The duplicate figures represent analyses of two independently prepared specimens.

The isopropylidene determination was carried out by the method of Kuhn and Roth.[20]

Acknowledgment.—The authors wish to thank Mr. E. Paredes for the preparation of the streptomycin used, and Mr. J. A. Alicino and his associates for the microanalytical determinations.

Summary

The preparation and properties of three hexaacetates, and of a pentaacetate of dihydrostreptobiosamine are described.

Catalytic reduction of the hydrochlorides of either dihydrostreptobiosamine or streptobiosamine yields an anhydro base, tetrahydroanhydrostreptobiosamine hydrochloride, which on acetylation forms a crystalline O-pentaacetyl base hydrochloride. A structural formula is suggested for this compound.

N-Methyl-L-glucosamine-β-pentaäcetate, isolated in the course of acetolysis studies on methyl pentaacetyldihydrostreptobiosaminide, and a dimeric product of unknown structure obtained by acetonation of methyl dihydrostreptobiosaminide and subsequent acetylation, are described.

NEW BRUNSWICK, NEW JERSEY RECEIVED MAY 19, 1947

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[20] R. Kuhn and H. Roth, *Ber.*, **65**, 1285 (1932).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ALBERTA]

Steric Inhibition of Resonance. The Reactivities of Some Halogenated Primary and Tertiary Aromatic Amines

BY REUBEN B. SANDIN AND JACK R. L. WILLIAMS[1]

During the past decade many important contributions have been made to the topic of steric inhibition of resonance and reaction rates.[2] Derivatives of aromatic tertiary amines have been examined extensively from this point of view. A careful study of hydrogen–deuterium exchange in aromatic amines has been carried out by Brown, Widiger and Letang.[3] Westheimer and Metcalf[4] have made an equally careful study of the rates of saponification of the esters of aminobenzoic acids. The authors of the present paper have examined in a qualitative way the behavior of *p*-iodoaniline, *p*-iododimethylaniline, 2,4,6-triiodoaniline and 2,4,6-triiododimethylaniline toward several electrophilic reagents (reactions with acid stannous chloride, bromine, and nitrous acid). The assumption was made that only in the case of 2,4,6-triiododimethylaniline should "damping" of resonance be marked and therefore should lead to a lowered reactivity of the halogen atoms, in the above reactions.

All the compounds needed for the investigation were known with the exception of 2,4,6-triiododimethylaniline. This substance was readily made from 2,4,6-triiodoaniline by the methylation procedure of Clarke, Gillespie and Weisshaus.[5] This process has also been used successfully by Emerson and co-workers.[6]

Experimental

Materials.—2,4,6-Triiodoaniline,[7] m. p. 185°, *p*-iodoaniline,[8] m. p. 62–63°, and *p*-iododimethylaniline,[9] m. p. 79–80°, were prepared without difficulty and in good yield.

2,4,6-Triiododimethylaniline.—Ten grams of 2,4,6-triiodoaniline, 300 cc. of 90% formic acid and 25 cc. of formalin was boiled under reflux for two hours. After the addition of 3–5 cc. of concentrated hydrochloric acid and removal of most of the formic acid under reduced pressure, the residue was made alkaline and distilled with steam. The methylated product passed over very slowly and was collected as a heavy oil. On long standing it solidified. It was found that much time could be saved by not doing a steam distillation. Instead, the reaction mixture after refluxing was diluted with 3 liters of water. It was then seeded with some of the solid compound and after standing for twelve hours, the material was collected. The yield of crude substance, m. p. 60–68°, was 9.2 g. After crystallization from acetic acid a 54% yield (6 g.) of pure compound was obtained as pale yellow needles, m. p. 69–70°.

Anal. Calcd. for $C_8H_8NI_3$: I, 76.32. Found: I, 76.14, 76.23.

Reaction with Hydrochloric Acid and Stannous Chloride.—The method employed was similar to the one used by Nicolet[10] and co-workers for the determination of so-called "positive" halogen. It was essential that the acid concentrations should be the same for all runs, since it is known that the rate of halogen removal is directly proportional to the acid concentration but is independent of the stannous chloride concentration.

In this work 0.001 mole of compound was dissolved in a boiling mixture of 50 cc. of glacial acetic acid and 10 cc. of concentrated hydrochloric acid. To this was added 0.025 mole of stannous chloride and the solution was refluxed for one hour. On boiling, some hydrogen chloride was lost. However, since all experiments were carried on as nearly as possible under the same conditions, no attempt was made to avoid this loss.

