responding acetals were formed as reaction products. This would indicate that the elimination of the ethyl radical (reaction 7) from the acetal radical proceeds to the complete exclusion of any hydrogen abstractions by this radical (reaction 8) even though hydrogen abstraction is apparently more facile than in the trimethyl orthoformate.

These free radical fragmentation reactions of ortho esters have short kinetic chain lengths. They are, however, reactions that might lead to the formation of by-products in other free radical reactions of ortho esters, for example, Kharasch additions to olefins.[3]

Experimental

Trimethyl orthoformate and triethyl orthoformate (Matheson, Coleman and Bell) were distilled before using. Triethyl orthoacetate was obtained as a research sample from Kay-Fries Chemicals, Inc., and was used without further purification as was the di-*t*-butyl peroxide (Lucidol). The compounds used as references for the gas chromatographic analyses were stock items which were purified when necessary to give a single chromatographic peak.

Trimethyl Orthoformate and Di-*t*-butyl Peroxide.—A reaction mixture consisting of trimethyl orthoformate (53 g., 0.50 mole) and di-*t*-butyl peroxide (4.4 g., 0.03 mole) was heated for 18 hr. at 135° in a stainless steel autoclave. After cooling, the gas formed in the reaction was released, passed through a Dry Ice trap and its volume (1.97 l. corrected to standard conditions) measured by means of a wet test meter. A sample of the gas was analysed on an 8-ft. silicon gel column (column temperature 77°, helium used as carrier gas) and proved to be 95% methane. Some of the formaldehyde produced in the reaction was condensed in the Dry Ice trap. The liquid portion of the reaction mixture was distilled and the fraction boiling up to 100° collected. Gas chromatographic analysis on an 8-ft. column packed with 15% di-2-ethylhexyl sebacate on Chromosorb P (column temperature 50°, helium used as carrier gas) indicated peaks with retention times identical with those of authentic samples of formaldehyde, methyl formate, methylal, acetone, and methyl carbonate as well as unchanged trimethyl orthoformate. The amounts of these components were determined by comparison of their peak areas, necessary correction factors having been previously determined from the chromatograms of known mixtures of these materials.

Triethyl Orthoformate and Di-*t*-butyl Peroxide.—A solution consisting of triethyl orthoformate (74 g., 0.50 mole) and di-*t*-butyl peroxide (4.4 g., 0.03 mole) was heated at 135° for 18 hr. in a flask equipped with a reflux condenser. The gases evolved during the course of the heating were passed through a Dry Ice trap and the volume (1.35 l. corrected to standard conditions) measured with a wet test meter. Gas chromatographic analysis of a sample of these gases on a silicon gel column (same conditions as in previous experiment) indicated the presence of ethane, a trace amount of ethylene, and a peak corresponding in retention time to that of methane and carbon monoxide. Some of the acetaldehyde formed was found in the Dry Ice trap (m.p. of 2,4-dinitrophenylhydrazone, 144–146°, reported, 147° [4]). The liquid portion of the reaction mixture was distilled and the fraction boiling up to 135° was collected. Gas chromatographic analysis on a 10-ft. column packed with 10% polyethylene succinate on Chromosorb W (column temperature, 53°, helium used as carrier gas) showed peaks with retention times the same as those of authentic samples of ethyl formate, *t*-butyl alcohol, and ethyl carbonate. Trace amounts of acetone and acetaldehye were also present. No peak with a retention time corresponding to diethoxymethane was found. The quantitative determination of the major components was made by comparison of their peak areas using toluene as an internal chromatographic standard. The necessary correction values of the mole ratios to area ratios of the reaction products with respect to toluene were determined from known mixtures.

