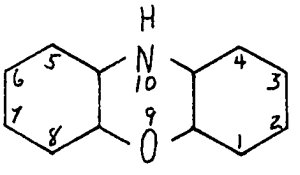

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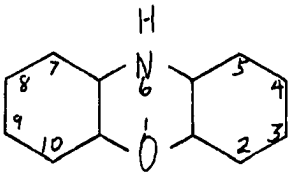

III

Preparation

The preparation of phenoxazine and its derivatives has been almost exclusively by cyclization reactions. These have been of several types, depending upon the starting materials and the conditions used. The discussion will be broken down by the nature of the reaction rather than by the type of product which is formed. By far the majority of cyclization reactions used in preparing phenoxazine and its derivatives has been a series of one step reactions rather than procedures

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[7]A. M. Patterson and L. T. Capell, "The Ring Index," Reinhold Publishing Corp., New York, N. Y., 1940, p. 250.

[8]F. Richter, ed., "Beilstein's Handbuch der Organischen Chemie," 4th ed., 2nd sup., Vol. XXVII, Springer-Verlag, Berlin, 1955, p. 31.

which first form an aryl amine or an aryl ether. There probably is little difference in the two methods except perhaps the extra work involved in isolation of the intermediate. The discussion will first consider the direct cyclization in one step, and then the cases in which an aryl amine or an aryl ether have been isolated.

Aminophenols with hydroxy compounds

The preparation of the parent compound, phenoxazine, was first reported by Bernthsen,[9] who reacted an intimate mixture of o-aminophenol and catechol in a tube for 40 hours at 260-280°. Later workers[10] reported that a modification of the method of Bernthsen produced yields of 50-60%. The preferred method now is one reported by Kehrmann and Niel[11] in which an equimolar mixture of o-aminophenol and o-aminophenol hydrochloride was heated to 240° in a stream of carbon dioxide rather than in a sealed tube. This method when used for 0.1 mole has given 40-50% yields,[12] even though only 30 minutes heating time was used.

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[9]A. Bernthsen, Ber., 20, 942 (1887).

[10]F. Kehrmann, C. Stampa, P. Thomas, W. Urech and G. Herrmann, Ann., 322, 9 (1902).

[11]F. Kehrmann and A. A. Niel, Ber., 47, 3102 (1914).

[12]S. Granick, L. Michaelis and M. P. Schubert, J. Am. Chem. Soc., 62, 1802 (1940).

One variation which has been used is the addition of a small amount of o-aminophenol hydrochloride to an equimolar mixture of o-aminophenol and catechol.[6] This is reported to have given 70% of phenoxazine when used to prepare small amounts.

Other means of preparing phenoxazine which have been claimed are the reduction of o-nitrophenyl phenyl ether with an oxygen acceptor[13] and the decomposition of 2-azidophenyl phenyl ether.[14] The product in the latter reaction was identified by its color with various reagents and by the formation of a green picrate which had no characteristic melting point. Also, phenoxazine has been prepared in about 12% yield by heating 2-amino-2'-hydroxy diphenyl ether in a sealed tube for 40 hours at 270-280°.[15]

Although less drastic methods are generally preferred for nuclear substituted phenoxazines, several have been prepared by the first cyclization described above. Generally this is used for stable groups of which the benzo and the alkyl groups are the most common. Phenanthazoxin has been prepared by heating 9,10-dihydroxyphenanthrene in a stream

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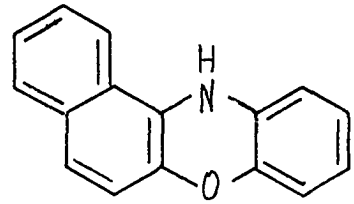
[13] H. C. Waterman and D. L. Vivian, U. S. Patent 2,292,808 [C. A., 37, 892 (1943)].

[14] P. A. S. Smith, B. B. Brown, R. K. Putney and R. F. Reinisch, J. Am. Chem. Soc., 75, 6335 (1953).

[15] N. M. Cullinane, H. G. Davey and H. J. H. Padfield, J. Chem. Soc., 1934, 716.

of ammonia.[16] In this reaction 9-amino-10-hydroxyphenanthrene seems to be an intermediate. By similar methods the three isomeric benzophenoxazines have been prepared. 1,2-benzophenoxazine, which is the parent compound of the Meldola



1,2-Benzophenoxazine

Blue and Nile Blue dyes, is prepared from o-aminophenol and 1-amino-2-naphthol hydrochloride at 260° under a carbon dioxide atmosphere.[17]

The 2,3-benzo-isomer is prepared from 2,3-dihydroxynaphthalene and o-aminophenol,[11] and the 3,4-isomer, the parent compound for the Isomeldola Blue dyes, from 1,2-dihydroxynaphthalene and o-aminophenol.[17]

Methyl substituted,[11,18a] and tert-octyl[18b] substituted phenoxazine have also been made, as well as 3-hydroxyphenoxazine.[12]

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[16] E. Bamberger and J. Grob, Ber., 34, 533 (1901).

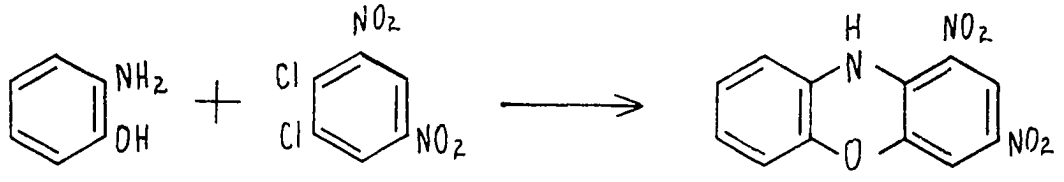
[17] H. Goldstein and Z. Ludwig-Semelitch, Helv. Chim. Acta, 2, 655 (1919).

[18a] F. Kehrmann, Ber., 34, 1623 (1901).

[18b] J. B. Niederl, U. S. Patent 2,483,838 [C. A., 44, 2035 (1950)].

Aminophenols with active halogen compounds

The reaction of o-aminophenol with an o-chloronitrobenzene has been used a great deal in forming diaryl amines which were later cyclized by loss of nitrous acid. Two cases have been reported in which hydrogen chloride was eliminated in the cyclization step,[19,20a] and one in which hydrogen iodide is eliminated.[20b] These results show that the elimination of hydrogen halide is favored over the elimination of nitrous acid in both neutral and basic solution.



Aminophenols with ortho-quinones and ortho-hydroxyquinones

The condensations of o-aminophenols with o-quinones and o-hydroxyquinones occur readily, usually within several hours at 80-100°. These reactions are generally run in the presence

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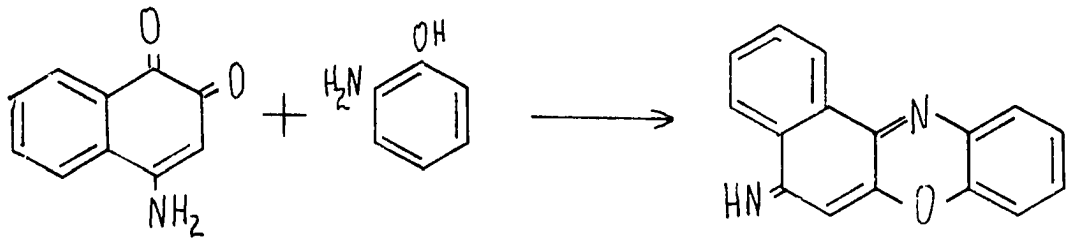
[19] O. L. Brady and C. Waller, J. Chem. Soc., 1930, 1218.

[20a] F. Ullman and S. M. Sane, Ber., 44, 3730 (1911).

[20b] S. S. Joshi and S. M. Sane, J. Indian Chem. Soc., 10, 459 (1933) [C. A., 28, 469 (1934)].

of acid, either using the salt of the reacting amine or adding a mineral acid to the reaction mixture. This type reaction was first reported in 1905,[21] and since a symmetrical quinone was used, there was no question of position of the nitrogen in relation to nuclear substituted groups in the heterocycle. Since that time, unsymmetrical quinones have been used in this reaction and it was shown that the condensation occurs in such a way that a para-quinoid structure is present in the product.[22,23,24,25] When an acetamido group is para to one of



the oxygens of the quinone then the main product is that in which the oxygen of the heterocycle is para to the acetamido

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[21] F. Kehrmann, Ber., 38, 2952 (1905).

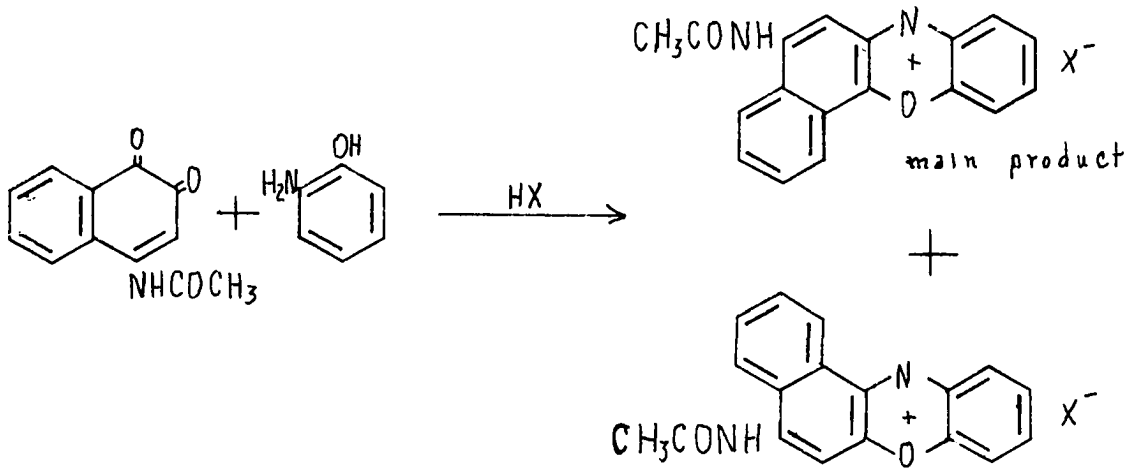
[22] F. Kehrmann, ibid., 38, 3605 (1905).

[23] F. Kehrmann, ibid., 40, 2071 (1907).

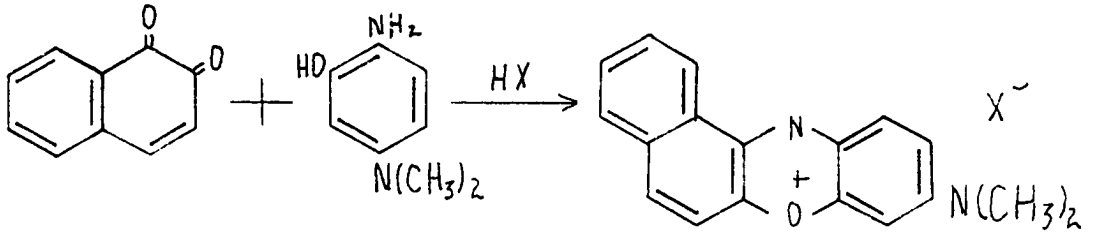
[24] F. Kehrmann, Ann., 414, 157 (1918).

[25] F. Kehrmann, E. Gillet and P. Borgeaud, Helv. Chim. Acta, 9, 866 (1926).

group.[22,24,25] In a few cases where no other hetero atom is



present in the quinone except the two oxygens, as for example in 1,2-naphthoquinone, the product which is formed is determined by whatever groups are present.[25] Recently this re-



action of o-aminophenol with o-quinones has been used to synthesize a series of phenoxazine derivatives as possible tumor retarding agents.[26,27,28]

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[26]M. L. Crossley, P. F. Dreisbach, C. M. Hofmann and R. P. Parker, J. Am. Chem. Soc., 74, 573 (1952).

[27]M. L. Crossley, R. J. Turner, C. M. Hofmann, P. F. Dreisbach and R. P. Parker, ibid., 74, 578 (1952).

[28]M. L. Crossley, C. M. Hofmann and P. F. Dreisbach, ibid., 74, 584 (1952).

Similar to the general class reaction discussed above is the reaction of 2,4,5-tris(toluino)toluene with sulfuric acid to form 3,7-dimethyl-2-toluinophenoxazine.[29]

Phenols with nitroso compounds

The first reported preparation of a phenoxazine derivative which was useful as a dye was by Meldola[30] in 1879. This preparation was effected by reacting p-nitrosodimethylaniline with beta-naphthol and gave a blue dye which was later identified as 7-dimethylamino-1,2-benzophenoxazonium chloride. A few years later the reaction of p-ditrosodimethylaniline with gallic acid was used for making a dye,[2] but it was several years before the structure of this compound was determined.[31]

Recently this reaction of a nitroso compound with a phenol has been used for the preparation of phenoxazine derivatives as possible growth retarding agents for tumors[27,28] and as possible antituberculosis agents.[32]

This reaction occurs very easily, requiring refluxing in acetic acid for several minutes. In one case spontaneous reflux occurred.[32] The reaction is acid catalyzed and both

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[29]E. Börnstein, Ber., 43, 2380 (1910).

[30]R. Meldola, ibid., 12, 2065 (1879).

[31]R. Nietzki and R. Otto, ibid., 21, 1736 (1888).

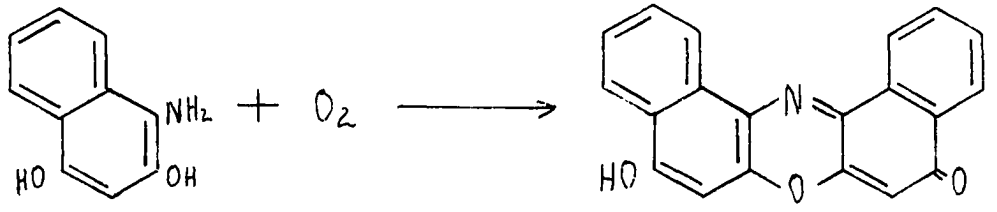
[32]R. A. Clapp, J. H. Clark, J. P. English, C. E. Fellows, R. E. Grotz and R. G. Shepherd, J. Am. Chem. Soc., 74, 1989 (1952).

zinc chloride and hydrochloric acid have been used successfully, however zinc chloride often causes difficulty in purification because of double salt formation.[27]

Nearly identical to the reaction described above is the reaction when an hydroxyl and a nitroso group are both on the same aromatic ring.[33] This condensation occurs under the same conditions as described above. This reaction has been used to prepare biological stains,[34] tuberculostatic agents,[32] and tumor-retarding agents.[26,27,28]

Oxidative cyclizations

Several cyclizations have been reported in which oxidation occurs during ring closure. Some have been where a condensation would normally be expected and oxidation of the expected phenoxazine derivative to a phenoxazone or to a phenoxazonium salt occurs.[35] In other cases the oxidation



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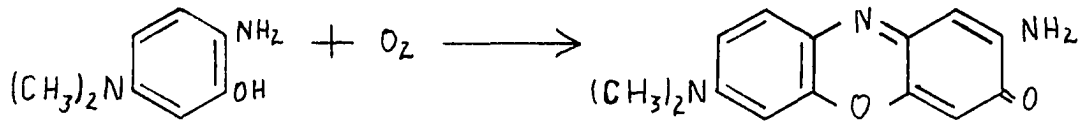
[33] R. Möhlau and K. Uhlmann, Ann., 289, 90 (1896).

[34] H. A. Slovitor, J. Am. Chem. Soc., 71, 3360 (1949).

[35] F. Kehrmann, Ber., 28, 353 (1895).

is necessary to obtain the phenoxazine derivatives.[36a] The nature of the product expected varies with the conditions used, for example, 4-dimethylamino-2-hydroxyaniline when heated in slightly acid solution produces 3,7-tetramethyldiaminophenoxazonium chloride[36b] but when heated in an alkaline solution forms 2-amino-7-dimethylaminophenoxazone.[36c]



Several reactions of o-aminophenols with p-quinones have been reported in which the corresponding phenoxazone has been produced.[23,35,37,38] Though no mention is made of oxidation, these reactions were run in the presence of air and oxidation has taken place.

In considering oxidative cyclizations it should be recognized that the nitroso-phenol cyclization actually involves oxidation-reduction between the two molecules.

[36a] H. Okamoto, Japan Med. J., 1, 422 (1948) [C. A., 45, 5757 (1951)].

[36b] F. Kehrmann and W. Poplawski, Ber., 42, 1275 (1909).

[36c] R. Möhlau, ibid., 25, 1055 (1892).

[37] F. Kehrmann and J. Messinger, ibid., 26, 2375 (1893).

[38] F. Henrich and P. Roters, ibid., 41, 4210 (1908).

Of important historical interest is the chemistry of resorufin and resozurin. These are, apparently, the first phenoxazine derivatives to be prepared, although they were not identified as such when they were prepared. Weselsky[39,40] in 1871 reported the reaction of resorcinol with nitric acid to give a complex compound comprising three resorcinol molecules and one of nitrogen trioxide. This he called diazoresorcin. He then prepared a second compound which was closely related except for the loss of water and this he called diazoresorufin. This same reaction has been used more recently and the products identified as 7-hydroxyphenoxazone (resorufin) and 7-hydroxyphenoxazone oxide (resozurin).[41,42] The structures of these compounds are given in the section on oxidation reactions of phenoxazine.

