unsatisfactory. The reaction mixture was filtered by suction, the solid washed free of copper salts with water, then with 5% sodium hydroxide to remove phenols, and finally with water.

Extraction and Purification of the Nitro Compounds.—The nitrobenzene, *o*- and *m*-dinitrobenzenes, *o*- and *m*-chloronitrobenzenes, *p*-nitrotoluene, *p*-nitrophenetole and α-nitronaphthalene were separated from the tarry residue by steam distillation and crystallized from alcohol. The *o*-chloronitrobenzene was distilled. The *p*-dinitrobenzene was extracted with benzene, the benzene evaporated, and the residue crystallized first from glacial acetic acid and then from alcohol. The ethyl *p*-nitrobenzoate, *p*-nitroazobenzene and *p*-nitrodiphenyl were extracted with alcohol, and water added to the hot alcoholic extractives nearly to the point of precipitation. The solutions were decolorized with charcoal, filtered hot and allowed to crystallize. The compounds were recrystallized from alcohol.

The experimental data are given in Table I. The nitro compounds obtained are well known and are described in various handbooks and lexicons; hence earlier reference to these are omitted. No figure is given for a yield of less than 10%.

Summary

A method applicable for the preparation of certain nitro compounds is described.

The rate of decomposition of the diazonium borofluoride, and the yield of the nitro compound obtained are influenced markedly by the group present.

BALTIMORE, MARYLAND RECEIVED MARCH 27, 1937

[FROM THE DEPARTMENT OF PATHOLOGY, HARLEM HOSPITAL, NEW YORK CITY]

The Iodination of *p*-Aminobenzenesulfonamide and Some Symmetrical Azobenzenesulfonamides

BY JOHN V. SCUDI

The preparation of the ortho mono- and diiodo derivatives of *p*-aminobenzenesulfonamide was studied in the hope that introduction of this radiopaque element might make it possible to visualize, roentgenologically, the course of this clinically important drug through various parts of the anatomy. Yields ranging from 90 to 100% of the desired compounds were obtained by the usual iodine monochloride methods.[1]

Upon evaporation of hydrochloric acid solutions of 1-amino-2-iodobenzene-4-sulfonamide (I) dismutation of the iodine occurred. *p*-Aminobenzenesulfonamide hydrochloride[2] was isolated and an equivalent weight of I was converted to 1-amino-2,6-diiodobenzene-4-sulfonamide (II). This reaction is suggestive of halogen dismutation of the Chloramine type.[3] However, the iodine is linked to carbon in I and II since they diazotize readily and do not liberate iodine when treated with silver nitrate. Further, II forms a sodio derivative in which both atoms of iodine are retained. Boiling the diiodo derivative (II) into solution in hydrochloric acid caused a cleavage of iodine, and *p*-aminobenzenesulfonamide hydrochloride, I, and some unchanged II were isolated. Dismutation of aryl iodine has been observed in the attempted sulfonation of iodobenzene,[4] iodophenols,[5] and iodoanilines.[6] Treatment of II with 75% sulfuric acid caused cleavage of iodine. Boiling glacial acetic acid is without any appreciable effect. These reactions of I and II may be interpreted as a cleavage of iodine with the formation of iodide and iodate in hydrochloric acid solution at equilibrium with iodine monochloride.[7]

The *p*-acetylamino derivatives were desired since these are expected to be less toxic than their prototypes.[8,9] Using acetic anhydride and limiting the time of heating, the acetyl derivatives of *p*-aminobenzenesulfonamide[2] and I were obtained although a similar derivative of II was not, as might be anticipated from space considerations.

Iodination of *p*-aminobenzenesulfonamide in sodium bicarbonate or sodium hydroxide media was unsatisfactory. Oxidation effects play a significant part since at least one-third of the arylamine was converted to azobenzene-4,4'-disulfonamide (III). In further analogy with the reactions of sulfanilic acid, this same product may be obtained using a variety of oxidizing agents: *e. g.*, the phenol reagent of Folin and Ciocalteu,[10] alkaline potassium ferricyanide,[11]

(1) Kalle and Co., German patent 129,808.
(2) P. Gelmo, *J. prakt. Chem.*, [2] **77**, 372 (1908).
(3) F. D. Chattaway, *J. Chem. Soc.*, **87**, 145 (1905).
(4) H. Hammerich, *Ber.*, **23**, 1635 (1890).
(5) G. S. Neumann, *Ann.*, **241**, 47 (1887).
(6) M. Boyle, *J. Chem. Soc.*, **95**, 1710 (1909).
(7) Gleu and Jagemann, *J. prakt. Chem.*, **145**, 257 (1936).
(8) M. Swick, *Surg. Gyn. Obstetr.*, **56**, 62 (1933).
(9) (a) A. T. Fuller, *Lancet*, **I**, 194 (1937); (b) Marshal, Emerson and Cutting, *J. Am. Med. Assoc.*, **108**, 953 (1937).
(10) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).
(11) F. Reitzenstein, *J. prakt. Chem.*, [2] **82**, 265 (1910).

acid, neutral or alkaline permanganate.[12] These afford possible methods for the colorimetric determination of *p*-aminobenzenesulfonamide in biological fluids. The alkaline permanganate method provides the simplest isolation of the azobenzene-4,4′-disulfonamide since the product is alkali soluble and may be precipitated as the azosulfonamide or its sodio derivative.