Triethyl Orthoacetate and Di-*t*-butyl Peroxide.—A reaction mixture consisting of triethyl orthoacetate (33.9 g., 0.21 mole) and di-*t*-butyl peroxide (11.9 g., 0.08 mole) was heated for 18 hr. at 135°. The gas produced was passed through a Dry Ice trap and collected over water (2.89 l. corrected to standard conditions). Analysis of the gas by gas chromatography on a 50-ft. column packed with propylene carbonate on alumina (column temperature, 0°, helium used as carrier gas) showed the presence of ethane in the amount shown in Table I and a peak corresponding in retention time to that of methane and carbon monoxide. Most of the acetaldehyde formed was collected in the Dry Ice trap. Gas chromatographic analysis of the materials in the liquid portion boiling up to the boiling point of the orthoacetate on the di-2-ethylhexyl sebacate column showed peaks with retention times identical with those of ethyl acetate and *t*-butyl alcohol. No peaks with retention times the same as those of acetone and acetal were found. Quantitative determination of ethyl acetate was made from its peak area using benzene as an internal chromatographic standard. The mole ratio to peak area ratio for the internal standard to ethyl acetate was previously determined from known mixtures of these components.

Acknowledgment.—We wish to thank Mr. Y. N. Tang for performing the chromatographic analyses of the gaseous products. Also, we gratefully wish to acknowledge the support of this work by the National Science Foundation.

(3) T. M. Patrick, U.S. Patent 2,716,660 (1955).

(4) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed., John Wiley & Sons, Inc., New York, N. Y., 1959, p. 283.

Aromatic Hydroxylation by the Model Peroxidase System[1]

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The "Model Peroxidase System," first investigated by Udenfriend and co-workers[3a,b] and later by Grinstead,[4] is capable of hydroxylating aromatic compounds at specific sites on the ring. This system uses oxygen or hydrogen peroxide in the presence of ascorbic acid, ferrous ion, and a complexing agent ethylene diamine tetraacetic acid (EDTA) in

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(3)(a) S. Udenfriend, C. T. Clark, J. Axelrod, and B. B. Brodie *J. Biol. Chem.*, **208**, 731 (1954); (b) B. B. Brodie, J. Axelrod, P. A. Shore, and S. Udenfriend, *ibid.*, **208**, 741 (1954).

(4)(a) R. R. Grinstead, *J. Am. Chem. Soc.*, **82**, 3464 (1960); (b) R. R. Grinstead *ibid.*, **82**, 3472 (1960).

TABLE I

TYPICAL YIELDS[a] OF *o*-HYDROXYACETANILIDE ISOLATED UNDER VARIOUS REACTION CONDITIONS

Oxidizing agent	Temp., °C.	Reaction time, hr.	Acetanilide recovered, g.	*o*-Hydroxyacetanilide isolated g.	*o*-Hydroxyacetanilide isolated %[c]
H_2O_2	100	2	Discarded[b]		
H_2O_2	75	2	3.5	3.5	22.0
H_2O_2	25	10	9.0	1.3	12.5
O_2	75	2	7.4	1.3	10.9
O_2	25	6	15.0	...	...

[a] Each mixture contained 18 g. of acetanilide, EDTA, Fe^{+2}, buffer pH 6.0, water (see Experimental). Solutions of ascorbic acid and hydrogen peroxide (where used) were each added dropwise. Oxygen (where used) was bubbled in rapidly from fritted-glass gas inlet tubes. [b] Each droplet of the hydrogen peroxide "exploded" as it came in contact with the hot solution, and consequently this mixture was discarded. [c] Per cent yield based on the amount of acetanilide which actually was reacted.

a neutral aqueous solution. The purpose of the present study is to utilize this system to provide a general synthetic method for various phenolic compounds.

Table I presents a summary of the various conditions studied in the hydroxylation of acetanilide. Although yields were somewhat variable, those listed in Table I are typical. The best results were obtained using hydrogen peroxide as the oxidant at 75°. Such conditions resulted in yields up to 46%, but they usually varied between 20 and 25%. At 100° the droplets of the hydrogen peroxide solution seemed to decompose upon contact with the hot solution; at room temperature the yields were markedly lowered. The lower temperature reaction would become important, however, if the system were to be applied to a substrate which is heat-labile or which produces heat-labile products.