Related to these oxidation reactions is the reaction of p-quinone-N,N-dichlorodiimide with phenols. This compound has been reacted with beta-naphthol to produce 1,2-benzo-7-phenoxazime,[23,31] and with resorcinol and orcinol to give aminophenoxazone derivatives.[42]

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[39]P. Weselsky, ibid., 4, 32 (1871).

[40]P. Weselsky, ibid., 4, 613 (1871).

[41]R. Nietzki, A. dietze and H. Maeckler, ibid., 22, 3020 (1889).

[42]R. Nietzki and H. Maeckler, ibid., 23, 718 (1890).

Reductive cyclizations

There is only one example of obvious reductive cyclization, and this is the reduction of o-aminophenol with hydrogen over platinum. There is produced from this reaction 85% of cyclohexyl amine and 15% of perhydrophenoxazine.[43]

Ring closure of diarylamines and diarylethers

The most common of the ring closure reactions involves the loss of nitrous acid from a 2-nitro-2'-hydroxydiaryl amine. This is often called Turpin's reaction because of his early work with it.[44] The intermediate amine can be isolated and then cyclized, or it can be formed and cyclized in a single step.

It has been generally assumed by many authors that the 6-position, meta to the nitro group, must be occupied by some bulky group such as nitro, methyl, carboxyl, or sulfonic acid.[19,20,45,46,47,48] This is generally true when the amine is a secondary amine. It is thought that this is a steric

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[43]A. Skita and H. Rolfes, ibid., 53B, 1242 (1920).

[44]G. S. Turpin, J. Chem. Soc., 59, 714 (1891).

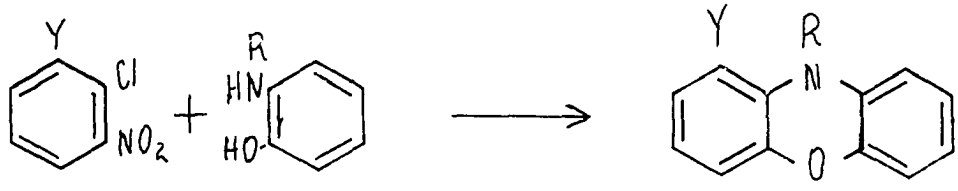
[45]Ger. Patent 200,736 [C. A., 3, 121 (1909)].

[46]Ger. Patent 396,514 [C. A., 5, 383 (1911)].

[47]F. Ullmann, G. Engi, N. Wosnessensky, E. Kuhn and E. Herre, Ann., 366, 79 (1909).

[48]E. Misslin and A. Bau, Helv. Chim. Acta, 2, 285 (1919).

effect, since both electron donating and electron withdrawing groups are effective as are groups which will chelate and those which will not.[19] This is further shown by the use of N-substituted aryl amines with only a hydrogen opposite to the nitro group. With methyl[49] or benzyl[50,51] on the nitrogen, the steric requirements are fulfilled and cyclization occurs, though no other groups are on the ring with the nitro group. Yields of over 50% have been obtained from the benzyl derivatives.



Another explanation which has been given[3] involves the concept of resonance. This is that the group opposite to the nitro group interferes with the resonance of the bond between the ring and the nitro group and thus weakens that bond, making displacement easier.

One case has been reported where ring closure has occurred with no group on the nitrogen amine and no substituent in the

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[49]K. C. Roberts and H. B. Clark, J. Chem. Soc., 1935, 1312.

[50]B. Boothroyd and E. R. Clark, ibid., 1953, 1499.

[51]B. Boothroyd and E. R. Clark, ibid., 1953, 1504.

6-position on the ring with the nitro group. This was the reaction of 2,4-dinitrochlorobenzene with o-aminophenol to produce a few per cent of 3-nitrophenoxazine.[52]

Although the majority of these reactions occurs through the diaryl amine, some must occur through the diaryl ether, because of the products isolated.[49,53,54] A study of the rearrangements of o-aminoaryl ethers has shown that these ethers generally undergo ready rearrangement to the diaryl amines before cyclization.[55]

The diaryl amine for cyclization is usually formed from an o-chloronitrobenzene and an o-aminophenol. This reaction is carried out by heating the reactants in a basic solution, and can be followed and stopped at the diaryl amine, or carried on to form the phenoxazine derivative. Not all 2-nitro-2'-hydroxydiaryl amines are formed by this means. Many, especially substituted compounds have been made from a substituted o-dinitrobenzene and an o-aminophenol.[53] The 2-nitro-2'-hydroxydiaryl amine thus produced is cyclized as discussed above.

Turpin[44] was the first to use this reaction in making

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[52]F. Kehrmann and M. Ramm, Ber., 53B, 2265 (1920).

[53]K. C. Roberts and C. G. M. deWorms, J. Chem. Soc., 1934, 727.

[54]K. C. Roberts and C. G. M. deWorms, ibid., 1935, 1309.

[55]K. C. Roberts, C. G. M. deWorms and H. B. Clark, ibid., 1935, 196.

1,3-dinitrophenoxazine. This reaction has since been repeated by other workers,[10,50,51,56,57,58] and there have been produced other nitrophenoxazines, carboxylic acids,[46,47,51] sulfonic acids,[46,47,50] acetamido compounds,[48] methyl compounds,[20,48,51] benzoyl compounds,[47] and halogen compounds.[20a, 20b,48,50,51,59]

In the cyclization of other diaryl amines, water[60,61] and methanol[62] have been eliminated.

N-Substitution Reactions

Acetylation of the heterocyclic nitrogen seems to occur quite readily, though it has been reported[44] that 1,3-dinitrophenoxazine does not form an acetyl derivative. Phenoxazine,[57,63] 3-nitro-,[52] 3-acetamido-,[63] 3-7-dimethyl-,[63] and

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[56]F. Kehrmann, Ber., 32, 2601 (1899)

[57]F. Kehrmann and A. Saager, ibid., 36, 475 (1903).

[58]F. Kehrmann and H. Goldstein, Helv. Chim. Acta, 4, 26 (1921).

[59]S. S. Joshi and R. S. Gupta, J. Indian Chem. Soc., 29, 193 (1952) [C. A., 47, 3255 (1953)].

[60]H. Leuchs and G. Theodorescu, Ber., 43, 1239 (1910).

[61]H. Leuchs, Ann., 460, 1 (1928).

[62]H. E. Fierz-David, J. Brassel and F. Probst, Helv. Chim. Acta, 22, 1348 (1939).

[63]F. Kehrmann and A. Boulis, Ber., 50, 1662 (1917).

2,3-benzophenoxazine[11,64] have all been acetylated by refluxing in acetic anhydride for a few hours.

Though most related heterocycles containing an N-H bond have been converted to N-alkyl and N-aryl compounds, there have been few of either alkyl or aryl derivatives of phenoxazine made. Morris[65] prepared some N-sulfolanyl- and N-sulfolenylphenoxazines by substitution. Some benzyl,[50,51] nitrophenyl[54] and a methylphenoxazine[49] have been made by cyclization for testing as tuberculostatic agents and in the study of the mechanism of ring closure.

A series of 10-substituted phenoxazines was prepared early in this study and has been reported elsewhere.[66] The reactivity of the nitrogen here seems to differ little from that of phenothiazine and carbazole, since the methods which have been used for these compounds were found to be adequate for phenoxazine. The alkyl derivatives were prepared in liquid ammonia using 10-sodiophenoxazine and an alkyl halide. The aryl compounds were prepared by heating phenoxazine with an aryl iodide or bromide in the presence of anhydrous potassium carbonate and copper-bronze powder. The aryl bromides require higher temperatures than do the aryl iodides and the

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[64]F. Kehrmann and A. A. Niel, ibid., 47, 3107 (1914).

[65]R. C. Morris and A. V. Snider, U. S. Patent 2,530,070 [C. A., 45, 2983 (1951)].

[66]H. Gilman and L. O. Moore, J. Am. Chem. Soc., 79 (1957), in press.

presence of iodine in the reaction when using an aryl bromide does not have any noticeable effect on the reaction.

Nuclear Substitution Reactions

The substitution reactions of phenoxazine have been mainly substitution of amines and oxygen into the 3-position in phenoxazonium salts. This type of reaction was first reported by Witt[67] in attempting to make Meldola's Blue, and studied by Hirsch and Kalckhoff[68] to make new dyes using aniline, p-toluidine, alpha-naphthyl amine, and p-dimethylaminoaniline. The last compound was used to prove the structure of the compound prepared by Witt. It has since been studied quite extensively by Kehrmann and co-workers,[10,18a,22,23,69,70] who showed that ammonia, aniline and oxygen would react with unsubstituted phenoxazonium salts as well as some nitro and amino substituted compounds.

Because of the unusual tissue staining properties of some of the phenoxazine dyes, Thorpe[71] studied the air oxidation of them and the hydrolysis of the amino group in the 3-position. It was found that the hydrolysis of this amino

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[67] O. N. Witt, Ber., 23, 2247 (1890).

[68] R. Hirsch and F. Kalckhoff, ibid., 23, 2992 (1890).

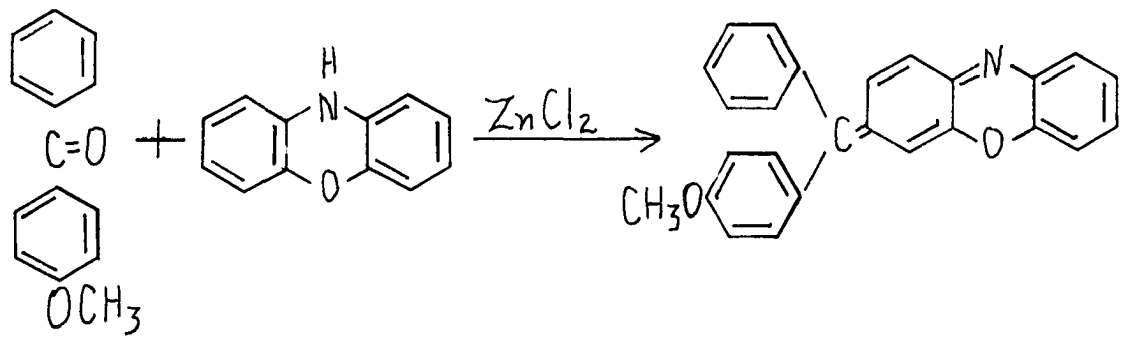
[69] F. Kehrmann, Ann., 322, 1 (1902).

[70] F. Kehrmann, Ber., 42, 347 (1909).

[71] J. F. Thorpe, J. Chem. Soc., 1907, 324.

group occurred rapidly in hot water when the amino nitrogen was unsubstituted or substituted with an alkyl group, but much slower when substituted with an aromatic or benzyl group. Oxidation of the 3-position occurs quite rapidly when no amino group is present and is acid catalyzed. The pure amino dyes generally do not fluoresce and thus, when a product fluoresces, it must be at least partially in the phenoxazone form.

Quite similar to the nitrogen and oxygen substitution reactions in the 3-position are the carbonyl condensations which occur also in the 3-position.[72] These and the related reaction of dichlorobenzophenone are Friedel-Crafts type reactions, in that a G. N. Lewis acid is used as a catalyst. These reactions were carried out in a study of spectra of phenoxazine and its derivatives, and compounds such as chromone and isochromone, which form stable radicals, were included.



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[72]R. Wizinger and S. Chatterjee, Helv. Chim. Acta, 35, 316 (1952).

Phenoxazine, because of the ease of oxidation, has never yet been nitrated directly. Bernthsen reported the nitration of phenoxazine with nitric acid to give a yellow compound, but this was not further characterized. Partially nitrated phenoxazine, which is much less susceptible to oxidation, can be nitrated.[57] Nitration of 10-acetylphenoxazine proceeds readily to produce 3,7-dinitro-10-acetylphenoxazine and a little 1,3,7,9-tetranitrophenoxazine.[57] Similarly, 2,3-benzo-10-acetylphenoxazine has been nitrated to give mono and tetranitro derivatives.[11,64] On the basis of the nitration products from 10-acetylphenoxazine, it was postulated that the mononitro-2,3-benzo-10-acetylphenoxazine was the 7-nitro derivative.

The first reported reaction of phenoxazine with bromine was by Bernthsen in 1887 who described the solution obtained as a dirty deep blue.[9] Since that time bromine has been used for oxidizing phenoxazines to the onium salts. Phenanthazoxin with bromine forms a perbromide,[18a] but only one nuclear bromination reaction has been made, and that is on resazurin to give a tetrabromo derivative.[41]

Oxidation Reactions

The simple oxidation of phenoxazine to a phenoxazonium salt occurs very rapidly[69,73] but this salt undergoes ready

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[73]F. Kehrmann and L. Löwy, Ber., 44, 3006 (1911).

oxidative substitution in the 3- and 7-positions,[23,69,73] para to the nitrogen. If the nitrogen is substituted such easy oxidation is precluded. This oxidation is reversible and a phenoxazine can be obtained from the onium salt by reduction with stannous chloride and hydrochloric acid.

In a study of the oxidation and the spectra of phenoxazine compounds,[63,74] it was shown that phenoxazine in concentrated sulfuric acid forms a "meri-quinonoid" monoacid salt. On the addition of hydrogen peroxide there was formed a "holo-quinonoid" diacid salt, which on dilution with acetic acid changes to the mono-acid salt. These various salts had various colors depending upon the groups on the phenoxazine nucleus, but their spectra were similar for the various types of salts. The holo-quinoid salts are stable only at 0-10° and decompose slowly, substituting the 3-position.

In 1940 Michaelis and co-workers[12,75,76] undertook a study to prove that:

> ... the reversibility of any bivalent oxidation-reduction depends on the fact that an intermediate univalent reaction product, a free radical, is capable of existence in sufficient concentration so that this concentration is not a limiting factor for the rate of the over-all process of the bivalent oxidation or reduction.[76]

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[74]F. Kehrmann and M. Sandoz, ibid., 50, 1667 (1917).

[75]L. Michaelis, S. Granick and M. P. Schubert, J. Am. Chem. Soc., 63, 351 (1941).

[76]L. Michaelis and S. Granick, ibid., 63, 1636 (1941).

In keeping with this main objective oxazine and thiazine derivatives were chosen since they form cations which can be oxidized to easily detectable free radicals in both acidic and basic media. The optical properties of the compounds were the means of detecting the free radicals, and very distinct potential curves over the pH range -8 to +14 were obtained. Oxonine, 3,7-diaminophenoxazine, was chosen for the main study because of its suitability over the entire range.

General studies of related heterocycles have shown that phenoxazines, phenothiazines and phenoselenazines, as diamino, monoamino and hydroxy compounds all form relatively stable semiquinone free radicals, of which the phenoxazine radicals are the least stable. Some of the compounds studied formed stable reduced forms or free radicals, but not stable holoquinoid forms.

The potentials for forming the free radicals for the three heterocycles are:[75]

phenoxazine	+0.724 volt
phenothiazine	+0.701 volt
phenoselenazine	+0.769 volt

A similar study of oxidation potentials was made on the resazurin-resorufin system[77] which, because of the colors

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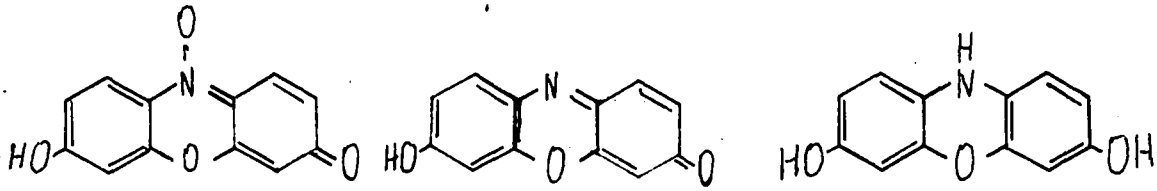
[77]R. S. Twigg, Nature, 155, 401 (1945).



resazurin (blue) | resorufin (pink) | dihydroresorufin (colorless)

produced, has been used for testing the bacterial content of milk. The two oxidation potentials were studied over a wide range of pH.

Oxidations have been carried out also by illumination in a rigid solvent.[78] The primary product is formed by the loss of an electron, and then this loses a proton to give a secondary product. The quantum yields were 0.1 for dilute and 0.04 for concentrated solutions. The authors state that a dimer of the leuco base exists and is responsible for the final products of the reaction. However, magnetic measurements indicate that there is no dimerization of the oxonine semiquinone.[79] How this might carry over to the compounds studied in the photo-oxidation is not known.

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[78] G. N. Lewis and J. Bigeleisen, J. Am. Chem. Soc., 65, 2419 (1943).

[79] L. Michaelis, ibid., 63, 2446 (1941).

Other Reactions

Some of the reactions which have been used on phenoxazine derivatives do not directly relate to the chemistry of the ring. The use of these reactions in the chemistry of phenoxazine will be discussed in this section.