Biochemical cleavage of sulfanilic acid from Orange I, II, III, IV, and phenylhydrazine-*p*-sulfonic acid has been demonstrated.[13] Recently the cleavage of *p*-aminobenzenesulfonamide from a number of azo derivatives has been demonstrated[14] to occur *in vivo*, but not *in vitro*.[14b,d,e,f] Comparative studies of the bactericidal and bacteriostatic activity of dyes of the type NH_2SO_2-$C_6H_4N{=}N{-}R$ have been made.[15] Comparisons of certain of these azo dyes with *p*-aminobenzenesulfonamide have indicated that the dyestuffs are the more toxic.[15c] The increased toxicity may be due in part to the formation of RNH_2 upon hydrogenation of the parent azo compound. Similar hydrogenation *in vivo* of azobenzene-4,4′-disulfonamide will give rise to two molecules of *p*-aminobenzenesulfonamide. Such symmetrical azobenzenesulfonamides seemed of sufficient interest to warrant their preparation for bacteriological evaluation.

The mono-iodo derivative (I) was oxidized to a small extent to 2,2′-diiodoazobenzene-4,4′-disulfonamide (IV) by one mole of iodine in caustic solution. No diiodo derivative (II) was obtained. Better yields of the azo compound (IV) were obtained by using a large excess of alkaline permanganate. It formed a sodio derivative like the iodine-free azo compound (III). Similar treatment of the diiodo derivative (II) yielded the nitroso compound. With iodine in caustic at room temperatures no reaction occurred, but, upon boiling, unchanged II and, among other things, a small amount of a purple sodio derivative (V) was obtained. The yield of this product was increased by oxidation with an excess of alkaline permanganate. Compound V on acidification gave a purple-red solution which precipitated green needles of 1-nitroso-2,6-di-iodobenzene-4-sulfonamide (VI), leaving a colorless filtrate.

(12) C. Laar, *J. prakt. Chem.*, [2] **20**, 264 (1879).

(13) (a) P. Sisley and C. Porcher, *Compt. rend.*, **152**, 1063 (1911); (b) P. Sisley, C. Porcher and L. Panisset, *ibid.*, 1794 (1911).

(14) (a) Levaditi and Vaisman, *Compt. rend. soc. biol.*, **119**, 946 (1935); (b) Nitti and Bovet, *Compt. rend.*, **202**, 1221 (1936); (c) Colebrook and Kenny, *Lancet*, **I**, 1279 (1936); **2**, 1319 (1936); (d) Colebrook, Buttle and O'Meara, *ibid.*, **2**, 1323 (1936); (e) Fuller, *ibid.*, **I**, 194 (1937); (f) Long and Bliss, *J. Am. Med. Assoc.*, **108**, 32 (1937).

(15) (a) Fourneau, Trefouel, Trefouel, Nitti and Bovet, *Compt. rend. soc. biol.*, **122**, 258, 652 (1936); (b) Nitti and Bovet, *Compt. rend.*, **202**, 1221 (1936); (c) Buttle, Gray and Stevenson, *Lancet*, **I**, 1286 (1936); (d) S. M. Rosenthal, *U. S. Pub. Health Reports*, **52**, 48, 192 (1937); (e) for extensive bibliography see P. Long and E. Bliss, *Arch. Surg.*, [II] **34**, 351 (1937).

Experimental

1-Amino-2-iodobenzene-4-sulfonamide (I). (A).—One-tenth mole of *p*-aminobenzenesulfonamide treated according to the method of Boyle[6] gave an immediate precipitation. After standing four hours, the product was filtered off and recrystallized from boiling water. Less than 1.0 g. of the diiodo derivative (II) was obtained as a water-insoluble residue: yellow plates, from water, yield, 28.0 g. or 94%; m. p. 179–180°.

B.—The reaction was repeated in glacial acetic acid maintained at 60–70° for two hours. The reaction mixture was cooled after twelve hours of standing, and the crystals were filtered off, washed with ether and dried. The yield was 90%. Diluting the acetic acid filtrate gave sufficient II to account for 100% of the *p*-aminobenzenesulfonamide.