Oxygen produced somewhat cleaner appearing mixtures, but the yields were lower. At the elevated temperatures it was necessary to bubble the oxygen very rapidly through the reaction mixture. In the initial experiments in which the oxygen was bubbled slowly into the mixture at a rate designed to keep the solution saturated with oxygen, almost no hydroxylation was observed. The best results with oxygen were obtained by admitting the oxygen as rapidly as possible through two fritted-glass gas inlet tubes.

The use of an aqueous solvent presents a serious limitation of this system since the rate of reaction depends on the solubility of the substrate in the solvent. This problem has not been satisfactorily solved. Although the reaction is not greatly affected by 10% solutions of ethyl alcohol, acetone, acetic acid, or dioxane, yields are generally lowered upon increasing the concentration of these organic solvents. However in the hydroxylation of pentamethylbenzene, which is quite insoluble even in the 10% organic solvents, it was necessary to increase the dioxane concentration to 33% to obtain hydroxylation. Anhydrous organic solvents such as benzene or two-phase systems such as water–benzene accompanied with violent stirring were unsatisfactory.

Although *o*-hydroxyacetanilide could be isolated readily from the reaction mixture, all attempts to isolate the *para* isomer failed. The evidence indicated, however, that the two isomers were formed in about equal amounts. Paper chromatograms of the reaction mixture showed the presence of a compound which exhibited the same R_f value as the *para* isomer, and which produced the same blue color as the *para* isomer when sprayed with the ferricyanide reagent. The compound after elution from the paper with ethyl alcohol exhibited the same ultraviolet absorption spectrum as the authentic *para* isomer.

p-Hydroxyacetanilide mixed with various combinations of the usual reactants could be recovered readily by extraction procedures. However, when the reaction was conducted in the normal manner and a known amount of the *para* isomer was added to the mixture at the completion of the reaction, no recovery could be obtained by the extraction. A possible explanation for this behavior is that the *para* isomer is being complexed by something which is being produced during the reaction. This complex apparently alters the solubility characteristics of the *para* isomer, preventing the isolation by extraction, but the complex either dissociates under the conditions of the paper chromatography, or else it exhibits the same R_f value, produced the same blue color with the developing reagent, and exhibits the same ultraviolet absorption spectrum as the *p*-hydroxyacetanilide.

Oxalic acid dihydrate was isolated from one reaction mixture. Oxalic acid has been reported[5] to be a degradation product of ascorbic acid in other systems.

Previous work and preliminary experiments in this research indicate that the hydroxylation by this system can be applied to many other compounds (see Table II). The hydroxylation of benzopyrene is especially intriguing since this carcinogen has been reported[6] to be hydroxylated "in vivo"; experiments with scorbutic guinea pigs are in progress to determine if ascorbic acid is a factor in tumor production by benzopyrene.

(5) P. B. Hawk, B. L. Oser, and W. H. Summerson, "Practical Physiological Chemistry," The Blakiston Co., New York, N. Y., 1951, p. 1133.

(6) J. W. Cook, R. S. Ludwiczak, and R. Schoental, *J. Chem. Soc.*, 1112 (1950).

TABLE II

PAPER CHROMATOGRAPHIC ANALYSIS OF PRODUCTS OF VARIOUS SUBSTRATES IN THE HYDROXYLATION REACTION

Substrate	Chromatographic solvent	Probable products	R_f
Benzopyrene	CO_2–H_2O	Unknown	0.0
Naphthalene	CO_2–H_2O	1-Hydroxynaphthalene	.41
		2-Hydroxynaphthalene	.38
		Unknown	.56
		Unknown	.73
Pentamethylbenzene	CO_2–H_2O	Pentamethylphenol	.23
Isatin	CO_2–H_2O	Unknown	.49
Tryptophane	CO_2–H_2O	Unknown	.65
Benzoic Acid	Isopropyl alcohol–ammonia–water	*o*-Hydroxybenzoic acid	.90
		p-Hydroxybenzoic acid	.30
		m-Hydroxybenzoic acid	.40
Acetanilide	Isopropyl alcohol–ammonia–water	*o*-Hydroxyacetanilide	.92
		p-Hydroxyacetanilide	.97
Quinoline	Benzene–acetic acid–water	3-Hydroxyquinoline	.50
		Unknown (white flour.)	.00
		Unknown (pink flour.)	.05
		Unknown (yellow flour.)	.10