Since the various nitrophenoxazines can be prepared quite readily by cyclization and there has been interest in the aminophenoxazines regarding their spectra as it relates to structure and dyeing properties, there has been some study on the reduction of the nitrophenoxazines to the corresponding amino compounds.

Bernthsen[9] was the first to report the reduction of a nitrophenoxazine. He reduced his yellow product from nitration and obtained a red-violet amino compound, identified only by its color with various reagents. This reduction was with tin and hydrochloric acid. This reagent was not used again until 1953, when Boothroyd and Clark[50] reduced 3-nitrophenoxazine-1-sulfonic acid in an excellant yield.

The most common reducing agent used for nitrophenoxazines was stannous chloride with hydrochloric acid. This was used to reduce 1-nitro-,[51,73] 3-nitro-,[52] 1-nitro-8-chloro-,[51] 1-nitro-3-carboxy-,[51] 3-nitro-1-carboxy-,[51] 1,3-dinitro-,[10] 3,7-dinitro-,[57] 1,3,7-trinitro-,[57] and 1,3,7,9-tetranitrophenoxazine.[57]

Zinc with ammonium chloride has been used to reduce

3-nitrophenoxazine-1-sulfonic acid.[47]

Catalytic reduction of the nitrophenoxazines is fairly recent, having been done only in 1953.[50,51] Hydrogen with palladium oxide was used at room temperature and atmospheric pressure to reduce 3-nitro-10-benzylphenoxazine nearly quantitatively. Use of a higher temperature or use of palladium chloride on carbon as catalyst reduced the benzyl group also to give a considerable amount of 3-aminophenoxazine.[50] The use of Adams catalyst gave good results with 1-methyl-3-nitro-, 1-methyl-3-nitro-8-chloro-, and 1,3-dinitrophenoxazine.[51]

Hydroxy and amino compounds form expected acetyl derivatives. 1-Chloro-2-hydroxyphenoxazone forms a monoacetyl derivative,[37] and 1,3-diaminophenoxazine forms either a monoacetyl[73] or a diacetyl[10] compound. This acetylation was assumed to occur on the amino groups. 1-Amino-[73] and 1,2-benzo-7-aminophenoxazine[23] both form monoacetyl compounds.

The acetyl derivatives are hydrolyzed easily by acids and by bases,[23] as is the acetyl on the heterocyclic nitrogen.[57]

The diazotization of aminophenoxazines was first reported as a proof on the quinoid form of the onium salts.[69] The conclusion which was made was that since the 3-amino group could be diazotized, there must exist an ortho-quinoid structure. However, nearly twenty years later the same author stated that in extremely strong acid the amine could

not be diazotized and so favored the *para*-quinoid structure.[80]

Reductive diazotizations using ethanol as the reducing agent have been reported in three instances.[23,36c,70]

Methyl sulfate has been used as an analytical tool for the quinoid forms of phenoxazine, since it adds to the *para*-quinoid forms.[10,69]

Picrates of phenoxazine,[10,18a] 2,7-dimethyl-, and 3,7-dimethylphenoxazine[18a] have been reported, and the failure of 3-methylphenoxazine to form a picrate has been noted.[10] The picrate of phenoxazine is a dark olive green and this fact has recently been used in the identification of phenoxazine as the product of the decomposition of 2-azidophenyl phenyl ether.[14] There was obtained only a tarry material which gave the same colors in various reagents as did phenoxazine and formed also a dark olive green picrate. The picrate, however, had no characteristic melting point.

Physical Properties

There have been relatively few studies on the physical properties of phenoxazine and its derivatives. Cullinane and Rees[81] have studied the molecular structure of phenoxazine by solid solution with similar heterocycles and by dipole. They

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[80] F. Kehrmann, *Helv. Chim. Acta*, 4, 527 (1921).

[81] N. M. Cullinane and W. T. Rees, *Trans. Faraday Soc.*, 36, 507 (1940).

conclude that phenoxazine has a small angle of fold.

Spectral studies of the oxazine compounds have been mainly in the visible region, where their dyeing properties are important. The first study of spectra, in 1896, describes the absorption bands, though without any assigned wavelengths.[33] There was noted a very pronounced relationship of spectra to the oxidation state of the molecule. The onium salts had double maxima and the reduced compounds had a single broad peak.

Kehrmann and co-workers[58,82] measured the spectra of several compounds which they prepared including a series of nitro substituted phenoxazines and a series of amino derivatives and their hydrochloride salts. Similarly a series of compounds prepared from the condensation of ketones with phenoxazine have been studied because of their intense colors.[72]

In a study of the oxidation state of phenoxazine and related compounds, it was concluded that the ortho- and the para-quinoid forms could be distinguished by the visible spectra. This was based upon a study of phenoxazine, 3,7-dimethyl-, 1-amino-, and 3-aminophenoxazine. In this study it was also found that most of the oxidation products of phenoxazine have a spectral maximum between 265 and 285 mμ, a fact which has been used recently in the identification

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[82]F. Kehrmann and M. Sandoz, Ber., 50, 1682 (1917).

of naturally occurring phenoxazine derivatives.

In 1940 another study showed that most phenoxazine derivatives have a spectral peak at 520-560 mμ, the exact position depending on what groups are present. Phenothiazine and phenoselenazine and their derivatives have no such simple spectra.[75]

One problem which exists in the study of some oxazine dyes is the occurrence of a color change in the staining process, which has been called metachromacy. This seems to be due to a loose polymerization of the dye molecules in solution, which breaks up slowly as they stain. This has been noted with Oxonine and Capri Blue, in the staining of nucleic acids and agar solutions.[83]

Though the ready preparation of phenoxazonium salts by bromine and ferric salts would indicate an aromatic character for the ortho-quinoid form, this form is not very stable over 10° but undergoes nuclear substitution in the 3-position, to produce a para-quinoid structure.

Though at one time it was thought that even the 3-aminophenoxazonium salts were ortho-quinoid,[69] it has since been shown by both spectral studies[74] and diazotization reactions[80] that the para-quinoid structure is at least the most important form. Much argument has been given on this

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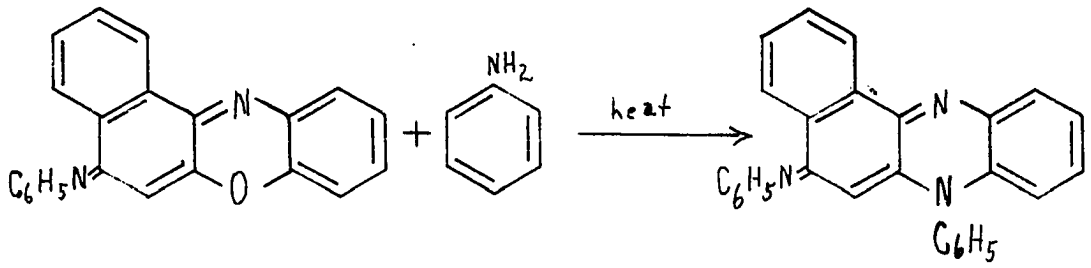
[83]L. Michaelis and S. Granick, J. Am. Chem. Soc., 67, 1212 (1945).

problem,[24,84] some based upon observation and some upon prejudice.

Lantz[85] states that the para-quinoid structure is the better since heating benzophenoxazimes with aniline in acid gives the benzophenazonium salts, replacing the nuclear oxygen with the nitrogen of a molecule of aniline. Yields of over 50% have been obtained for dibenzophenoxazimes.



Uses of Phenoxazine and Its Derivatives

The uses of phenoxazine derivatives actually are not many. The most important at present are biological staining and the related treatment of cancer and tuberculosis. Phenoxazines have been patented for use as pharmaceuticals,[86] bactericides,[18b] antiallergy agents,[87] insecticides,[88] cellulose

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[84]F. Kehrmann and H. de Gottrau, Ber., 38, 2574 (1905).

[85]R. Lantz, Ann. chim., 11 2, 168 (1934).

[86]W. Schulemann, F. Mietzsch and A. Wingler, U. S. Patent 1,879,541[C. A., 27, 1094 (1933)].

[87]Swiss Patent 262,432[C. A., 45, 4746 (1951)].

[88]W. A. Knapp, U. S. Patent 2,385,284[C. A., 39, 5372 (1945)].

fiber dye,[89] and nickel plating additive.[90] Reports in the journals indicate the testing of various derivatives as bactericides,[91] antitetanus agents,[92] anti-horse strongyle agents,[93] antioxidant,[94] and as an indicator for both acid constants of carbonic acid.[95]

Because of the ready reduction of resazurin, a blue compound, to resorufin, a pink compound, and then to dihydroresorufin, a colorless compound, resazurin has been used for testing the hygienic quality of milk. Bacteria which are present reduce this compound rapidly, and the shade of color obtained is an indication of bacterial content.[77]

The more important uses will be discussed under their separate headings below.

Biological staining

The use of phenoxazine derivatives in biological staining

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[89]H. C. Olpin and P. B. Law, U. S. Patent 2,464,885 [C. A., 43, 4488 (1949)].

[90]R. Lind, W. J. Harshaw and K. E. Long, U. S. Patent 2,291,590[C. A., 37, 568 (1943)].

[91]T. Yuasa, Ann. Rept. Research Inst. Tuberc., Kanazawa Univ., 11, 265 (1953)[C. A., 48, 12900 (1954)].

[92]M. Heki, T. Nishikawa and M. Jujii, Japan J. Med. Sci. and Biol., 5, 89 (1952)[C. A., 46, 10412 (1952)].

[93]N. D. Levine and V. Ivens, Am. J. Vet. Research, 15, 349 (1954).

[94]C. M. Murphy, H. Ravner and N. L. Smith, Ind. Eng. Chem., 42, 2479 (1950).

[95]G. Mannelli and E. Martini, Ann. chim., 41, 68 (1951) [C. A., 45, 8394 (1951)].

is closely related to their use in the treatment of cancer and tuberculosis and is discussed some under these headings. The general uses of these dyes can be found in reference books[1,2,96] and so will not be discussed here.

Two studies relating to the biological staining have been made which involve the chemistry of the molecule. In early work it was found that with certain oxazine dyes that the protein tissue was stained blue and the fatty tissue red. Thorpe[71] studied this problem and found that the red dye was the hydrolyzate of the original dye. An oxygen had replaced one of the amino groups to give a phenoxazone. The phenoxazone thus produced was fat soluble, and could be isolated by extraction with toluene, and did indeed stain fat a red color, but had no effect on protein, and the remaining purified amino dye would stain protein a blue color and have no effect on fat. Another observation of certain oxazine dyes was that they stained tissue a different shade than the solution of the dye, and that the shade would change on aging. This process was called metachromacy. Oxonine, for example, in a freshly made agar solution would dye the agar a color with an absorption maximum at 580 mμ, but after aging for several hours the absorption maximum was 460 mμ.[83] This is thought to be caused by some type of loose polymer complex of the dye which breaks up on the protein, either agar or nucleic acids,

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[96]H. J. Conn, "Biological Stains," 6th ed., Biotech Publications, Geneva, N. Y., 1953, pp. 117-125.

to give a monomer, which has a different shade.

Treatment of cancer

Possible use of phenoxazine derivatives in the treatment of cancer is based mainly upon their property of selective staining. Nile Blue A, Nile Blue 2B and related compounds have been shown to selectively stain tumor tissue and also to retard growth of the cancer.[97a,97b,98,99,100,101,102] A series of 3-benzylamino-7-diethylamino-1,2-benzophenoxazonium salts with halogen on the benzyl ring has been prepared for investigation.[34] Testing of these compounds has shown that the 1,2-benzophenoxazines are much superior to the simple phenoxazines, though some simple phenoxazines do inhibit growth of tumors in mice. The presence of a benzylamino or arylamino group in the 3-position improves the effectiveness of the compound, as does an amino or substituted amino group

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[97a] M. R. Lewis, H. A. Slovitor and P. P. Goland, Anat. Record, 95, 89 (1946).

[97b] M. R. Lewis, P. P. Goland and H. A. Slovitor, ibid., 96, 201 (1946).

[98] M. R. Lewis and P. P. Goland, ibid., 99, 369 (1947).

[99] J. F. Riley, Cancer Research, 8, 183 (1948).

[100] M. R. Lewis, Anat. Record, 102, 37 (1948).

[101] M. R. Lewis, M. L. Crossley and P. F. Dreisbach, Proc. Soc. Exptl. Biol. and Med., 78, 406 (1951).

[102] M. R. Lewis and P. P. Goland, Cancer Research, 13, 130 (1953).

in the 7-position.[26,27,28,103]

Though these compounds show good promise, they are still undergoing experimental study and are not recognized as a means of chemotherapy for cancer.

Treatment of tuberculosis

The first testing of a phenoxazine derivative for tuberculosis showed Nile Blue Sulfate to have no curative properties,[104] but soon after New Methylene Blue GG showed some effect in decreasing the extent of infection in test animals.[105] A study by Hollande[106,107,108] established a tuberculostatic effect for Nile Blue Chloride which varied with the strain of microorganism and the medium on which it grows.

Other studies have indicated promising results,[26,27,28, 32,36a,50,51,109,110] but as yet none of the compounds has

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[103]M. R. Lewis, P. P. Goland and H. A. Slovitor, ibid., 9, 736 (1949).

[104]H. J. Corper, J. Infectious Diseases, 11, 373 (1912).

[105]L. M. Dewitt, ibid., 13, 378 (1913).

[106]A. C. Hollande and G. Cremieux, Compt. rend. soc. biol., 98, 1379 (1928).

[107]A. C. Hollande and G. Cremieux, ibid., 99, 542 (1928).

[108]A. C. Hollande and G. Hollande, ibid., 102, 546 (1929).

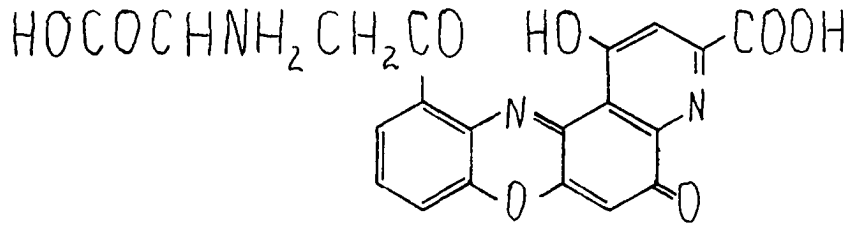
[109]H. G. Wells and E. R. Long, "Chemistry of Tuberculosis," Williams and Wilkins, Baltimore, Md., 1932, p. 437.

[110]Toshio Murata, Ann. Rept. Research Inst. Tuberc., Kanazawa Univ., 12, 45 (1954) [C. A., 49, 13366 (1955)].

found clinical use since better drugs are available.

Naturally Occurring Phenoxazine Derivatives

Although there are not a large number of naturally occurring phenoxazine derivatives when compared with some of the alkaloid heterocycles, there is a class called the ommochromes which are found principally in the eyes of orthoptera. These have the phenoxazone nucleus, substituted in the 2-position with an amino group and in the 1- and 9-positions with organic radicals. The first structure identified and synthesized was xanthommatine.



Xanthommatine

These compounds are formed from tryptophan as shown by C^{14} radioactive tracer studies. A very good review on these compounds and their chemistry has just appeared.[111]

The antibiotic actinomycin is related to these compounds

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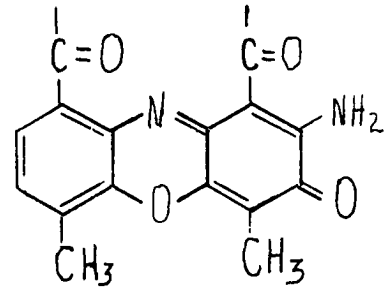
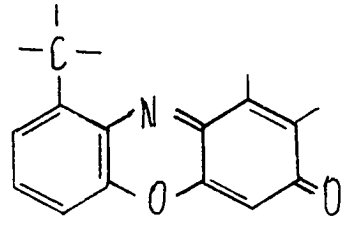
[111] A. Butenandt, Angew. Chem., 69, 16 (1957).

and has been given the partial structure:[112]



Another naturally occurring compound of the phenoxazone group is polystictin or cinnibarin, which is a red pigment isolated from the wood rotting fungus Coriolus sanguineus and from Trametes cinnabarina. This compound, on the basis of its spectra and reactions which are similar to actinomycin has been assigned the partial structure:[113,114]



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[112] H. Brockmann and H. Muxfeldt, ibid., 68, 69 (1956).

[113] G. W. K. Cavill and J. R. Tetaz, Chem. Ind., 1956, 986.

[114] J. Gripenberg, P. Honkanen and O. Patoharju, ibid., 1956, 1505.

Boronic Acids

The chemistry of organoboron compounds has just been reviewed[6] and so only the chemistry since that article, and that very closely related to the research which has been carried on, will be reviewed.

A series of papers has been published by K. Torsell[115,116,117,118,119,120] on the transformations of arylboronic acids involving nitration, oxidation of methyl groups, formation of acid chlorides, esterification of carboxylic acids, Wohl-Ziegler bromination of side chains, and formation of a complex with fructose. The stability of the carbon-boron bond was considered for these reactions and the effect of many of the boronic acids on microorganisms and enzyme systems was studied.