The product is very soluble in alcohol and acetone, slightly soluble cold and soluble in hot water, slightly soluble in hot benzene and toluene, insoluble in ether and chloroform. It is insoluble in sodium carbonate, soluble in sodium hydroxide and hydrochloric acid, it diazotizes readily, and does not precipitate silver iodide from silver nitrate solutions.

Anal. Calcd. for $C_6H_7O_2N_2SI$: N, 9.39. Found: N, 9.35.

1-Amino-2,6-diiodobenzene-4-sulfonamide (II). (A).—This was prepared as in IA using 33.0 g. of iodine monochloride. After standing for four hours at 40–50° the product was filtered from the reaction mixture and washed with hot water until the filtrate was colorless. The yield was 41–42 g. or 96–99%.

B.—Nineteen and five-tenths grams of iodine monochloride were added to 10.0 g. of *p*-aminobenzenesulfonamide in 200 ml. of hot glacial acetic acid and the reaction held at 80–90° for two hours. The reaction mixture was boiled with two liters of water, the product was filtered off, and washed with hot water until the filtrate was colorless. The yields were 18–19 g. or 73–77%. The filtrate gave 1.0 g. of crude II on cooling, and small amounts of I. These conditions appear to be optimal for this method, but the yield is lower, and the product less pure than in the aqueous hydrochloric acid method.

The product is difficultly soluble in the usual solvents; insoluble in dilute acids and sodium carbonate; soluble in alkalies and precipitates the sodium or potassium salt as white needles from hot concentrated solutions of alkalies. This is an excellent method of purification of the product since the alkaline filtrates precipitate II unchanged on acidification. The product diazotizes readily and does not precipitate silver iodide: white needles from glacial acetic acid; m. p. 265°, with decomposition.

Anal. Calcd. for $C_6H_6O_2N_2SI_2$: N, 6.60. Found: N, 6.61, 6.74. Calcd. for $C_6H_5O_2N_2SI_2K$: K, 8.64. Found: K, 8.28.

***p*-Acetaminobenzenesulfonamide and 1-Acetamino-2-iodobenzene-4-sulfonamide.**—Quantitative yields of the

p-acetamino derivatives were obtained by boiling *p*-aminobenzenesulfonamide or I into solution in a minimal volume of acetic anhydride. The reaction may be boiled for one to five minutes but not thirty minutes. Crystallization occurred on cooling. The products were filtered off, washed with ether and recrystallized from dilute alcohol. The diiodo derivative II could not be acetylated under a variety of conditions. Acetaminobenzenesulfonamide[2]: white needles; m. p. 214°. 1-Acetamino-2-iodobenzene-4-sulfonamide: white needles; m. p. 216°.

Anal. Calcd. for $C_8H_9O_3N_2SI$: N, 8.25. Found: N, 8.29.

The Conversion of I to II and *p*-Aminobenzenesulfonamide Hydrochloride.—Boiling 2.0 g. of I for ten to twenty minutes in 10% hydrochloric acid caused the crystallization of 0.8 g. of II from the hot mixture. This proved to be identical with the products prepared by the above methods, m. p. 264–265°. The filtrate on evaporation to dryness gave 0.6 g. of *p*-aminobenzenesulfonamide hydrochloride which was further purified by precipitation with ether from alcoholic solution. This was compared with an authentic specimen, m. p. 235–237°, with decomposition, and further identified by analysis. Small amounts of unchanged I were obtained from the alcohol-ether filtrate.

Anal. Calcd. for $C_8H_9O_2N_2SCl$: N, 13.46. Found: N, 13.30.

The Conversion of II to I and *p*-Aminobenzenesulfonamide Hydrochloride.—One gram of II dissolved in 30 ml. of 20% hydrochloric acid after thirty minutes of boiling to give a yellow solution. The solution was boiled to small volume and evaporated to dryness on a water-bath. The residue was taken up in alcohol and addition of ether caused the precipitation of 0.15 g. of *p*-aminobenzenesulfonamide hydrochloride which melted at 235–237° and showed no depression in melting point when mixed with an authentic specimen. Its identity was further confirmed by analysis. The alcoholic–ether extract was evaporated to dryness, and the residue extracted with 40 ml. of boiling water. On cooling, this gave 0.27 g. of I which melted at 177–179° and at 178–180° when mixed with a pure sample of I. The residue from the water extraction, recrystallized from dilute acetic acid, gave 0.36 g. of II melting at 262–265° and at the same temperature when mixed with a pure sample of II.

Anal. Calcd. for $C_8H_9O_2N_2SCl$: N, 13.46. Found: N, 12.99.