Experimental

Procedure for Hydroxylation.—A mixture of 18 g. (0.13 mole) of acetanilide, 3.8 g. (0.01 mole) of EDTA (tetra sodium salt), 0.2 g. (0.0004 mole) of ferrous sulfate heptahydrate, 50 ml. of phosphate buffer, pH 6 (prepared from 1 *M* stock solutions), and 100 ml. of water was heated at 75° with vigorous stirring. Solutions of ascorbic acid [35.5 g. (0.2 mole) dissolved in 150 ml. of water] and hydrogen peroxide [46 ml. of 30% solution (0.4 mole) diluted to 200 ml.] were added dropwise simultaneously over a period of 90 min. After permitting the dark mixture to react for an additional 30 min., it was cooled to room temperature. During the reaction 6 *M* sodium hydroxide was added as needed to maintain the pH between 6.5 and 7.0.

Variations in this procedure are presented in Table I. In the experiments in which oxygen replaced the hydrogen peroxide an additional 200 ml. of water was added to maintain constant volume, and the oxygen was bubbled through the reaction mixture from a fritted-glass gas inlet tube during the entire reaction period.

Isolation of Products.—Sodium hydroxide was added until the reaction mixture was distinctly alkaline, and this solution was extracted with several portions of chloroform. The chloroform extracts were combined, dried with anhydrous sodium sulfate, and upon removal of the solvent by vacuum distillation yielded 3.5 g. of unchanged acetanilide, m.p. 112–113° (lit.,[7] m.p. 113°). The melting point of a mixture of this product with authentic acetanilide showed no depression.

The aqueous phase after adjusting its pH to 5 by the addition of 3 *M* hydrochloric acid was extracted with several portions of ether. The combined etheral extracts after drying with anhydrous sodium sulfate and removal of the solvent by vacuum distillation yielded crude *o*-hydroxyacetanilide. Recrystallization from ethanol yielded 3.5 g. of product, m.p. 200–203° (lit.,[8] m.p. 203°).

The *p*-hydroxyacetanilide in the aqueous phase from the above could be extracted into *n*-butyl alcohol, or, if the aqueous phase were first saturated with ammonium sulfate, into ether. These results were determined by paper chromatography. However, all attempts to isolate the para isomer from these solvents yielded intractable oils.

Variations of Solvent.—The hydroxylation procedure was modified in order to study more conveniently the effect of changing the solvent. In each of sixteen Erlenmeyer flasks were placed 3.5 g. of ascorbic acid, 1.8 g. of acetanilide, 0.4 g. of EDTA, and 0.02 g. of ferrous sulfate heptahydrate. One hundred milliliters of the solvent to be tested was added to a flask, and a solution of 4.6 ml. of 30% hydrogen peroxide diluted to 15 ml. was added dropwise. A magnetic stirrer was used to agitate the mixture for 2 hr. The temperature of each mixture was approximately 40°. At the completion of the reaction period aliquots were removed for paper chromatographic analysis.

Solvents consisting of 10, 50, and 100% of each of the following were tested: ethyl alcohol, acetone, acetic acid, dioxane, and benzene (two-phase system, accompanied by very violent stirring). The paper chromatograms were compared with a chromatogram obtained from a reaction conducted under the same conditions, but in which water was used as a solvent, and were examined for both of the possible isomeric products.

The reactions which contained only 10% of the ethyl alcohol, acetone, acetic acid, or dioxane were found to be about equally as effective in hydroxylation as the pure water solvent; however, the other mixtures contained almost no hydroxylated products.