The three isomeric hydroxybenzeneboronic acids have been prepared by halogen-metal interconversion[121] and the ortho isomer has been used for coupling with diazonium salts to

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[115] K. Torsell, Arkiv för Kemi, 10, 473 (1957).

[116] K. Torsell, ibid., 10, 497 (1957).

[117] K. Torsell, ibid., 10, 507 (1957).

[118] K. Torsell, ibid., 10, 513 (1957).

[119] K. Torsell, ibid., 10, 529 (1957).

[120] K. Torsell, ibid., 10, 541 (1957).

[121] H. Gilman, L. Santucci, D. R. Swayampati and R. O. Ranck, J. Am. Chem. Soc., 79 (1957), in press.

prepare several azo boronic acids.[122] Various bromine and sulfur containing boronic acids have been prepared,[123] and cleavage studies have been made on benzeneboronic acid and o-hydroxybenzeneboronic acid.[124]

These last studies were made in an attempt to prepare organoboron compounds for possible brain cancer therapy. This use of organoboron compounds was postulated first in 1940[125] because boron-10,[126] although not radioactive, decomposes when irradiated with slow neutrons to give off an alpha particle which is effective for only about 9 microns. Borax was tested as the source of this *in vivo* radiation,[127] but the differential uptake between normal and abnormal tissue was not great enough. Boron-containing azo dyes which were prepared by

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[122] H. Gilman, L. Santucci, D. R. Swayampati and R. O. Ranck, ibid., 79 (1957), in press.

[123] L. Santucci and H. Gilman, ibid., 79 (1957), in press.

[124] H. Gilman, D. R. Swayampati and R. O. Ranck, to be published.

[125] P. G. Kruger, Proc. Nat. Acad. Sci., 26, 181 (1940).

[126] Naturally occurring boron contains 18.83% of this isotope. See NBS Circular No. 499, p. 7.

[127] L. E. Farr, W. H. Sweet, J. S. Robertson, C. G. Foster, H. B. Locksley, D. L. Sutherland, M. L. Mendelsohn and E. E. Stickley, Am. J. Roentgenol. Radium Therapy Nuclear Med., 71, 279 (1954).

Snyder and co-workers[128,129] were tested[130] and found to be much better than borax in differential uptake and in effectiveness on the tumor.

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[128] H. R. Snyder and C. Weaver, J. Am. Chem. Soc., 70, 232 (1948).

[129] H. R. Snyder and S. L. Meisel, ibid., 70, 774 (1948).

[130] P. G. Kruger, Radiation Research, 3, 1 (1955).

EXPERIMENTAL[131]

Nitration

Nitration of phenoxazine

To a stirred solution of 9.16 g. (0.05 mole) of phenoxazine in 100 ml. of glacial acetic acid at 0-10° was added 5 ml. of nitric acid in 40 ml. of glacial acetic acid. A red color developed immediately and a red solid separated. Addition took 1/2 hour, then the mixture was stirred for 15 minutes and poured into 200 g. of ice. There separated 10.0 g. of red solid melting over the range 180-240°. Extraction with petroleum ether (b. p. 60-71°) dissolved 2.20 g. of phenoxazine, melting over the range 128-134° (identified by mixture melting point). Fractional crystallization from ethanol and from pyridine gave 3.00 g. (22%) of red needles which decomposed above 240°. The infrared spectrum had the bands expected for 3,7-dinitrophenoxazine and this compound formed an acetyl derivative which was identical to 3,7-dinitro-10-acetylphenoxazine, which was prepared as described in the literature, see Preparation of 3,7-dinitro-10-acetyl-

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[131]All melting points are uncorrected. All infrared spectra were obtained by use of the Baird double beam infrared spectrophotometer of the Institute for Atomic Research, Iowa State College. The writer expresses his appreciation to Robert McCord and E. Miller Layton for the determination of the spectra.

phenoxazine.

No 3-nitrophenoxazine was isolated, and the oxidation products were not identified.

Preparation of 3-nitro-10-ethylphenoxazine (attempted)

A 10.56 g. (0.05 mole) sample of 10-ethylphenoxazine in 250 ml. of glacial acetic acid was nitrated with 5 ml. of nitric acid in 40 ml. of glacial acetic acid for 5 minutes at room temperature and then poured into 1 l. of water. This mixture stood for 1 hour to allow the colloid to aggregate, then the mixture was filtered and the solid distilled to give 6.5 g. of 10-ethylphenoxazine, m. p. 46-48° (mixture melting point was undepressed). No mononitro-10-ethylphenoxazine was isolated.

Preparation of 3,7-dinitro-10-ethylphenoxazine

To 10.56 g. (0.05 mole) of 10-ethylphenoxazine in 250 ml. of glacial acetic acid was added 10 ml. of nitric acid in 40 ml. of glacial acetic acid. An immediate red precipitate formed and after stirring for two minutes the mixture was poured into 1 l. of distilled water. There separated 15.2 g. of a blood red solid, melting over the range 234-254°. This solid was refluxed twice with 1 l. portions of ethyl acetate to leave 8.0 g. of red needles, m. p. 264.0-265.5°. The ethyl acetate was concentrated to 500 ml. and chilled to

give 2.14 g. of red needles, m.p. 263-265°. Distillation of the filtrate to dryness left a red solid which on careful fractional crystallization with ethyl acetate gave 1.0 g. of 3-nitro-10-ethylphenoxazine, melting over the range 143-147°. The mixture melting point with an authentic sample prepared from 3-nitrophenoxazine was undepressed.

Preparation of 3-nitro-10-acetylphenoxazine[52]

In a beaker 11.26 g. (0.05 mole) of 10-acetylphenoxazine[132] was suspended in 120 ml. of glacial acetic acid at room temperature and then 6 ml. of nitric acid in 8 ml. of glacial acetic acid was added. The mixture turned dark orange and all the solid went into solution. The mixture stood for 15 minutes and then was poured into 500 ml. of water. A large tarry mass and some gelatinous material separated. The gel was filtered to give 2.31 g. of red solid, m.p. 134.0-135.5°, and the tarry mass was heated with water and broken with a stirring rod to give 10.75 g. of red-orange solid, m.p. 131-133°. This is a 97% crude yield. This solid was recrystallized from benzene and then from ethanol to give 9.0 g. of red needles, m.p. 132-135°. This represents an 84.4% yield.

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[132]The preparation of 10-acetylphenoxazine has been previously reported. See references 57 and 63.

Preparation of 3,7-dinitro-10-acetylphenoxazine[57]

In a beaker, 11.26 g. (0.05 mole) of 10-acetylphenoxazine was suspended in 80 ml. of glacial acetic acid and 13.2 ml. (0.2 mole) of nitric acid in 16 ml. of glacial acetic acid was added at room temperature. After standing for a few minutes all the solid dissolved and the mixture turned dark red and warmed considerably. Brown fumes began to evolve, and so the mixture was set in an ice-bath for 24 hours. Red needles had separated at the end of this time and filtration gave 6.6 g. of product, melting over the range 182.5-187.0°. Recrystallization from benzene gave 5.88 g. of red needles, m.p. 185-188°.

Bromination

Reaction of phenoxazine with N-bromosuccinimide

To 13.74 g. (0.075 mole) of phenoxazine in 300 ml. of carbon tetrachloride was added slowly with stirring 14.69 g. (0.085 mole) of N-bromosuccinimide. The solution became deep violet. After stirring for 72 hours, the solid present was filtered. This solid was shown to be succinimide by mixture melting point. The carbon tetrachloride was distilled and the residue recrystallized from an ethanol-water mixture, and then from chloroform to yield 0.56 g. of bromophenoxazonium bromide, m.p. 152-153°.

Anal. Calcd. for $C_{12}H_7Br_2NO$: C, 42.3; H, 2.36; N, 4.11. Found: C, 42.85, 43.04; H, 2.36, 2.47; N, 4.08, 4.12.

Reaction of phenoxazine with three moles of bromine

In a 500 ml. flask with a stirrer and a condenser attached by a tube to a funnel over water, was placed 9.16 g. (0.05 mole) of phenoxazine in 200 ml. of carbon tetrachloride. To this mixture was added 24.0 g. (7.75 ml., 0.15 mole) of bromine in 80 ml. of carbon tetrachloride. The mixture became violet-brown immediately and an increase in temperature was noted. The mixture was heated to reflux for 3 1/2 hours until all color of bromine was gone from the refluxing vapors, and allowed to stir for 10 hours. The mixture on filtration gave 12.60 g. of a green solid, melting over the range 225-235°. Recrystallization from pyridine gave a cream solid which decomposed sharply at 254°.

Anal. Calcd. for $C_{12}H_7Br_4NO$: N, 2.80; Br, 63.80. Found: N, 3.05, 3.12; Br, 63.36, 63.34.

The probable structure of a 3,7-dibromophenoxazonium bromide hydrobromide has been assigned on the basis of infrared spectrum and the analysis. There is ionic bromide present as indicated by an immediate precipitate on the addition of silver nitrate to a solution of the compound, this could be the onium bromide or the hydrobromide. Reduction studies have indicated that this compound is very difficult to reduce, which may be due to the fact that it is a hydrobromide

salt, since most of the known onium salts are easily reduced.

Reaction of phenoxazine with five moles of bromine

In a 500 ml. flask were mixed 9.16 g. (0.05 mole) of phenoxazine in 200 ml. of glacial acetic acid and 40.0 g. (12.9 ml., 0.25 mole) of bromine in 30 ml. of glacial acetic acid. The temperature rose very rapidly during addition. The color was red at the start, turned deep violet and then green. The mixture was heated to reflux for 7 hours until the bromine color was no longer present in the vapors. Filtration of the cooled mixture gave 24.0 g. of green solid, melting over the range 228-245°. Refluxing with pyridine left 2.2 g. of dark solid, not melting to 300°. From the pyridine was obtained, after two recrystallizations, 10.0 g. of tan solid, m.p. 238-239°, which darkened in the air.

Anal. Calcd. for $C_{12}H_4Br_4NO$: N, 2.80. Found: N, 2.75, 2.81.

The infrared spectrum of this compound was different from that of 3,7-dibromophenoxazonium bromide hydrobromide. This compound does have ionic bromide as indicated by an immediate precipitate on addition of silver nitrate to a solution of the compound.

Reaction of phenoxazine with twelve moles of bromine

In a 500 ml. flask set in a well ventilated hood, 9.16 g. (0.05 mole) of phenoxazine in 150 ml. of glacial acetic acid

was reacted with 96 g. (0.6 mole) of bromine. There was a distinct rise in temperature and a green solid separated. The mixture was stirred and heated to reflux for 66 hours, cooled and a gray solid, melting over the range 270-275°, was filtered off. Recrystallization several times from pyridine using Norit A gave finally 2.57 g. of fine needles, m.p. 304-306°.

Anal. Calcd. for $C_{12}H_5Br_6NO$: N, 2.13; Br, 72.66. Found: N, 2.20; Br, 71.88.

On the basis of this analysis and the infrared spectrum, which has some similarities to that of the compound assigned the structure 3,7-dibromophenoxazonium bromide hydrobromide, this compound has tentatively been assigned the structure of 1,3,7,9-tetrabromophenoxazonium bromide hydrobromide.

Reaction of 10-ethylphenoxazine with N-bromosuccinimide

To a stirred solution of 10.56 g. (0.05 mole) of 10-ethylphenoxazine in 300 ml. of carbon tetrachloride was added 9.79 g. (0.055 mole) of N-bromosuccinimide over a 20 minute period. The color changed from yellow to violet. The mixture was stirred for 18 hours, during which time a violet solid separated. This solid was filtered and recrystallized from water to give 6.5 g. of white powder, melting over the range 116-122° (mixture melting point with succinimide was undepressed). The solution was distilled to dryness to leave a tarry residue, and after several attempts to crystallize this, it was distilled over the range 165-175°/0.4 mm., to

give 10.0 g. of a red liquid (69%). This was redistilled to give 8.6 g. (59%) of a red viscous liquid boiling over the range 120-140°/0.01 mm. which darkened rapidly in the air.

Anal. Calcd. for $C_{14}H_{12}BrNO$: C, 57.94; H, 4.17; N, 4.83. Found: C, 58.60, 58.79; H, 4.31, 4.22; N, 5.13, 5.05.

Reaction of 10-ethylphenoxazine with two moles of N-bromosuccinimide

To 2.64 g. (0.0125 mole) of 10-ethylphenoxazine in 35 ml. of carbon tetrachloride was added 4.45 g. (0.025 mole) of N-bromosuccinimide and the mixture stirred for 48 hours under a nitrogen atmosphere. The succinimide was filtered off (3.5 g.), the carbon tetrachloride solution distilled to dryness, and the residue recrystallized from petroleum ether (b.p. 60-71°) to give 3.0 g. of product, m.p. 100.5-104.0°. A mixture melting point with the product from the reaction of 10-ethylphenoxazine with bromine was undepressed. This represents 65% of the theoretical yield.

Reaction of 10-ethylphenoxazine with two moles of bromine

To a stirred solution of 5.28 g. (0.025 mole) of 10-ethylphenoxazine in 50 ml. of carbon tetrachloride was added 8.0 g. (2.58 ml., 0.05 mole) of bromine in 15 ml. of carbon tetrachloride. During addition a violet liquid formed and hung momentarily to the side of the flask, but disappeared on continued stirring. The color slowly turned gray then gray-

green. A solid appeared to be in suspension in the liquid. Filtration after a few minutes gave 9.30 g. of green solid, melting over the range 125-130° dec., which fumed in the air. The fumes were acid to moist litmus, and formed white clouds with ammonia vapor. When the fuming ceased there remained 7.0 g. (75.8%) of a brown solid, melting over the range 89-98° dec. Several recrystallizations from petroleum ether (b.p. 60-71°) gave 4.45 g. of product, m.p. 104-104.5°.

Anal. Calcd. for $C_{14}H_{11}Br_2NO$: N, 3.80. Found: N, 3.87, 3.79.

Reaction of 10-ethylphenoxazine with four moles of bromine

In a flask with a stirrer and a reflux condenser were placed 5.28 g. (0.025 mole) of 10-ethylphenoxazine and 150 ml. of carbon tetrachloride. Into this was dropped slowly 16.0 g. (0.1 mole) of bromine in 50 ml. of carbon tetrachloride. Warming was noticed and a violet color formed which disappeared on stirring. After 1/3 of the bromine had been added a green color formed and a green solid separated. Completion of the addition left a violet color. The mixture was heated to reflux with stirring for 14 hours until there was no color of bromine in the vapors. A small amount of black solid was filtered and the solution was distilled to 50 ml. from which 10.0 g. of solid, melting over the range 70-85°, separated on cooling. The product was distilled over the range

170-176°/0.03 mm., to give 2.66 g. of tribromo-10-ethylphenoxazine melting over the range 90-94°.

Anal. Calcd. for $C_{14}H_{10}Br_3NO$: N, 3.12; Br, 53.52. Found: N, 3.21, 3.17; Br, 54.49, 54.59.

Reaction of 10-phenylphenoxazine with N-bromosuccinimide

To a stirred solution of 19.4 g. (0.075 mole) of 10-phenylphenoxazine in 300 ml. of carbon tetrachloride was added 14.69 g. (0.0825 mole) of N-bromosuccinimide. There developed a deep violet color which changed to yellow after 1 hour. The reaction was stirred for 22 hours, then filtered to give 8.40 g. of gray solid, m.p. 123-126° (succinimide by mixture melting point) and a yellow-brown solution. The solution was distilled to 50 ml. and chilled to give 7.84 g. of green crystals, melting over the range 80-88°. This was recrystallized from ethanol several times using Norit A to give 4.80 g. of yellow crystals, m.p. 83-86°.

Anal. Calcd. for $C_{18}H_{12}BrNO$: N, 4.14. Found: N, 4.26, 4.10.

Reaction of 10-acetylphenoxazine with N-bromosuccinimide

At room temperature (attempted). A 5.63 g. (0.025 mole) sample of 10-acetylphenoxazine and 4.45 g. (0.025 mole) of N-bromosuccinimide were stirred in 55 ml. of carbon tetrachloride for 48 hours. Filtration left a solid which, on drying

under a heat lamp, decomposed to a black tar which could not be crystallized. From the carbon tetrachloride solution was isolated 2.90 g. of unchanged 10-acetylphenoxazine (mixture melting point).

At reflux temperature with a catalyst (attempted). A 5.63 g. (0.025 mole) sample of 10-acetylphenoxazine with 4.45 g. (0.025 mole) of N-bromosuccinimide in 100 ml. of carbon tetrachloride was refluxed for 20 hours. There was no evidence of reaction. To the cooled mixture was then added 3.4 g. (0.025 mole) of anhydrous zinc chloride and this mixture then heated slowly. Near the reflux temperature there began to appear a violet solid and a light brown solution. The cooled mixture was filtered, the solid extracted with 300 ml. of ether and the ether evaporated to give only a trace of tarry solid. Water extracted from this same solid nearly the quantity of succinimide expected for complete reaction. From the carbon tetrachloride there was obtained 3.6 g. of 10-acetylphenoxazine (identified by mixture melting point). This represents a 64% recovery of starting material, though all the N-bromosuccinimide seemed to have reacted. No other products could be isolated from the solid mass which was left from the ether and water extractions.