After refluxing, the reaction mixture was made alkaline, extracted with carbon tetrachloride and steam distilled.

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[2] See the excellent discussions on this subject by Wheland in Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 272–279; and by Bartlett in Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, pp. 211–213; see also Birtles and Hampson, *J. Chem. Soc.*, 10 (1937); Ingham and Hampson, *ibid.*, 981 (1939).

[3] Brown, Widiger and Letang, THIS JOURNAL, **61**, 2597 (1939); see also Brown, Kharasch and Sprowls, *J. Org. Chem.*, **4**, 442 (1939).

[4] Westheimer and Metcalf, THIS JOURNAL, **63**, 1339 (1941).

[5] Clarke, Gillespie and Weisshaus, *ibid.*, **55**, 457 (1933).

[6] (a) Emerson, Neumann and Moundres, *ibid.*, **63**, 972 (1941); (b) Emerson, *ibid.*, **63**, 2023 (1941).

[7] Jackson and Whitmore, *ibid.*, **37**, 1522 (1915).

[8] Brewster, "Organic Syntheses," Coll. Vol. II, 1943, p. 347.

[9] Reade and Sim, *J. Chem. Soc.*, 157 (1924).

[10] (a) Nicolet, THIS JOURNAL, **43**, 2081 (1921); (b) Nicolet and Sampey, *ibid.*, **49**, 1796 (1927); (c) Nicolet and Ray, *ibid.*, **49**, 1801 (1927); (d) Nicolet and Sandin, *ibid.*, **49**, 1806 (1927); (e) Nicolet, *ibid.*, **49**, 1810 (1927).

The reaction mixture thus freed from organic material was acidified with sulfuric acid and treated with excess ferric chloride. The liberated iodine was steam distilled into a cold solution of potassium iodide in water, and was then titrated with standard sodium thiosulfate. The percentage of iodine removed for each compound is shown in Table I. The authors make no claim to great quantitative accuracy. Aniline was characterized as acetanilide and dimethylaniline as the picrate.

Reaction with Bromine.—A solution of 0.001 mole of compound in 100 cc. of glacial acetic acid was treated with 0.003 mole of bromine in 10 cc. of acetic acid.[11] After standing for twenty-four hours at room temperature, the reaction mixture was made alkaline, extracted with carbon tetrachloride and steam distilled. The amount of displaced iodine was determined by a process similar to the above. The results are shown in Table I.

Reaction with Nitrous Acid.—This reaction was carried out according to the procedure used by Emerson[6b] for the preparation of N-nitroso-N-methyl-2,4,6-tribromoaniline. Seven grams of 2,4,6-triiododimethylaniline afforded an almost quantitative yield (7.2 g.) of practically pure N-nitroso-N-methyl-2,4,6-triiodoaniline, m. p. 122-123°. It was crystallized from alcohol, pale yellow crystals, m. p. 123-124°.

Anal. Calcd. for $C_7H_5ON_2I_3$: I, 74.10. Found: I, 74.20, 73.80.

Aitken and Reade[12] have shown that the reaction of *p*-iododimethylaniline with nitrous acid affords iodine, *p*-nitrodimethylaniline and 4-iodo-2-nitrodimethylaniline. They also found that no nitrosoamine was produced.

Discussion of Results

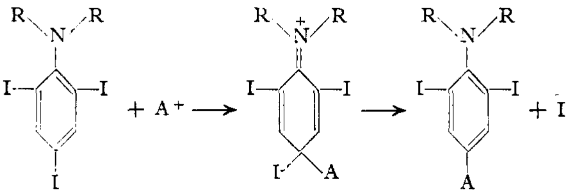
The results obtained are in accordance with the original assumption. This is especially noticeable in Table I. Under the same experimental conditions, compounds I and II (not ortho substituted), and compound III (ortho substituted) show a marked halogen reactivity. On the other hand, compound IV (ortho substituted and N-methylated) shows a percentage of halogen removal which is almost negligible. In the reaction with stannous chloride the contrast between III and IV, where the ratio of iodine removed is approximately 30:1, is very definite.