In the attempted hydroxylation of pentamethylbenzene, the substrate seemed to be almost completely insoluble even in the 10% organic solvents, and no hydroxylation was observed. However, some hydroxylation did occur in a solvent consisting of approximately 33% dioxane.

Paper Chromatography Procedures.—Paper chromatography was used to identify the products of the reaction. In some instances positive identification of products was not attempted since the presence of phenolic material in the reaction mixture was sufficient to demonstrate that hydroxylation had occurred. The "ferric ferricyanide" spray reagent, as described by Barton, Evans, and Gardner,[9] was used to locate the nonfluorescent products on the developed chromatogram, while the fluorescent products of quinoline were located by irradiation with ultraviolet light.

The solvent, consisting of isopropyl alcohol, ammonia, and water[10] (8:1:1) was used in the paper chromatography of products of benzoic acid and acetanilide. Water to which had been added sufficient carbon dioxide to adjust the pH to 4.3[9] was employed for the products of benzopyrene, naphthalene, pentamethylbenzene, isatin, and tryptophane. Benzene, acetic acid, and water[11] (2:2.2:1) was used for the chromatography of the products of quinoline. The R_f values of each product are shown in Table II.

Further Proof of the Formation of *p*-Hydroxyacetanilide.—The presence of the *p*-hydroxyacetanilide in the reaction mixture was further confirmed by the elution of the com-

(7) J. H. Mathrews, *J. Am. Chem. Soc.*, **39**, 1131 (1917).

(8) J. B. Tingle and L. F. Williams, *Am. Chem. J.*, **37**, 51 (1907).

(9) G. M. Barton, R. S. Evans, and J. A. F. Gardner, *Nature*, **170**, 239 (1952).

(10) M. D. Armstrong, K. N. F. Williams, and P. E. Wall, *J. Biol. Chem.*, **218**, 293 (1956).

(11) C. Mitoma, H. S. Posner, H. C. Reitz, and S. Udenfriend, *Arch. Biochem Biophys.*, **61**, 433 (1956).

pound from the paper chromatogram with ethyl alcohol and examining the ultraviolet absorption spectrum of this solution. This spectrum was identical to one obtained from an alcoholic solution of an authentic sample of the *para* isomer.

Boron–Nitrogen Compounds. VIII.[1,2] 2-Dimethylamino-1,3,2-benzodiazaboroline

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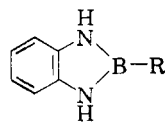
Schupp and Brown[3] first investigated the reaction of boron compounds with *o*-phenylenediamine. On interaction of dichlorophenylborane, for instance, compound I with R = C_6H_5 was obtained. Considerable interest has since developed

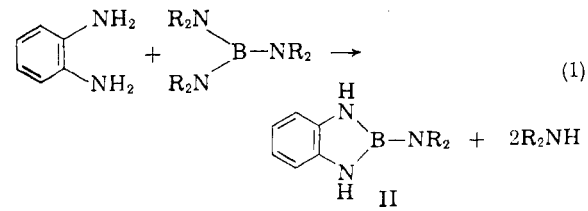


I

in compounds of this aromatic, boron-containing heterocyclic system[4] and several preparative approaches have been investigated.[5–9] However, no 2-amino-1,3,2-benzodiazaborolines (I. R = NR_2') have been reported. In connection with our previous work on the diazaboroline system[10] and on B-aminoborazines[11,12] the preparation of such compounds seemed of interest.