Reaction of 10-acetylphenoxazine with two moles of bromine

10-Acetylphenoxazine (11.26 g., 0.05 mole) was reacted with 5.16 ml. (0.10 mole) of bromine in 200 ml. of carbon tetrachloride at reflux temperature. There separated first a red oil which clung to the side of the flask, then the mixture turned violet and the oil disappeared. This mixture was refluxed 3 hours until all the bromine color had gone from the vapors. A green-blue solid was filtered which fumed in the air. Washing with water left 9.0 g. of a pink solid, m.p. 121-126°. Extraction with petroleum ether (b.p. 60-71°) dissolved only part of the solid, and on chilling gave 1.8 g. of tan powder, melting over the range 101-124°. Recrystallization several times from petroleum ether (b.p. 60-71°) gave 0.1 g. of gray powder, melting over the range 115-130°. No pure sample could be obtained, but the infrared spectrum indicated this compound to be a monosubstituted phenoxazine. The residue which was left was recrystallized from 95% ethanol to give 1.0 g. of bromophenoxazonium bromide, m.p. 158-159° dec. The infrared spectrum was identical with that of the compound prepared from phenoxazine and N-bromosuccinimide, and a mixture melting point of the two was undepressed.

Anal. Calcd. for $C_{12}H_7Br_2NO$: Br, 46.92. Found: Br, 46.20, 45.92.

Distillation of the carbon tetrachloride solution gave 9.0 g. of solid melting over the range 153-175°, but multiple

recrystallizations from petroleum ether (b.p. 60-71°) failed to give a pure sample for analysis. The infrared spectrum of this fraction is very similar to that of 3,7-dibromophenoxazonium bromide hydrobromide.

Azo Coupling

Reaction of phenoxazine with p-nitrobenzene diazonium chloride

In a beaker, 2.76 g. (0.02 mole) of p-nitroaniline was diazotized at 0° with 12 ml. of concentrated hydrochloric acid, 12 ml. of water, and 1.52 g. (0.022 mole) of sodium nitrite in 5 ml. of water. Twenty ml. of etanol was added with 5 g. of ice and the mixture was allowed to stand for 15 minutes. This diazonium salt was added slowly to 3.66 g. (0.02 mole) of phenoxazine in 200 ml. of ethanol. A red color developed and a red solid separated. Filtration gave 4.13 g. of a tarry solid, melting over the range 84-104°. From the solution there was obtained 0.60 g. of red powder, melting over the range 119-130°. The combined solids were extracted with petroleum ether (b.p. 60-71°) to give on chilling 0.40 g. of phenoxazine (identifed by mixture melting point). The solids remaining were dissolved in chloroform and chromatographed through a column of alumina (35 X 300 mm.). Using chloroform as eluent two brown bands were washed off which contained only traces of solid, then using ethyl acetate as eluent a deep violet band washed off and then a green band.

From the deep violet fraction was obtained 1.07 g. of tarry solid, melting over the range 140-160°, which was recrystallized twice from ethanol to give 0.20 g. of crude 3-(p-nitrophenylazo)phenoxazine, melting over the range 185-195°, and some even more crude material. The infrared spectrum supported the structure assigned.

Reaction of 10-ethylphenoxazine with p-nitrobenzene diazonium chloride

In a beaker, 2.76 g. (0.02 mole) of p-nitroaniline was diazotized as described above and was added slowly to 4.23 g. (0.02 mole) of 10-ethylphenoxazine in 100 ml. of ethanol at room temperature with vigorous stirring. There developed an immediate red color which darkened as a solid separated. The suspension was stirred for 1 1/2 hours, poured into 1 l. of water from which an oil separated. This mixture was extracted with three 200 ml. portions of ether. The ether solution was washed with 50 ml. of 10% potassium hydroxide and with 50 ml. of water, then distilled from a red oil. This oil was distilled to give 2.6 g. of 10-ethylphenoxazine (identified by mixture melting point). The distillation residue was dissolved in chloroform and chromatographed through a column of alumina. First a deep red band then a pink band and finally a brown were washed off. From the first fraction was obtained a tarry solid which crystallized from ethanol to give 0.50 g. of red platelets, m.p. 130-132°. Recrystalliza-

tion gave 0.3 g. of product, melting over the range 141-147°.

The infrared spectrum has the expected bands for 3-(p-nitrophenylazo)-10-ethylphenoxazine.

Reaction of 10-phenylphenoxazine with p-nitrobenzene diazonium chloride (attempted)

In a beaker, 2.76 g. (0.02 mole) of p-nitroaniline was diazotized as described above and was added slowly to 5.18 g. (0.02 mole) of 10-phenylphenoxazine in 200 ml. of ethanol and 200 ml. of acetic acid. A deep red color developed immediately. Filtration gave 4.47 g. of a red solid, melting over the range 121-134°. Addition of water gave no more solid. This solid was chromatographed on a column of alumina (35 X 300 mm.) with ethyl acetate as the eluent. There came first a red band and then a yellow. Distillation of the ethyl acetate left 4.20 g. of 10-phenylphenoxazine (identified by mixture melting point).

Metalation

General procedure

All preparations of organometallic compounds were carried out in an atmosphere of dry, oxygen-free nitrogen. The solvent used was sodium dried diethyl ether. The metalation reaction was carried out in a three-necked, round-bottomed flask of a size to allow efficient stirring.

The metalating agents, n-butyllithium[133] and methyllithium,[134] were prepared by methods reported in the literature. As the metalation reaction proceeded it was checked by means of color test IIa[135] for the presence of the metalating agent. After metalation was complete, the mixture was poured jet-wise into a Dry Ice-ether slurry and then allowed to warm to room temperature. Water was added to dissolve the salt of the acid, and the two layers were separated. The organic layer was washed with 10% potassium hydroxide, and the combined aqueous solution was washed with fresh ether. The water solution was warmed on a steam bath to remove any dissolved ether and then cooled before acidification with dilute hydrochloric acid. The acid which separated was then recrystallized from a suitable solvent.

Reaction of phenoxazine with n-butyllithium

A 9.16 g. (0.05 mole) sample of phenoxazine was metalated with 0.12 mole of n-butyllithium for 110 hours. Work up of the mixture as described in the "General procedure" gave 7.8 g. of crude acid, melting over the range 200-215°. From the

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[133]H. Gilman, J. A. Beel, C. G. Brannen, M. W. Bullock, G. E. Dunn and L. S. Miller, J. Am. Chem. Soc., 71, 1449 (1949).

[134]H. Gilman, E. A. Zoellner and W. M. Selby, ibid., 55, 1252 (1932).

[135]H. Gilman and J. Swiss, ibid., 62, 1847 (1940).

ether layer was obtained 4.2 g. of phenoxazine (identified by mixture melting point). The acid was refluxed with 95% ethanol to leave a yellow solid melting over the range 315-320°. This appears from its neutralization equivalent to be a mixture of mono and dibasic acids, but attempts to purify the dibasic acid failed. From the ethanol solution was obtained 3.18 g. of the monobasic acid, melting over the range 230-240°. This was further purified by recrystallization from toluene using Norit A to give 0.50 g. of phenoxazine carboxylic acid, m.p. 244-245°.

Anal. Calcd. for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.31; Neut. equiv., 227.2. Found: C, 68.89, 68.63; H, 3.88, 4.00; N, 6.14, 6.11; Neut. equiv., 228.6.

Reaction of 10-ethylphenoxazine with n-butyllithium

A 10.56 g. (0.05 mole) sample of 10-ethylphenoxazine was metalated with 0.07 mole of n-butyllithium at reflux for 42 hours. Work up was done as described in the "General procedure". There was obtained 8.5 g. of crude acid. This was refluxed with absolute ethanol, leaving a residue, melting over the range 300-305°. Attempts to recrystallize this high melting solid failed, as the neutral equivalent after several recrystallizations and triturations with acetic acid indicated that some monocarboxylic acid was still present. From the ethanol was obtained, after several recrystalliza-

tions, 3.90 g. of 10-ethylphenoxazinecarboxylic acid, m.p. 163.5-165.0°.

Anal. Calcd. for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49; Neut. equiv., 255.26. Found: C, 70.45, 70.33; H, 5.38, 5.33; N, 5.27, 5.42; Neut. equiv., 253.9.

From the ether solution was obtained 3.5 g. of 10-ethylphenoxazine (identified by mixture melting point).

Reaction of 10-ethylphenoxazine with methyllithium

A 5.28 g. (0.025 mole) sample of 10-ethylphenoxazine was metalated with 0.05 mole of methyllithium at ether reflux for 26 hours. Using the "General procedure" there was obtained 0.20 g. of yellow solid, melting over the range 106-206°. Dissolution in base and acidification gave 0.10 g. of product, m.p. 160-163°. A mixture melting point with the product from the metalation of 10-ethylphenoxazine with n-butyllithium was undepressed. From the ether layer was isolated 4.97 g. of 10-ethylphenoxazine (identified by mixture melting point).

Reaction of 10-phenylphenoxazine with n-butyllithium

A 10.0 g. (0.039 mole) sample of 10-phenylphenoxazine was metalated with 0.07 mole of n-butyllithium for 23 hours. After work up as described in the "General procedure" there was obtained 8.20 g. of crude acid. The acid was recrystallized from ethanol and toluene to give 0.47 g. of 10-phenyl-

phenoxazinecarboxylic acid, m.p. 208-210°.

Anal. Calcd. for $C_{19}H_{13}NO_3$: N, 4.62; Neut. equiv., 303.3. Found: N, 4.55, 4.68; Neut. equiv., 306.1.

The solid which was insoluble in the ethanol used for the recrystallization of the monocarboxylic acid was recrystallized from acetic acid three times to give 1.38 g. of dicarboxylic acid, m.p. 332.5-334.0°.

Anal. Calcd. for $C_{20}H_{13}NO_5$: N, 4.03; Neut. equiv., 173.66. Found: N, 3.83, 3.99; Neut. equiv., 176.9.

From the ether there was obtained 1.10 g. of 10-phenylphenoxazine (identified by mixture melting point).

Reaction of 10-phenylphenoxazine with methyllithium

10-Phenylphenoxazine (6.48 g., 0.025 mole) was metalated with 0.05 mole of methyllithium at ether reflux for 26 hours. Acidification of the solution after work up as described in the "General procedure" gave 0.20 g. of crude acid which was dissolved in base and reprecipitated with hydrochloric acid to give 0.10 g. of product melting over the range 197-205°. A mixture melting point with 10-phenylphenoxazinecarboxylic acid from the metalation of 10-phenylphenoxazine with n-butyllithium was undepressed. From the ether solution was recovered 6.05 g. of 10-phenylphenoxazine (identified by mixture melting point).

Reduction of Nitro Derivatives

Reduction of 3,7-dinitro-10-ethylphenoxazine

In a thick-walled flask, 8.0 g. (0.0265 mole) of 3,7-dinitro-10-ethylphenoxazine was suspended in 80 ml. of 95% ethanol with 2 g. of palladium-calcium carbonate catalyst and this flask mounted in a shaker and attached to a hydrogen tank with a pressure gauge (Parr apparatus). The mixture was shaken for 18 hours until the red color had disappeared. The pressure drop corresponded to the reduction of two nitro groups. The mixture was filtered from the catalyst and the solution immediately turned green in the air. The solution was concentrated to 90 ml. and then diluted with 80 ml. of water. This gave 6.4 g. of dark green needles melting over the range 80-84°. This represents a crude yield of 100%. Recrystallization gave 4.24 g. of green needles, m.p. 83-85°.

Anal. Calcd. for $C_{14}H_{15}N_3O$: N, 17.42. Found: N, 16.33, 16.10.

The poor analysis is probably due to oxidation of the compound by the air.

Reduction of 3-nitro-10-acetylphenoxazine

In a thick-walled flask, 5 g. (0.019 mole) of 3-nitro-10-ethylphenoxazine was suspended in 100 ml. of absolute ethanol and 0.5 g. of palladium-calcium carbonate catalyst was added. This mixture was shaken under 45 pounds pressure

of hydrogen until the theoretical amount had been absorbed, and the red color had disappeared. The catalyst was filtered off and the solution concentrated by distillation under nitrogen atmosphere to a low volume. Filtration of the chilled solution gave 3.78 g. of 3-amino-10-acetylphenoxazine melting over the range 140.0-145.5°.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: N, 11.67. Found: N, 11.38, 11.40.

Cleavage Reactions

Reaction of 10-ethylphenoxazine with hydriodic acid

In a 100 ml. flask, 5.0 g. (0.022 mole) of 10-ethylphenoxazine was heated to reflux for 27 hours with 9.0 ml. (15.0 g., 0.06 mole) of hydriodic acid. The color became light brown. The mixture was neutralized with 10% potassium hydroxide which caused a tan solid to separate. This was filtered to give 6.00 g. of a tarry solid. Extraction with petroleum ether (b.p. 60-71°) left a small residue melting over the range 283-291°. Concentration and chilling of the solution gave 2.54 g. of product, m.p. 149-153°. A mixture melting point with phenoxazine was not depressed.

Reaction of 10-ethylphenoxazine with lithium

In a 500 ml. flask, 10.56 g. (0.05 mole) of 10-ethylphenoxazine and 1.04 g. (0.15 g.-atom) of lithium were heated

to reflux for 24 hours in 70 ml. of ether. Color test I[136] was positive and color test IIa[135] weakly positive. The mixture, cooled to room temperature, was poured jet-wise into a Dry Ice-ether slurry, and allowed to warm to room temperature. Hydrolysis was effected with 100 ml. of water, the layers separated, the organic layer extracted with 50 ml. of 10% potassium hydroxide and the combined water layers acidified to give 4.11 g. of an acid, m.p. 170-173° dec. Recrystallization from ethanol gave 2.30 g. of nearly white platelets, m.p. 175-177°. The ether layer was extracted with two 50 ml. portions of 10% sulfuric acid which on neutralization gave no product. Distillation of the ether left a residue which distilled at 142-150°/2.0 mm. This was identified as 10-ethylphenoxazine by a mixture melting point.

The acid product is thought to be N-ethyl-2-hydroxy-2'-carboxydiphenyl amine. An attempt to synthesize this acid by an unambiguous method has not yet been successful.

Reaction of dibromo-10-ethylphenoxazine with hydriodic acid

Method I (attempted). In a 100 ml. flask, 2.0 g. of dibromo-10-ethylphenoxazine (from the bromination of 10-ethylphenoxazine) and 9.0 ml. (0.06 mole) of hydriodic acid were refluxed in 50 ml. of 80% acetic acid for 30 hours. The

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[136] H. Gilman and F. Schulze, ibid., 47, 2002 (1925).

mixture was cooled and poured into 500 ml. of water. Ammonium nitrate was added to break up the colloid and the mixture was allowed to stand. Filtration gave 2.16 g. of yellow solid, melting over the range 99-120°. Recrystallization from petroleum ether (b.p. 60-71°) gave 1.55 g. of starting material (identified by mixture melting point). No other products were isolated.

Method II. In a 100 ml. flask, 2.0 g. of dibromo-10-ethylphenoxazine was reacted with 70 g. of 50% hydriodic acid at reflux temperature for 19 hours. The dark mass was extracted with ether and with water to leave 0.25 g. of a green solid, melting over the range 178-190°. The layers were separated, and the ether layer was distilled to dryness. Extraction with petroleum ether (b.p. 60-71°), and chilling gave 0.30 g. of brown solid, melting over the range 115-120°. This material contained both bromine and nitrogen. The infrared spectrum was nearly identical with that of the product from the reduction of dibromophenoxazonium bromide hydrobromide with lithium aluminum hydride.

Reaction of 10-phenylphenoxazine with hydriodic acid (attempted)

In a 200 ml. flask, 5.0 g. (0.02 mole) of 10-phenylphenoxazine, 9.0 ml. (0.06 mole) of hydriodic acid, and 30 ml. of glacial acetic acid were refluxed for 27 hours. Water was added and 5.0 g. of 10-phenylphenoxazine separated (iden-

tified by mixture melting point).

Reaction of 10-phenylphenoxazine with lithium (attempted)

In a 500 ml. flask a vigorously stirred mixture of 5.0 g. (0.02 mole) of 10-phenylphenoxazine, 1.28 g.-atom of lithium and 100 ml. of ether was refluxed for 53 hours. The lithium became bright and appeared to have reacted partially. The mixture was carbonated by pouring jet-wise into a Dry Ice-ether slurry. Work up yielded only a trace of acidic material and 3.58 g. of 10-phenylphenoxazine (identified by mixture melting point).

Reduction of Onium Salts

The product which was obtained from the reaction of three moles of bromine with phenoxazine has been tentatively identified as 3,7-dibromophenoxazonium bromide hydrobromide. It was thought that this salt would reduce easily as does phenoxazonium chloride. However, several different reducing agents were tested before one was found which would reduce this compound. The following experiments were run on this compound.