TABLE I

	Substance	Per cent. halogen removed by HCl-$SnCl_2$	By Br_2
I	*p*-Iodoaniline	46[a]	58[d]
II	*p*-Iododimethylaniline	55[b]	65[d]
III	2,4,6-Triiodoaniline	90[a]	100[e]
IV	2,4,6-Triiododimethylaniline	3[c]	18[d]

[a] Aniline recovered. [b] Dimethylaniline recovered. [c] Unchanged material recovered. [d] No definite organic compound recovered. [e] Practically pure tribromoaniline, m. p. 120-122°, recovered.

The reaction with acid stannous chloride[13] is probably an electrophilic attack by hydrogen ion on nuclear carbon, the iodine being displaced as positive ion.[14] A decrease in halogen reactivity is the normal result of a steric damping of resonance by ortho substituents. The mechanism of this effect can be represented in the following way[15]



(1)

where A^+ is the electrophilic reagent Similarly A^+ will react with the original, to form the intermediate (I) where A is now located at the ortho carbon atoms. With the proper ortho substituents, if R is large it will prevent the ability of R_2N and the benzene ring to become coplanar and so inhibit resonance. As a result the tendency for the formation of intermediate (I) is lowered. In the case of compound IV, the effective dimensions of R (methyl) and the ortho substituents (iodine) are such that this has been brought about.

The authors believe that the reactions with bromine and nitrous acid can be explained on the same basis. In the former reaction, compound IV shows the least amount of iodine displaced. In the latter reaction, compound IV shows no evidence of iodine reactivity at all. The nature of A^+ is not so obvious in these reactions. The authors would like to think of A^+ as being Br^+ [16] in the bromine reaction, and NO^+ [16] (or the positive end of the dipole in $:\overset{\overset{-}{\cdot\cdot}}{\underset{\cdot\cdot}{O}}:\overset{+}{\underset{\underset{H}{\cdot\cdot}}{N}}::\underset{\cdot\cdot}{O}:$[17] in the reaction with nitrous acid.

In connection with the relatively high degree of reactivity of triiodoaniline compared with compounds I and II, it is interesting to note that Emerson, Dorf and Deutschman[18] have shown that in the reductive alkylation of 2,4,6-tribromoaniline with amalgamated zinc and hydrochloric acid as the reducing agent, an 88% yield of N,N-dimethyl-*p*-bromoaniline was obtained. Similarly a 68% yield of N,N-dimethyl-4-bromo-2-methylaniline was obtained from 4,6-dibromo-2-methylaniline. Evidently in the reactions with electrophilic reagents, halogen which is ortho substituted is more reactive than halogen which is para substituted.

Arnold, Pierce and Barnes[19] have used the concept of steric inhibition of resonance to account for the fact that the melting points of various

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(11) In the case of 2,4,6-triiododimethylaniline a small amount of very pale yellow precipitate was produced (salt or perbromide?). It was not examined further.

(12) Aitken and Reade, *J. Chem. Soc.*, 1896 (1926).

(13) The presence of the stannous chloride obviates the effects due to resubstitution and oxidation.

(14) Remick, "Electronic Interpretations of Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1940, pp. 420-421.

(15) Brown and Fried, THIS JOURNAL, **65**, 1841 (1943).

(16) (a) Bartlett in Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, p. 210; (b) Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 258-259.

(17) Hurd in Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, p. 615.

(18) Emerson, Dorf and Deutschmann, THIS JOURNAL, **62**, 2159 (1940).

(19) Arnold, Pierce and Barnes, *ibid.*, **62**, 1627 (1940).

alkylated amino nitro compounds decrease markedly with increasing size of the alkyl groups. The melting points of the trihalogeno derivatives of aniline, monomethylaniline and dimethylaniline show the same decrease; thus, the melting points for the trichloro derivatives are 78.5, 32 and < 25°; for the tribromo, 122, 39 and < 25°, respectively; and for the triiodoaniline and dimethylaniline, 185 and 69–70°, respectively. The authors believe that these decreases are another example of the same phenomenon recorded by Arnold, Pierce and Barnes.

Summary

1. 2,4,6-Triiododimethylaniline has been synthesized.

2. *p*-Iodoaniline, *p*-iododimethylaniline, 2,4,6-triiodoaniline and 2,4,6-triiododimethylaniline have been examined from the standpoint of halogen reactivity, in reactions with acid stannous chloride, bromine and nitrous acid.