Results and Discussion

Recently the preparation of the 1,3,2-benzodiazaboroline (I) with R = Cl has been reported.[13] Attempts to aminolize the boron–chlorine linkage under mild conditions did not yield the desired 2-



(1)

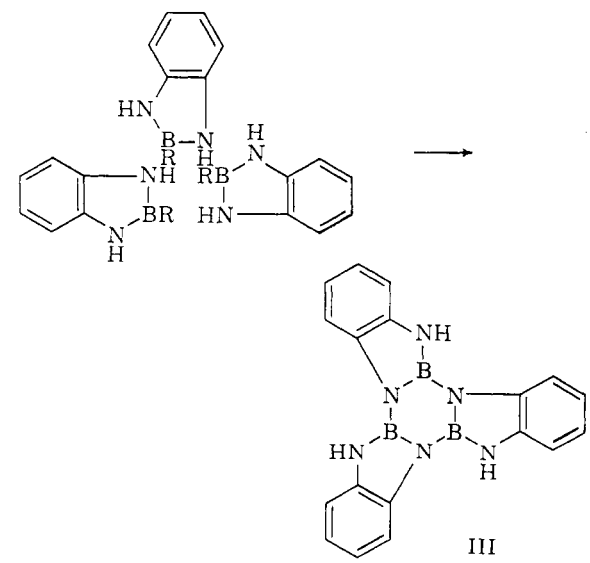
II

amino-1,3,2-benzodiazaboroline (II). Therefore a transamination reaction as illustrated in equation 1 was used to synthesize this compound. On refluxing *o*-phenylenediamine and tris(dimethylamino)-borane, in ether, compound II was obtained in good yield and could be isolated from the crude reaction mixture by vacuum sublimation.

The infrared spectrum of 2-dimethylamino-1,3,2-benzodiazaboroline shows a distinct N—H stretching frequency near 3390 cm.$^{-1}$. Two extremely strong absorptions were recorded at 1468 cm.$^{-1}$. and 1378 cm.$^{-1}$ and consequently assigned as B—N absorptions. This seems to be in general agreement with our previous observation[10] that boron–nitrogen compounds with one central boron atom linked to two different nitrogen atoms affords two different B—N absorptions.

When a higher boiling solvent, for instance xylene, was employed in the preparation described above, compound II was not obtained but III could be isolated in nearly quantitative yield.

It has been shown previously,[14,15] that the final product from the interaction of boron trichloride and *o*-phenylenediamine likewise is the borazine derivative (III). Similarly, III was obtained on refluxing tris(ethoxy)borane with *o*-phenylenediamine in xylene.[9]



III

(1) Part VII, K. Niedenzu, *Inorg. Chem.*, **1**, 943 (1962).

(2) Supported by the U. S. Army Research Office (Durham).

(3) L. J. Schupp and C. A. Brown, Abstracts of Papers, 128th National Meeting of the American Chemical Society, Minneapolis, Minn., 1955, p. 48-R.

(4) Several nomenclatures have been introduced into the literature to refer to compounds of type I. The benzodiazaboroline designation is one recommended by the Committee on the Nomenclature of Boron Compounds of the American Chemical Society.

(5) M. J. S. Dewar, V. P. Kubba, and R. Pettit, *J. Chem. Soc.*, 3076 (1958).

(6) R. L. Letsinger and S. B. Hamilton, *J. Am. Chem. Soc.*, **80**, 5411 (1958).

(7) E. Nyilas and A. H. Soloway, *ibid.*, **81**, 2681 (1959).

(8) M. F. Hawthorne, *ibid.*, **81**, 5836 (1959).

(9) R. J. Brotherton and H. Steinberg, *J. Org. Chem.*, **26**, 4632 (1961).

(10) K. Niedenzu, H. Beyer, and J. W. Dawson, *Inorg. Chem.*, **1**, 738 (1962).

(11) K. Niedenzu and J. W. Dawson, *J. Am. Chem. Soc.*, **81**, 3561 (1959).

(12) K. Niedenzu, D. H. Harrelson, and J. W. Dawson, *Chem. Ber.*, **94**, 671 (1961).

(13) L. F. Hohnstedt and A. M. Pellicciotto, Final Report, Office of Naval Research Contract Nonr 2793(00), 1961.

(14) C. A. Brown, Final Report, Office of Naval Research Contract Nonr 1439(02), 1956.

(15) B. Rudner and J. J. Harris, Abstracts of Papers, 138th National Meeting of the American Chemical Society, New York, N. Y., 1960, p. 61-P.