Reduction by sodium bisulfite (attempted)

3,7-Dibromophenoxazonium bromide hydrobromide (1.15 g.) was refluxed with 50 ml. of a 15% solution of sodium bisulfite for 24 hours. From the work up only starting material could

be isolated (identified by mixture melting point).

Reduction by hydrogen (attempted)

A suspension of 11.45 g. of 3,7-dibromophenoxazonium bromide hydrobromide in 200 ml. of 95% ethanol, with 1 g. of palladium-calcium carbonate catalyst was shaken at 46 pounds pressure of hydrogen for 24 hours. There was no drop in pressure, and filtration of the chilled mixture gave 10.0 g. of tan solid, m.p. 250-252°, identified by a mixture melting point with the starting material.

Reduction by stannous chloride and hydrochloric acid (attempted)

In a 250 ml. flask, 2.1 g. (0.005 mole) of 3,7-dibromophenoxazonium bromide hydrobromide was suspended by vigorous stirring in 100 ml. of 95% ethanol, and a solution of 1.13 g. (0.005 mole) of stannous chloride and 5 ml. (0.06 mole) of concentrated hydrochloric acid in 30 ml. of water was added. The faint green tint of the organic salt disappeared with the first few drops. The mixture was stirred for 1 1/2 hours, then refluxed for 68 hours. Filtration of the hot mixture gave 1.30 g., and concentration and chilling gave an additional 0.13 g. of the starting material (identified by mixture melting point).

Reduction by lithium in ether (attempted)

In a 500 ml. flask, 2.1 g. (0.005 mole of 3,7-dibromophenoxazonium bromide hydrobromide and 0.104 g. (0.015 g.-atom) of lithium in 250 ml. of ether were stirred at room temperature for 144 hours. There was recovered 1.75 g. of starting material (identified by mixture melting point).

Reduction by lithium aluminum hydride in ether (attempted)

In a 250 ml. flask, 2.1 g. (0.005 mole) of 3,7-dibromophenoxazonium bromide hydrobromide was suspended in 50 ml. of anhydrous ether and 0.1 g. (0.0038 mole) of lithium aluminum hydride in 70 ml. of anhydrous ether was added slowly. On addition of a few drops of the hydride suspension a deep green color developed, which on further addition and stirring changed to yellow. The mixture was sitrred for 30 hours at room temperature, 20 ml. of ethyl acetate was added to destroy the excess hydride, and 20 ml. of hydrochloric acid in 100 ml. of water was added to dissolve all the inorganic compounds. There was recovered 1.85 g. of starting material (identified by mixture melting point).

Reduction by lithium aluminum hydride in tetrahydrofuran

In a 250 ml. flask, 2.1 g. (0.005 mole) of 3,7-dibromophenoxazonium bromide hydrobromide was mixed with 1.0 g.

(0.025 mole) of lithium aluminum hydride in 100 ml. of dry tetrahydrofuran.[137] A green color developed and a gas was evolved. The reaction mixture was refluxed for 66 hours, and the excess lithium aluminum hydride was destroyed with 20 ml. of ethyl acetate. A spontaneous reflux indicated that excess of the hydride was still present. Ten milliliters of water and 20 ml. of hydrobromic acid were added and all of the solid dissolved. Dilution of the mixture with 1 l. of water caused 1.24 g. of a gray solid, melting over the range 134-142°, to separate. This solid was extracted with petroleum ether (b.p. 60-71°) to leave 0.65 g. of gray solid, m.p. 154-156° dec., and on chilling the petroleum ether, 0.5 g. of a white powder, m.p. 115-117°, separated. This white powder, which darkened very rapidly in the air, was dried under a stream of nitrogen. The infrared spectrum indicated this compound to be a dibromophenoxazine. The spectrum was nearly identical to that of dibromophenoxazine isolated from the cleavate of dibromo-10-ethylphenoxazine by hydriodic acid. These are probably both 3,7-dibromophenoxazine.

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[137] The tetrahydrofuran was purified by distillation under a nitrogen atmosphere, first from sodium wire and then from lithium aluminum hydride.

Reduction by sodium borohydride and aluminum trichloride[138]

To 2.1 g. (0.005 mole) of 3,7-dibromophenoxazonium bromide hydrobromide in 50 ml. of anhydrous ethylene glycol dimethyl ether was added dropwise 1.0 g. (0.025 mole) of sodium borohydride and 1.3 g. (0.01 mole) of anhydrous aluminum trichloride in 45 ml. of the same solvent. The mixture was heated to 85° for 42 hours, and hydrolyzed by the addition of 50 ml. of water. Some hydrogen gas was evolved. A white solid melting above 360° was removed by filtration. After cooling, the filtrate deposited a green solid, melting over the range 99-125°. Both solids were extracted with ether, which on distillation left 0.3 g. of gray powder, melting over the range 99-125°. A mixture melting point with the product from the reduction by lithium aluminum hydride was undepressed.

Miscellaneous Reactions of Phenoxazine

Reaction of 3,7-dibromophenoxazonium bromide hydrobromide with ethyl magnesium bromide (attempted)

In a 125 ml. flask, 2.1 g. (0.005 mole) of 3,7-dibromophenoxazonium bromide hydrobromide was reacted with 0.005 mole of ethyl magnesium bromide[139] at ether reflux for 90

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[138]See H. C. Brown and B. C. Subba Rao, J. Am. Chem. Soc., 78, 2582 (1956) for a discussion of the use of this reagent.

[139]Kindly provided by M. Hughes.

hours. At the end of this time there appeared to have been no reaction so the ether was removed by distillation. The remaining paste was heated for 7 hours at 100°. Starting material (1.83 g.) was recovered by hydrolysis and recrystallization (identified by mixture melting point).

Hydrolysis of 3-nitro-10-acetylphenoxazine

To 3.0 g. (0.012 mole) of 3-nitro-10-acetylphenoxazine in 160 ml. of ethanol was added 25 ml. of 10% potassium hydroxide diluted to 100 ml. with water. A green color developed which changed rapidly to a violet color. The mixture was warmed to 50° for one hour, and 80 ml. of 10% sulfuric acid was added. This caused the separation of 2.5 g. (93%) of a red solid, m.p. 200° dec. This is the melting point reported by Kehrmann and Ramm for 3-nitrophenoxazine.[52]

Preparation of 3-nitro-10-ethylphenoxazine

A 1.5 g. (0.0067 mole) sample of 3-nitrophenoxazine was refluxed for 3 hours with 1.0 ml. (0.01 mole) of ethyl iodide in a solution of 0.57 g. (0.01 mole) of potassium hydroxide in 25 ml. of acetone. After one hour the violet color of the potassium salt was nearly gone, and 1.0 ml. more of ethyl iodide was added. When the color was blood red, the mixture was poured into 800 ml. of water and filtered to give 1.58 g. of red solid, melting over the range 140-155°. Recrys-

tallization from ethanol using Norit A gave 0.50 g. of blood red needles, m.p. 158.5-160.0°. A mixture melting point with the mono-nitroproduct isolated from the nitration of 10-ethylphenoxazine was undepressed.

Anal. Calcd. for $C_{14}H_{12}N_2O_3$: N, 10.93. Found: N, 11.08, 10.86.

Reaction of phenoxazinecarboxylic acid with methanol

A solution of 3.0 g. of phenoxazinecarboxylic acid in 200 ml. of absolute methanol saturated with gaseous hydrogen chloride, was refluxed for 36 hours. From this mixture separated 2.0 g. of a yellow solid, melting over the range 100-112°. Recrystallization from an ethanol-water mixture using Norit A gave 1.0 g. of yellow needles, m.p. 112.5-114.0°.

Anal. Calcd. for $C_{14}H_{11}NO_3$: N, 5.81. Found: N, 6.08, 6.18.

Reaction of methyl phenoxazinecarboxylate with ethyl iodide

Method I (attempted). In a 100 ml. flask, 0.2 g. of potassium hydroxide in 50 ml. of acetone was mixed with 0.30 g. of methyl phenoxazinecarboxylate and 10 ml. of ethyl iodide and the mixture refluxed for 4 hours. The mixture was cooled, filtered, and the tarry solid which separated was extracted with ether. The ether solution was dried over anhydrous calcium chloride and distilled to a low volume to give, on chill-

ing and filtration, 0.12 g. of starting material (identified by mixture melting point).

Method II. In a 250 ml. flask, 125 ml. of ammonia was condensed and to this was added 0.5 g. of sodium and a crystal of ferric nitrate. The mixture was stirred until the blue color was replaced by gray. To this solution was added 0.50 g. of methylphenoxazinecarboxylate and the color became reddish brown. The solution was stirred for 20 minutes and 25 ml. of ethyl iodide was added. The color became nearly yellow with a separate dark red liquid phase in the bottom of the flask. The ammonia was allowed to evaporate, and the solid mass which remained was extracted with ether, the solid inorganic salts dissolved in water and this water solution extracted with ether. Distillation of the combined ether solution under reduced pressure left a yellow glass which was recrystallized from an ethanol-water mixture to give 0.15 g. of yellow needles melting over the range 90-94°. A mixture melting point with the starting material was depressed.

The analysis of this compound indicated 9.21 and 9.29% of nitrogen present. This could fit quite well for N-ethylamido-10-ethylphenoxazine or for N,N-diethylamidophenoxazine, each of which would have 9.52% of nitrogen. N-ethylamido-10-ethylphenoxazine has been prepared and is quite different from this product.

Preparation of (N-ethylamido)-10-ethylphenoxazine

A 1.0 g. sample of 10-ethylphenoxazinecarboxylic acid (prepared by metalation) was reacted with 10 ml. of thionyl chloride (colorless, C. P. grade) by heating on a steam bath for 15 minutes. This mixture was then added slowly to 50 ml. of 30% ethyl amine. The resulting mixture was cooled and filtered to give 1.75 g. of product melting over the range 186-195°. The infrared spectrum had the bands expected for this compound. Attempts to purify part for an analytical sample were unsuccessful.

Reaction of 10-ethylphenoxazinecarboxylic acid with methanol

To 200 ml. of absolute methanol saturated with hydrogen chloride was added 0.8 g. of 10-ethylphenoxazinecarboxylic acid (prepared by metalation) and the mixture was refluxed for 48 hours. The cooled mixture was neutralized with 10% potassium hydroxide, and the yellow oil which separated was extracted with ether. Distillation of the ether left only a small amount of yellow oil which would not crystallize. The infrared spectrum had the bands expected for methyl 10-ethylphenoxazinecarboxylate.

Preparation of methyl x-nitro-10-ethylphenoxazinecarboxylate

In a 500 ml. flask, 250 ml. of absolute methanol was

saturated with anhydrous hydrogen chloride and to this was added 2.0 g. of 10-ethylphenoxazinecarboxylic acid (prepared by metalation). This was stirred at reflux temperature for 2 hours and subsequently at room temperature overnight. The mixture was poured into 500 ml. of water, from which a yellow oil separated. This mixture was extracted with ether to give a yellow solution which was washed with 50 ml. of 5% potassium hydroxide and 50 ml. of 5% nitric acid. When the ether solution was distilled to a low volume the color became red, and a solid separated. Addition of 200 ml. of ether failed to dissolve all of the solid, and 1.5 g. of red powder melting over the range 141-150° was filtered off. Distillation of the ether extract gave 1.15 g. of bright red solid melting over the range 135-150°. Several recrystallizations from ethyl acetate gave 0.70 g. of fine red needles, m.p. 173.5-175.0°. A qualitative test for both an ester and a nitro group were positive.

Anal. Calcd. for $C_{16}H_{14}N_2O_5$: N, 8.93. Found: N, 8.87, 8.91.

The nitration in this reaction seemed to occur when the ether solution was distilled to a low volume. Apparently some nitric acid remained from the wash solution.

Reaction of bromo-10-ethylphenoxazine with n-butyllithium

To a stirred solution of 4.35 g. (0.015 mole) of bromo-

10-ethylphenoxazine (from the bromination of 10-ethylphenoxazine) in 100 ml. of anhydrous ether was added 0.015 mole of n-butyllithium. The mixture turned yellow immediately, was allowed to stir for 30 minutes, then poured jet-wise into a Dry Ice-ether slurry. After allowing the mixture to warm to room temperature, water was added to dissolve the solid. The layers were separated, the ether layer washed with 50 ml. of 10% potassium hydroxide, and this wash solution combined with the water layer. There separated on acidification of the aqueous layer 2.30 g. of crude acid, melting over the range 175-190°. This crude acid was recrystallized from an ethanol-water mixture and a toluene-petroleum ether (b.p. 60-71°) mixture to give 0.60 g. of acid melting over the range 207.5-210.5°.

Anal. Calcd. for $C_{15}H_{13}NO_3$: N, 5.49; Neut. equiv., 255.26. Found: N, 5.42, 5.29; Neut. equiv., 258.6.

This acid is different in all properties from the acid obtained by metalation of 10-ethylphenoxazine, indicating that the position of attack of the N-bromosuccinimide is, as expected, different from that of n-butyllithium.

Reaction of 10-(p-bromophenyl)-phenoxazine with n-butyllithium

To a solution of 2.62 g. (0.0078 mole) of 10-(p-bromophenylphenoxazine[66] in 100 ml. of ether and 100 ml. of benzene (this compound was not readily soluble in ether) was added

0.0126 mole of n-butyllithium. An immediate white precipitate formed and then a yellow color developed which faded with subsequent stirring. The mixture was stirred for 30 minutes, then poured jet-wise into a Dry Ice-ether slurry and allowed to warm to room temperature. Water was added, the layers separated, and the organic layer washed with 50 ml. of 10% potassium hydroxide. The combined water solution was acidified to yield 1.1 g. of crude acid, melting over the range 245-255°. The acid was recrystallized twice from an ethanol-water mixture to give 0.56 g. of a yellow powder, m.p. 258.5-260.5°.

Anal. Calcd. for $C_{19}H_{13}NO_3$: N, 4.62; Neut. equiv., 303.3. Found: N, 4.54, 4.41; Neut. equiv., 307.6.

Preparation of Boronic Acids

Boronic acids of 10-ethyl- and 10-phenylphenoxazine have been prepared, and an attempt made to prepare a phenoxazine boronic acid. These experiments will be given first, followed by the experiments on the general methods of preparing boronic acids, and finally the experiments on the preparation of boronic acid dyes.

Preparation of phenoxazineboronic acid (attempted)

In a 500 ml. flask, 9.16 g. (0.05 mole) of phenoxazine in 200 ml. of anhydrous ether was reacted with 0.126 mole of

n-butyllithium at reflux temperature for 100 hours. The metalation mixture was added slowly from a dropping funnel to 70 ml. (0.25 mole) of n-butyl borate in 400 ml. of anhydrous ether at -70°. The mixture was stirred for 10 minutes after addition was completed and then 100 ml. of 10% sulfuric acid was added. After stirring overnight, the layers were separated and the ether solution extracted with 8-50 ml. portions of 10% potassium hydroxide. The basic solution was acidified and 4.05 g. of dark green solid, melting over the range 133-140°, separated. This solid was recrystallized and sublimed to yield only phenoxazine (identified by mixture melting point). No other product was isolated from either the acid solution or the ether. It is possible that some of the boronic acid was formed and was decomposed by the addition of the mineral acid, though this does not usually decompose aromatic boronic acids.

Preparation of 10-ethylphenoxazineboronic acid anhydride

In a dry 500 ml. flask, 10.56 g. (0.05 mole) of 10-ethylphenoxazine was metalated with 0.07 mole of n-butyllithium at ether reflux for 8 hours (until color test IIa[135] was negative). The metalation mixture was then added slowly to 35 ml. (0.125 mole) of n-butyl borate in 300 ml. of anhydrous ether cooled to -70°. The mixture became gray during addition. Color test I[136] was negative immediately after addition so

the mixture was hydrolyzed with 110 ml. of 10% sulfuric acid. A white solid separated which dissolved as the reaction mixture warmed to room temperature. The layers were separated and the aqueous layer washed with 250 ml. of ether in two portions. The combined ether solution was extracted with 130 ml. of 10% potassium hydroxide in three portions, and the resulting basic solution was acidified. A white solid separated and was filtered and dried. This gave 10.43 g. (82%) of the boronic acid, melting over the range 141-145°. Recrystallization several times from toluene using Norit A gave 1.98 g. of product, melting over the range 146-149°.

Anal. Calcd for $C_{14}H_{12}BNO_2$: B, 4.57; Neut. equiv., 237.06. Found: B, 4.88, 4.94; Neut. equiv., 240.86.