3. As predicted from the theory of damped resonance, the halogen atoms in 2,4,6-triiododimethylaniline show a relatively low order of reactivity.

Edmonton, Alberta, Canada Received April 28, 1947

[Contribution from the Research Laboratories of Merck and Co., Inc.]

Isolation of Rhizopterin, A New Growth Factor for *Streptococcus Lactis R*

By Edward L. Rickes, Louis Chaiet and John C. Keresztesy[1]

Experiments performed in this Laboratory very early demonstrated the presence, in some natural materials, of more than one factor capable of supporting the growth of *Streptococcus lactis R*[2] on a "folic acid" deficient medium.[3] Differential assays of concentrates from several natural sources indicated that some possessed a much higher activity for *S. lactis R* than for *Lactobacillus casei*.[4] This property was shown to a striking degree by a charcoal adsorbate[5] derived from the purification of *Rhizopus nigricans* fumaric acid fermentation liquors. The fractionation of an eluate of this adsorbate by means of chromatographic adsorption procedures led to the isolation, in crystalline form, of a compound highly active for *S. lactis R* and substantially inactive for *L. casei*.[6]

Subsequent investigations have shown that this new growth factor is a pterin.[7,8] Because of the nature and source of the factor, the name rhizopterin has been adopted for this compound, formerly referred to as the "S.L.R. factor."[9]

It was found that rhizopterin was relatively weakly adsorbed on alumina and appeared in the initial eluates. It could be distinguished from succeeding more strongly adsorbed fractions, which were active for both organisms. The more strongly adsorbed material which appeared in the later eluate fractions corresponded closely in chromatographic adsorption behavior and biological activity to other concentrates of vitamin B_c (pteroylglutamic acid).

To obtain rhizopterin in crystalline form, it was necessary to effect a 200,000-fold purification from the initial charcoal eluate. This was accomplished through the following sequence of steps: elution of the charcoal adsorbate, readsorption by norit A and elution, adsorption by fuller's earth and elution, precipitation at *p*H 7, chromatographic adsorption on alumina, and crystallization as the free acid or ammonium salt.

The microanalytical data for rhizopterin are in best agreement with the formula $C_{15}H_{12}N_6O_4$. The pterin-like nature of the compound is indicated by a comparison of the absorption spectra of rhizopterin and xanthopterin (Figs. 1 and 2). The pterin nature is further demonstrated by the potentiometric titration data for these two compounds (Table I). An equivalent weight of 167.5 was calculated for rhizopterin. Since the potentiometric titration indicated that it is a dibasic acid, one may assume a molecular weight of 335, which is in agreement with the value of 340 calculated for $C_{15}H_{12}N_6O_4$. Other physical properties of rhizopterin, such as its insolubility in water and common organic solvents, its solubility in acid and alkali, and the failure of the compound to melt up to 300° further indicate the correlation of rhizopterin with the pterins. Later chemical and physico-chemical studies confirmed the pterin-like nature of the compound.[7,8]

Table I

	Rhizopterin	Xanthopterin
Initial *p*H	4.3	5.0
Midpoint	7.2	9.0
Neutralization point	10.3	10.8

It was found that 0.000034 γ of rhizopterin per ml. of culture medium is necessary to produce half maximum growth of *S. lactis R*. It is essentially inactive for *L. casei*.

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(1) Present address: National Institute of Health, Bethesda, Md.

(2) This organism is also known as *Streptococcus faecalis R*.

(3) Assay method of Mitchell, Snell and Williams, This Journal, **63**, 2284 (1941).

(4) Norit eluate factor assays using *L. casei*: Hutchings, Bohonos and Peterson, *J. Biol. Chem.*, **141**, 521 (1941).

(5) This adsorbate was obtained from Chas. Pfizer and Co. *Cf.* J. H. Kane, A. Finlay, P. F. Amann, U. S. Patent 2,327,191 (1943).

(6) Keresztesy, Rickes and Stokes, *Science*, **97**, 465 (1943).

(7) Wolf, Anderson, Kaczka, Harris, Arth, Southwick, Mozingo and Folkers, This Journal, **69**, 2753 (1947).

(8) Rickes, Trenner, Conn and Keresztesy, *ibid.*, 2751 (1947).

(9) Stokes, Keresztesy and Foster, *Science*, **100**, 522 (1944).