Preparation of 10-phenylphenoxazine-boronic acid anhydride

In a 500 ml. flask, 12.96 g. (0.05 mole) of 10-phenylphenoxazine was metalated with 0.07 mole of n-butyllithium at ether reflux for 23 hours (until color test IIa[135] was negative). The metalation mixture then was added from a dropping funnel to 27 ml. (0.1 mole) of n-butyl borate in 300 ml. of ether at -70°. Immediately after addition, color test I[136] was negative, so the reaction was hydrolyzed by the addition of 50 ml. of 10% sulfuric acid and 50 ml. of water. The mixture was allowed to warm to room temperature, the layers separated, the ether layer washed with water, and then ex-

tracted with 200 ml. of 10% of potassium hydroxide in four portions. The basic solution was golden yellow and a turbidity formed on standing. This mixture was washed with 50 ml. of ether, but the layers separated only slowly on standing. A solid which was present was filtered off, and the aqueous solution was made just acid to give 10.0 g. of product, melting over the range 155-165°. Extraction of this solid with 100 ml. of boiling toluene left 5.5 g. of solid, melting over the range 187-197°. This was recrystallized from pyridine, and then from acetone four times to give 0.24 g. of 10-phenylphenoxazineboronic acid anhydride, m.p. 300.0-301.5°.

Anal. Calcd. for $C_{18}H_{12}BNO_2$: Neut. equiv., 286. Found: Neut. equiv., 276.

Reaction of n-butyl borate with organometallic compounds

The preparation of boronic acids by the reaction of a borate ester with a Grignard reagent has been known for a long time, however, many boronic acids can not be prepared by this method because of side reactions which occur. A series of reactions was run to explore the possibility of using a less reactive organometallic reagent, which might allow the synthesis of new boronic acids, and particularly of boronic acid dyes.

General procedure. To an ether solution of 0.1 equivalent of an organometallic reagent (0.1 mole for aryl matallic

halides, and 0.05 mole for diaryl metallic compounds) under an atmosphere of dry nitrogen in a three-necked, round-bottomed flask, was added 0.25 mole of n-butyl borate (150% excess). The reaction mixture was stirred for 6 hours and then was hydrolyzed by the addition of sufficient 10% sulfuric acid to obtain a clear aqueous layer. The layers were separated and the water layer extracted with 200 ml. of ether in three portions. The combined ether solution was then extracted with 200 ml. of 10% potassium hydroxide in three portions, and the basic solution acidified with 10% sulfuric acid to obtain the boronic acid.

Table 1 shows the results that were obtained from a series of reactions. The temperature is the temperature at which the n-butyl borate was added to the organometallic reagent and which was maintained until the mixture was hydrolyzed. The per cent yield is based upon the crude yield. In all cases the purity of the crude acid seemed to be the same, since the melting points were nearly the same.

Reactions of boron trichloride with organomercury compounds

General procedure. The organomercury compound (0.05 mole) was suspended in 500 ml. of chlorobenzene in a three-necked, round-bottomed flask with a stirrer, a Dry Ice-acetone condenser attached to a mercury pressure relief valve, and an inlet tube which extended below the level of the solvent. The

Table 1. Benzeneboronic acid from n-butyl borate and organometallic reagents

ArM	Temp., °C	Yield, %
$(C_6H_5)_2Zn$	25	7
$(C_6H_5)_2Zn$	-70	1
$(C_6H_5)ZnCl$	25	7
$(C_6H_5)_2Cd$	25	17
$(C_6H_5)_2Cd$[a]	25	20
$(C_6H_5)_2Cd$	35	18
$(C_6H_5)_2Cd$	-12	14
$(C_6H_5)CdCl$	25	21
$(C_6H_5)_2Hg$	35	0
$(C_6H_5)_2Hg$[b]	110	0

[a]Reacted for 48 hours with 0.5 mole of n-butyl borate.

[b]Reacted for 144 hours in refluxing toluene.

inlet tube was connected through a mercury trap to a tank of boron trichloride. Boron trichloride was bubbled slowly into the mixture until the boron trichloride tank had decreased in weight the amount desired for the reaction. After stirring for the desired length of time, the mixture was filtered by suction to remove the mercuric chloride and the filtrate was hydrolyzed in an ice bath by the slow addition of ice. The hydrolysis appeared to be catalyzed by hydrogen chloride and so once started was auto-catalytic. The rate of hydrolysis

could be controlled only by the amount of water present. The acid which was formed was extracted with 250 ml. of 10% potassium hydroxide in four portions. The aqueous solution was washed with 100 ml. of ether and then acidified. The cream colored solid which separated was filtered and recrystallized from water.

The results are listed in Table 2.

Reaction of benzeneboronic acid with Indulin (attempted)

In a 500 ml. flask, 6.0 g. (0.01 mole) of Indulin (spirit soluble) and 3.6 g. (0.03 mole) of benzene boronic acid were refluxed for 4 hours in ether. On distillation to a very low volume and chilling, benzenboronic acid crystallized out, and evaporation to dryness left nearly pure Indulin (identified by mixture melting point).

Reaction of benzeneboronic acid with Sudan Black B

In a 2 l. flask, 5.54 g. (0.01 mole) of Sudan Black B was dissolved in 1 l. of dry ether. To this solution was added 3.63 g. (0.03 mole) of benzeneboronic acid in 800 ml. of ether. The mixture was refluxed for 2 hours, and then distilled to a volume of 200 ml. On chilling and filtration there was found 6.8 g. of black crystals, melting over the range 190-225°. This solid was recrystallized from ether to

Table 2. Reaction of boron trichloride and organomercury compounds

ArM	Excess BCl_3, %	Reaction time[a] (hrs.)	Yield, %[b]
$(C_6H_5)_2Hg$	100	1/2	70.5
$(C_6H_5)_2Hg$	100	0	55.0
$(C_6H_5)_2Hg$	50	2 1/2	52.0
$(C_6H_5)_2Hg$	50	3	57.0
$(C_6H_5)_2Hg$	200	6	70.0
p-$ClHgC_6H_4OH$[c]	100	10	0.0[d]
p-$CH_3COOHgC_6H_4NH_2$	100	10	0.0[e]
p-$ClHgC_6H_4COOH$	100	4	0.0
p-$ClHgC_6H_4COOH$[f]	1000	12	0.0
Merbromin	50	18	0.0

[a]This represents the time after the addition of the boron trichloride was complete.

[b]The yield is based upon the recrystallized boronic acid.

[c]Used 0.2 mole of organomercury compound.

[d]There was separated 54% of phenol, and 92% of mercuric chloride.

[e]Isolated nearly 100% of mercuric chloride.

[f]This reaction was run at 120°.

give 0.65 g. of fine black needles melting over the range 165-170°. This product had a neutralization equivalent of 236.1, which corresponds to a complex of 5 molecules of benzeneboronic acid with one molecule of Sudan Black B, which would have a neutral equivalent of 232.82. Attempts to separate the benzeneboronic acid by extraction with petroleum ether (b.p. 60-71°), in which it is very soluble, were unsuccessful.

The residue which was left from the recrystallization of the above complex was triturated with petroleum ether (b.p. 60-71°) and ether to leave 0.20 g. of black powder, melting over the range 135-139°. This solid had a neutral equivalent of 300.9, which corresponds quite closely to the neutral equivalent of a complex of 3 molecules of benzeneboronic acid with one molecule of Sudan Black B, which would be 306.9.

The infrared spectra of the two complexes are very similar to the spectrum of Sudan Black B but with the expected bands for benzeneboronic acid superimposed on it.

Preparation of 5-(3-trifluoromethylphenylazo)-2-hydroxybenzeneboronic acid anhydride

In a 250 ml. flask, 16.11 g. (0.10 mole) of m-aminobenzotrifluoride[141] was diazotized[142] at 0-5° with 22.5 ml.

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[141]This compound and the amines used in the following reactions were kindly provided by Dr. James Straley of the Tennessee Eastman Co.

[142]D. Aeloney, J. Am. Chem. Soc., 56, 2063 (1934).

of concentrated hydrochloric acid, 20 ml. of water, and 7.6 g. (0.11 mole) of sodium nitrite in 20 ml. of water. The reaction mixture was stirred for 45 minutes at 5° and then added slowly to a solution of 16.50 g. (0.138 mole) of o-hydroxybenzeneboronic acid[143] and 11 g. (0.275 mole) of sodium hydroxide in 150 ml. of water. The temperature was kept below 5° at all times, and the pH after completion of addition was 9. The coupling mixture stood for 3 1/2 hours with occasional stirring as it warmed to room temperature. Filtration left 26.6 g. (91%) of yellow brown solid, melting over the range 75-115°. This solid was recrystallized four times from petroleum ether (b.p. 60-71°) to give 7.5 g. of the desired yellow azo dye, m.p. 110-112°.

Anal. Calcd. for $C_{13}H_8BF_3N_2O_2$: B, 3.71; Neut. equiv., 292.02. Found: B, 1.58, 1.19; Neut. equiv., 288.2.

The poor boron analysis may be due to the fluorine in the molecule.

Preparation of 5-(2,6-dichloro-4-nitrophenylazo)-2-hydroxybenzeneboronic acid anhydride

A 10.35 g. (0.05 mole) sample of 2,6-dichloro-4-nitroaniline[141] in 37 ml. of pyridine was added slowly at 10° to nitrosylsulfuric acid (prepared by the addition of 7.5 g.

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[143]Part of the o-hydroxybenzeneboronic acid used in these experiments was kindly provided by A. Mitchell.

(0.11 mole) of sodium nitrite to 75 ml. of sulfuric acid in 37.5 ml. of water at 0°).[144] The diazonium salt was stirred for 30 minutes at 10°, 5 g. of urea in 200 ml. of water was added, and the mixture then stirred until foaming ceased. The yellow solution was filtered by suction and then added slowly to 6.9 g. (0.05 mole) of o-hydroxybenzeneboronic acid[143] in 30 ml. of pyridine. The mixture stood for 2 hours and 15.0 g. (84%) of red-orange solid melting over the range 92-100° separated. This material was recrystallized five times from a benzene-petroleum ether (b.p. 60-71°) mixture to give 0.3 g. of the azo dye, melting over the range 170-175°.

Anal. Calcd. for $C_{12}H_6BCl_2N_3O_4$: Neut. equiv., 337.9. Found: Neut. equiv., 322.2.

Further analyses will be made on this compound when a more pure sample has been prepared.

Preparation of 5-(4-nitro-2-methylsulfonylphenylazo)-2-hydroxybenzeneboronic acid

A 10.11 g. (0.05 mole) sample of 4-nitro-2-methylsulfonylaniline[141] was diazotized by the method described in the previous experiment. The clear yellow solution of the diazonium salt was added to a solution of 6.9 g. (0.05 mole) of o-hydroxybenzeneboronic acid[143] in 30 ml. of pyridine at 0°. The mixture was allowed to stir for 10 hours warming slowly

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[144]This procedure of diazotization was used by C. DeMilt and G. VanZandt, J. Am. Chem. Soc., 58, 2044 (1936).

to room temperature. Filtration left 13.0 g. of the azo dye, melting over the range 140-155°. Recrystallization of 3.0 g. of this product from 95% ethanol four times gave 0.15 g. of an orange powder, melting over the range 195-200°.

Anal. Calcd. for $C_{13}H_{12}BN_3O_7S$: Neut. equiv., 365.1. Found: Neut. equiv., 377.

Further analyses will be made when more of this compound is purified.

The product of this reaction is the acid, although the other two dyes reported here are acid anhydrides. This probably is due to the water in the ethanol.

DISCUSSION

Nitration

The direct nitration of phenoxazine and the identification of the products obtained has never been carried out before. Bernthsen[9] reported that phenoxazine formed a yellow nitration product which he reduced to a red amino compound. Neither of his products was further identified. Nitration of phenoxazine at 0-10° in glacial acetic acid produced 22% of 3,7-dinitrophenoxazine. No mononitrophenoxazine could be isolated. 3,7-Dinitrophenoxazine has been prepared previously by hydrolysis of 3,7-dinitro-10-acetylphenoxazine which was obtained by nitration of 10-acetylphenoxazine.[57] Following these directions there was prepared a product identical with the nitration product of phenoxazine. 10-Ethylphenoxazine reacted readily with nitric acid to yield 67% of 3,7-dinitro-10-ethylphenoxazine and 7% of 3-nitro-10-ethylphenoxazine. An attempt to prepare 3-nitro-10-ethylphenoxazine as the major product by use of a lower concentration of nitric acid was not successful. With an acetyl group on the 10-position, the nitration is more easily controlled. 10-Acetylphenoxazine was nitrated readily at room temperature in 15 minutes to produce 84.4% of 3-nitro-10-acetylphenoxazine. This compound is known.[52] It was prepared by acetylation of 3-nitrophenoxazine, available from cyclization.

3-Nitro-10-acetylphenoxazine was hydrolyzed to 3-nitro-

phenoxazine and this compound reacted with ethyl iodide in base to yield the same mononitro compound as that isolated from the nitration of 10-ethylphenoxazine.

The nitration of phenoxazine gives a means of measuring the competitive orientation by nitrogen and oxygen. Previous studies have shown that acylation of the nitrogen reduces its orienting power, but not below the orienting power of oxygen.[145] Acylation is used to reduce the basicity of nitrogen so that it will not form a salt and become then a meta directing group. In phenoxazine and 10-ethylphenoxazine, the same effect is produced by an aryl group which also reduces the basicity of nitrogen. The nitration using N,O-diacetyl-o-aminophenol, in which both the oxygen and the nitrogen have a reduced directive influence, gave a product substituted entirely ortho and para to the nitrogen.[145] Phenoxazine and its derivatives similarly have groups attached to both the oxygen and nitrogen which reduce their directive influence, and, as in the situation noted before, nitration was found to be directed entirely by the nitrogen.

Nitration of related heterocycles might be compared here.

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[145]C. K. Ingold, "Structures and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 239-240.

Carbazole[146] and dibenzothiophene[147] nitrate readily in the position para to the hetero atom, but dibenzofuran[148] nitrates meta to the oxygen. It would seem from this, that nitrogen and sulfur have a greater directive influence than does oxygen, which appears here to be meta directing. Nitration of this compound, however, is anomalous, since bromination, acylation and other electrophilic substitution reactions give products substituted para to the hetero atom in dibenzofuran.

The series of nitration reactions performed show that in phenoxazine and in the 10-ethyl and 10-acetyl derivatives, the nitrogen does have a greater directive influence than the oxygen on electrophilic aromatic substitution.

Bromination

The reaction of phenoxazine with bromine has been used to prepare phenoxazonium bromide.[69] To this time, all substitution reactions which are known to occur with phenoxazonium salts involve oxygen, water, amines, or carbonyl compounds

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[146]W. C. Sumpter and F. M. Miller, "Heterocyclic Compounds with Indole and Carbazole Systems," Interscience Publishers, Inc., New York, N. Y., 1954, pp. 70-109.

[147]H. D. Hartough and S. L. Meisel, "Compounds with Condensed Thiophene Rings," Interscience Publishers, Inc., New York, N. Y., 1954, pp. 225-282.

[148]W. E. Parham in R. C. Elderfield, "Heterocyclic Compounds," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1951, pp. 123-145.

(see the "HISTORICAL" section). It seemed logical that bromine would also attack phenoxazonium bromide to produce bromo derivatives. Reactions were performed using various amounts of bromine with phenoxazine and its derivatives. When three moles of bromine reacted with one of phenoxazine, a compound was produced which contained four atoms of bromine. This compound had ionizable bromide, and reduced with difficulty to the same compound which was formed by the cleavage with hydriodic acid of dibromo-10-ethylphenoxazine (prepared by bromination of 10-ethylphenoxazine). The product from the reduction appeared to be a dibromophenoxazine, but no sample could be purified for analysis because of the small amount available which darkened in the air. The 12.0-13.5 μ region of the infrared spectra of this compound, of dibromo-10-ethylphenoxazine, and the known 3,7-dinitro-10-ethylphenoxazine, were nearly identical.

On the basis of the spectral comparison, of the known reactivity of the 3- and the 7-positions in phenoxazonium salts, and the similarity of mechanism of nitration and bromination, these compounds have been assigned structures substituted in the 3- and the 7-positions.

In order to explain the presence of four atoms of bromine in the product of the bromination of phenoxazine with three moles of bromine, it appears that this product is a phenoxazonium bromide hydrobromide. The formation of an onium bromide hydrobromide has been noted with phenothiazine, which

forms phenothiazonium bromide hydrobromide on treatment with bromine.[149,150] More evidence is needed to show the complete structure of these compounds.

The reaction of phenoxazine with five moles of bromine produced a compound which also contained four atoms of bromine. Since this differs in its properties from 3,7-dibromophenoxazonium bromide hydrobromide, it has been tentatively identified as 1,3,7-tribromophenoxazonium bromide.

Bromination with twelve moles of bromine per mole of phenoxazine produced a compound containing six atoms of bromine. There are only four positions which would be especially activated in the phenoxazonium ion, and so it would be expected that substitution would occur at these positions. It seems plausible that this compound is 1,3,7,9-tetrabromophenoxazonium bromide hydrobromide.

N-Bromosuccinimide as a bromination agent for heterocycles has not been investigated extensively, but generally aromatic bromination with this reagent gives the same products as the use of bromine.[151] The reaction of N-bromosuccinimide with 10-ethylphenoxazine proceeded smoothly to give a monobromo- or a dibromo-10-ethylphenoxazine, depending upon the

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[149] R. Pummerer and S. Gassner, Ber., 46, 2310 (1913).

[150] F. Kehrmann and L. Diserens, ibid., 48, 318 (1915).

[151] C. Djerassi, Chem. Revs., 43, 247 (1948).

amount used. The dibromo compound is identical with the product from the reaction of 10-ethylphenoxazine with two moles of bromine. 10-Phenylphenoxazine also was brominated smoothly by N-bromosuccinimide to produce a monobromo product. Phenoxazine reacted with N-bromosuccinimide to yield a complex mixture from which a compound containing two atoms of bromine was isolated. This product appears to be a bromophenoxazonium bromide.

For the reasons given previously, these compounds have been assigned structures substituted in the 3- and the 7-positions.

The attempted dibromination of 10-acetylphenoxazine with bromine did not proceed easily and gave a complex mixture of products. Interestingly, the acetyl group was not present in any of the purified products. From this reaction was isolated 3,7-dibromophenoxazonium bromide hydrobromide, 3-bromophenoxazonium bromide, and what seemed to be a monobromophenoxazine, probably 3-bromophenoxazine. The spectrum of this latter compound had bands expected for an N-H bond, for 1,2,4-trisubstituted benzene, and for ortho-disubstituted benzene. The fact that this last compound was isolated may be an indication of the mechanism. The reaction may conceivably proceed by attack of the bromonium ion on the 3-position giving an intermediate which could either lose a proton from the 3-position, or the acetyl group. In the bromination of 10-acetylphenoxazine, the acetyl carbonium ion seems to be the

leaving group.

The loss of an acyl group has also been noted in nitration reactions. The nitration of 10-acetylphenoxazine is reported to have given a small amount of 1,3,7,9-tetranitrophenoxazine with the main product of 3,7-dinitro-10-acetylphenoxazine,[57] and the reaction of 10-chloroacetylphenothiazine with nitric acid produced 10-chloroacetylphenothiazine-5-oxide and 3-nitrophenothiazine-5-oxide.[152] Since both bromine and nitric acid are oxidizing agents, and the electrophilic substitution of the two are similar, the mechanism of the observed reactions may be nearly the same.

Azo Coupling

The azo coupling reactions, since they involve the attack of a positive ion, should yield phenoxazine derivatives substituted in the 3-position. A small amount of an azo dye was isolated from the reaction of phenoxazine with p-nitrobenzenediazonium chloride, but purification failed. The 12.0-13.5 μ region of the infrared spectrum of this compound was nearly identical to the spectra of known derivatives substituted in the 3-position. The coupling reaction involving 10-ethylphenoxazine produced a greater yield than did that involving phenoxazine, and it also had an infrared spectrum similar to

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[152] H. Gilman and R. D. Nelson, J. Am. Chem. Soc., 75, 5422 (1953).

other 3-substituted derivatives of phenoxazine. This compound will be analyzed further when it has been purified. 10-Phenylphenoxazine did not couple under the conditions employed. As a general rule for heterocycles related to phenoxazine, azo coupling reactions are carried out on hydroxy derivatives, since the oxygen, nitrogen, and sulfur in the heterocycle are not sufficiently activating to produce good yields from the azo coupling reaction.[146,148] Considering the difference in yields obtained from the azo coupling reactions, it appears that the order of reactivity for the three compounds is: 10-ethylphenoxazine phenoxazine 10-phenylphenoxazine, though evidence of the side reactions involved is needed before this can be taken as proof.

Metalation

Phenoxazine, 10-ethylphenoxazine, and 10-phenylphenoxazine are metalated quite easily with n-butyllithium to yield, after carbonation, the monocarboxylic acid of each, with a small amount of dicarboxylic acid. An attempt was made to produce only the monometalated product by the use of methyllithium,[153] but under the conditions tried, only a very small amount of metalation occurred.

The position of metalation was not proven, but it seems quite safe, since much comparative metalation has been

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[153]S. H. Eidt, unpublished Ph.D. Thesis, Iowa State College Library, 1955, p. 215.

done,[154] to assume that metalation would occur in the 4-position, next to the oxygen.

It is possible that unsubstituted phenoxazine may metalate ortho to the nitrogen, since phenothiazine metalates next to the nitrogen, even though the order of ease of metalation reported for dibenzofuran, dibenzothiophene and 9-ehtylcarbazole is: O S N. However, the failure of methyl phenoxazinecarboxylate to react with ethyl iodide in base is evidence against metalation in the 1-position. If metalation had occurred in the 1- or the 3-position, then the ester group would be in conjugation with the heterocyclic nitrogen and enhance the acidity of the hydrogen attached to it. This would make the reaction with ethyl iodide in base easier. This type of ethylation proceeds readily with 3-nitrophenoxazine and has been reported for 3-nitrocarbazole[155] where conjugation with the nitrogen is possible, but phenoxazine is not ethylated by this method.[66]

The infrared spectra of all the monometalation products are quite similar, especially in the region 12.0-13.5 u. The band expected for a vicinal trisubstituted benzene was weak in the spectra of the carboxylic acids but quite strong in

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[154]H. Gilman and J. W. Morton, Jr., in Roger Adam's "Organic Reactions," Vol. VIII, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 258.

[155]T. S. Stevens and S. H. Tucker, J. Chem. Soc., 123, 2140 (1923).

those of the boronic acids.

The conversion of phenoxazinecarboxylic acid and 10-ethylphenoxazinecarboxylic acid to a common derivative, methyl 10-ethylphenoxazinecarboxylate, was not successful. Phenoxazinecarboxylic acid formed a methyl ester, but attempts to convert this to methyl 10-ethylphenoxazinecarboxylate by reaction with ethyl iodide failed. Reaction of the ester with sodium amide followed by ethyl iodide gave a product which was not the desired methyl 10-ethylphenoxazinecarboxylate, but a compound containing more nitrogen. The analysis could fit quite well either (N-ethylamido)-10-ethylphenoxazine or (N,N-diethylamido)phenoxazine. The first was prepared from 10-ethylphenoxazinecarboxylic acid and was different from the above product.

A halogen-metal interconversion reaction of 3-bromo-10-ethylphenoxazine followed by carbonation, produced an acid which was different from the 10-ethylphenoxazinecarboxylic acid prepared by metalation.

Reduction of Nitro Derivatives

The nitro groups in both 3,7-dinitro-10-ethylphenoxazine and 3-nitro-10-acetylphenoxazine were easily reduced by hydrogen using a palladium-calcium carbonate catalyst. This was expected since other nitro derivatives have been similarly reduced.[50,51]

Cleavage Reactions

10-Ethylphenoxazine reacted readily with hydriodic acid in 80% acetic acid to yield phenoxazine. The 3,7-dibromo derivative reacted more sluggishly, giving a phenoxazine derivative only when refluxed with 50% hydriodic acid with no dilution. 10-Phenylphenoxazine is not effected by this reagent. These results were expected on the basis of previous studies using hydriodic acid on phenothiazine derivatives,[156] in which the ethyl group but not the phenyl was removed. 10-Ethylphenoxazine was cleaved by metallic lithium at the oxygen bridge to produce, after carbonation, an acid, probably 2-carboxy-2'-hydroxydiphenyl ethyl amine. An attempt to prepare this acid by an unambiguous synthesis has not yet been successful. The failure of 10-phenylphenoxazine to be cleaved by lithium was surprising, since the phenyl group is removed readily from 9-phenylcarbazole in tetrahydrofuran, and dibenzofuran is cleaved at the oxygen bridge in both ether and tetrahydrofuran.[157]

Reduction of Onium Salts

The oxidation-reduction reaction of the onium salts of

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[156] H. Gilman, P. R. Van Ess and D. A. Shirley, J. Am. Chem. Soc., 66, 1214 (1944).

[157] H. Gilman and J. J. Dietrich, J. Org. Chem., 22 (1957) in press.

phenoxazine has been studied somewhat before. It was thought in this study that bromo derivatives of phenoxazonium bromide could be easily reduced to yield bromophenoxazines. The reduction of 3,7-dibromophenoxazonium bromide hydrobromide, however, was very difficult. This difficulty may be due to either the insolubility of the compound, or to the fact that this compound seems to be a hydrobromide salt. Either of these explanations, or perhaps both, seem possible from the evidence, since the only reductions which were successful were by basic reagents in ethylene glycol dimethyl ether and tetrahydrofuran. All other reductions were attempted in diethyl ether or aqueous ethanol.

Preparation of Boronic Acids

The boronic acids of 10-ethyl- and 10-phenylphenoxazine were prepared in essentially the same manner as the boronic acids of other heterocycles.[123] No reason is apparent for the failure of phenoxazine to form a boronic acid.

The preparation of benzeneboronic acid from n-butyl borate using organometallic reagents, less reactive than phenylmagnesium bromide or phenyllithium, do not produce good yields. It was found that the use of diphenylcadmium gave better yields than did the use of diphenylzinc, and diphenylmercury did not react. The relative reactivity of the organic derivatives of these three metals toward active hydro-

gen has been reported to be Zn>Cd>Hg.[158] No explanation is available why greater yields are obtained using diphenylcadmium than using diphenylzinc in this reaction. Reactions at temperatures below 0° produced lower yields than those at room temperature, but reactions at higher temperatures did not give improved yields.

Since the use of n-butyl borate with diphenylmercury did not yield any boronic acid, a more reactive boron compound was used. The reaction of diarylmercury compounds with boron halides is known,[159,160,161,162] and has been used to prepare several boronic acids including ortho- and para-anisylboronic acid and ortho- and para-phenetylboronic acid.

All of the previous reactions were run in sealed tubes at high temperatures (180-240°). It has now been shown that the reaction between diphenylmercury and boron trichloride occurs readily at room temperature in chlorobenzene. It is necessary to allow the reaction to stir for at least 30 minutes before it is worked up, since work up immediately after addition of the boron trichloride was completed, gave a

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[158] J. F. Nelson, Iowa State Coll. J. Sci., 12, 145 (1937).

[159] A. Michaelis and P. Becker, Ber., 13, 58 (1880).

[160] A. Michaelis and P. Becker, ibid., 15, 180 (1882).

[161] A. Michaelis and M. Behrens, ibid., 27, 244 (1894).

[162] A. Michaelis, Ann., 315, 19 (1901).

lowered yield. A 100% excess of boron trichloride is necessary for the best yield. The mercuric chloride which is produced must be filtered off before the phenylboron dichloride is hydrolyzed, since mercuric salts cleave aromatic boronic acids to give arylmercury compounds.[128,163,164]

The yield of benzeneboronic acid by this method is about the same as that obtained from n-butyl borate with phenylmagnesium bromide.

Attempts to use other organomercury compounds were unsuccessful. p-Chloromercuriphenol appeared to react with the boron trichloride, but only phenol and mercuric chloride were isolated. Similarly p-acetoxymercurianiline reacted to produce nearly a quantitative amount of mercuric chloride, but no boronic acid. When there was a carboxyl group on the aromatic ring of the organomercury compound, no reaction occurred.

The preparation of boron compounds for possible use in brain tumor therapy, as was discussed in the "HISTORICAL" section, can be approached several ways. One possible method is to form boron complexes with known biological stains. Benzeneboronic acid is known to form complexes with aliphatic

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[163] A. D. Ainley and F. Challenger, J. Chem. Soc., 1930, 2171.

[164] T. S. West, Metallurgia, 47, 97 (1953) [C. A., 47, 4791 (1953)].

amines and with pyridine, but not with weaker bases.[165,166] An ether solution of benzeneboronic acid was mixed with an ether solution of Indulin and with one of Sudan Black B. No complex was formed with Indulin, but two complexes were isolated from the Sudan Black B, one with three molecules of benzeneboronic acid per molecule of Sudan Black B and one with five molecules of benzeneboronic acid per molecule of Sudan Black B.

Several boron containing azo dyes have been prepared as a continuation of previous studies.[122,128,129] The aniline derivatives which were used to prepare these dyes were chosen because of the substituted groups present. Some azo dyes are carcinogenic in the liver, but this activity is decreased by the presence of such groups as the bromo, chloro, nitro, and trifluoromethyl.[167] These amines were diazotized by methods reported in the literature. The coupling reactions for 5-(2,6-dichloro-4-nitrophenylazo)-2-hydroxybenzeneboronic acid and 5-(4-nitro-2-methylsulfonylphenylazo)-2-hydroxybenzeneboronic acid were carried out in pyridine, which neutralized the excess acid but did not give a strongly basic solution.

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[165] G. E. K. Branch and D. L. Yabroff, J. Am. Chem. Soc., 54, 2569 (1932).

[166] D. L. Yabroff and G. E. K. Branch, ibid., 55, 1663 (1933).

[167] J. P. Greenstein, "Biochemistry of Cancer," 2nd ed., Academic Press, New York, N. Y., 1954, pp. 88-103.

Pyridine has been known for its exaltation of the coupling power of diazonium salts, but the reasons for this are not entirely known, since it does not seem to be due to just a buffering action.[168]

The melting points of these dyes are dependent upon the rate of heating and the temperature at which the sample is inserted into the melting point bath, as has been noted previously for boronic acids.[122,123] The purification of the dyes was, therefore, most easily followed by frequent neutral equivalents.

Suggestions for Future Research

The nitration of phenoxazine should be studied more thoroughly to determine the best condition for nitration, and the oxidation products should be identified.

The products from the bromination of phenoxazine have not been identified unambiguously. These should be studied more thoroughly, as should also the conditions under which the hydrobromide salts are formed.

It would be interesting to know the fate of the acetyl group in the bromination of 10-acetylphenoxazine. If it forms acetyl bromide, this should be rather easily identified. The mechanism would be of interest since this might be related to

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[168] K. H. Saunders, "The Aromatic Diazo Compounds," 2nd ed., Edward Arnold and Co., London, 1949, p. 224.

general electrophilic aromatic substitution as a good illustration of a reaction involving an addition intermediate.

Synthesis of either phenoxazine-4-carboxylic acid or 10-ethylphenoxazine-4-carboxylic acid should be carried out by some unambiguous method, possibly by a cyclization reaction.

The proof of the structure of most of the derivatives of 10-phenylphenoxazine will depend upon some means of converting them to known compounds or synthesizing them unambiguously. Therefore, studies should be carried out on the cleavage of 10-phenylphenoxazine and its derivatives, on the cyclization to prepare 10-phenylphenoxazine derivatives, and on the conversion of phenoxazine derivatives to the corresponding 10-phenyl derivatives.

Further experiments should be carried out on the reaction of various organomercury compounds with boron trichloride. It would be interesting to know the effects which various groups on the aryl radical would have on this reaction, and which groups would aid the displacement of the mercury, but still not cause displacement of the boronic acid group. Since both the formation of the phenylboron dichloride and the subsequent cleavage of the carbon-boron bond are electrophilic reactions, weak ortho-para directing groups may serve this purpose. Complex organomercury compounds, particularly those derived from biological stains, may react with boron trichloride to produce boronic acids which could be used in brain tumor therapy.

The complexes of boronic acids with dyes could prove useful in brain tumor therapy, however, as yet nothing is known of their stability in water. Other dyes should be tested for the formation of possible complexes.

More azo dyes containing boronic acid groups should be synthesized if definite correlations are to be made of the relationship of the structure of these dyes to their physiological activity. It would certainly be interesting to make a direct comparison of the preparation of these azo dyes, with and without pyridine, to determine if this solvent aids the coupling of o-hydroxybenzeneboronic acid with the various diazonium salts.

Some studies have been made on the stability of the carbon-boron bond, but none yet on this bond in azo dyes. A study of the stability of this bond, particularly in a solution resembling plasma in concentration of salts and in pH, would be beneficial.

SUMMARY

A historical review of phenoxazine and its derivatives was presented.

The nitration of phenoxazine, 10-ethylphenoxazine, and 10-acetylphenoxazine was studied and the products identified by interconversion and comparison to some known derivatives. This is the first time phenoxazine and 10-ethylphenoxazine have been nitrated directly and the products identified. It was shown that the nitrogen has a greater directive influence on electrophilic substitution than the oxygen.

Studies on the bromination of phenoxazine, 10-ethylphenoxazine, 10-phenylphenoxazine, and 10-acetylphenoxazine were made and the products tentatively identified. A mechanism was proposed to account for the products obtained from the reaction of two moles of bromine with one of 10-acetylphenoxazine.

Azo coupling reactions of phenoxazine, 10-ethyl- and 10-phenylphenoxazine were reported.

The formation of carboxylic acids by metalation of phenoxazine and its ethyl and phenyl derivatives with n-butyllithium, and an attempt to use methyllithium to obtain only the monometalation product were reported. Attempts were made to prove the structures of the acids.

Reduction of nitro derivatives and onium salts and cleavage reactions were used in attempts to prove the struc-

tures of some of the products from the substitution reactions.

Comparisons were made on the means of preparing boronic acids using various organometallic reagents with n-butyl borate and boron trichloride.

A complex of benzeneboronic acid with Sudan Black B was reported.

Several boronic acid azo dyes were prepared for testing in brain tumor chemotherapy.

Suggestions for future research on both phenoxazine and boronic acids were made.

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