Azobenzene-4,4′-disulfonamide (III). (A).—To 1.7 g. of *p*-aminobenzenesulfonamide warmed into solution in 200 ml. of water containing 4.2 g. of sodium bicarbonate, 2.5 g. of iodine was added. The reaction was stirred at 50–60° for three hours, cooled, the crystalline product was filtered off, and washed with hot water until the filtrate was colorless. Recrystallized from large volumes of acetic acid 0.4 g. or 25% of orange plates or needles, melting above 270°. The product is difficultly soluble in the usual solvents but may be purified easily as the sodio derivative which crystallizes in golden yellow crystals from hot 15% sodium hydroxide.

(B).—To 1.7 g. of *p*-aminobenzenesulfonamide dissolved in 100 ml. of 5% sodium hydroxide, 2.5 g. of iodine was added and the reaction was boiled for ten minutes. A slight turbidity occurred but this dissolved on boiling to give a deep red solution. To the hot filtered solution, 100 ml. of 7.5% sodium bicarbonate solution was added and the orange crystalline precipitate was filtered off and recrystallized from acetic acid. The yield of 0.5 g. was increased by 0.1 g. by acidifying the sodium carbonate filtrate with acetic acid. This method gave the best yields (35%) and the purest products.

(C).—To 5.0 g. of *p*-aminobenzenesulfonamide in 100 ml. of boiling 5% sodium hydroxide, 5.6 g. of potassium permanganate in 100 ml. of boiling water was added, and the reaction was boiled until the manganate green was discharged. The reaction mixture was filtered and the manganese dioxide washed with hot water. The combined filtrates were acidified and yielded 1.5 g. or 30% of III. Better yields were obtained from alkaline media than from neutral or acidic media. Increasing the permanganate concentration did not increase the yields appreciably.

Anal. Calcd. for $C_{12}H_{10}O_4S_2N_4$: N, 16.57. Found: N, 16.49. Calcd. for $C_{12}H_{10}O_4S_2N_4Na_2$: Na, 9.50. Found: Na, 9.58, 9.95.

2,2′-Diiodoazobenzene-4,4′-disulfonamide (IV).—This was prepared from I in 20% yields using method IIIB. Using method IIIC, yields of 50% were obtained using twice the amount of permanganate. Large amounts of unchanged I were obtained with smaller amounts of permanganate. The product is difficultly soluble in the common solvents. It is slightly soluble in hot alcohol and separates from it as an orange powder, which melts above 270°. It is easily soluble in caustic, and readily forms an orange colored sodio derivative which separates from hot 10–15% NaOH in plate-like crystals.

Anal. Calcd. for $C_{12}H_{10}O_4S_2N_4I_2$: N, 9.46. Found: N, 9.53 (average of 2). Calcd. for $C_{12}H_8O_4S_2N_4I_2Na_2$: Na, 7.23. Found: Na, 7.59.

1-Nitroso-2,6-diiodobenzene-4-sulfonamide.—To 4.2 g. of II in 150 ml. of 3% sodium hydroxide, 200 ml. of 5% potassium permanganate was added and the reaction mixture was boiled for ten minutes. The mixture was filtered, the manganese dioxide washed with 50 ml. of hot 3% sodium hydroxide, and the combined filtrates were made up in strength to 10% sodium hydroxide by the addition of solid caustic. The purple crystalline product (V) was filtered off and recrystallized from boiling 15% sodium hydroxide. The yield was 1.5 g. or 31%. The sodio derivative dissolved in dilute acetic acid gave a purple red solution from which a quantitative yield of the free nitroso compound precipitated leaving a practically colorless solution: green needles which turned brown at 250° and melted with decomposition above 270°.

Anal. Calcd. for $C_6H_4O_3N_2I_2S$: N, 6.39. Found: N, 6.25. Calcd. for $C_6H_3O_3N_2I_2SNa$: Na, 5.00. Found: Na, 5.03.

Summary

1. The mono and diiodo derivatives of *p*-aminobenzenesulfonamide have been prepared with a view to roentgenological visualization of the drug *in vivo*.

2. Dismutation of aryl iodine in boiling hydrochloric acid has been demonstrated.

3. Iodine in alkaline media has been shown to produce azo- and nitroso-benzenesulfonamides. These products are of bacteriological interest.

4. The bacteriological and roentgenological properties of these substances are under investigation.

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Densities of Mixtures of Light and Heavy Water

BY L. G. LONGSWORTH

In the preparation of some H_2O–D_2O mixtures for use in a series of transference measurements with such mixtures as solvents, it was observed that the mole fraction of D_2O in the solvent computed from the weights of H_2O and D_2O deviated by as much as 0.5% from the value obtained with the aid of the relation

$$N_{D_2O} = 9.377\ \Delta S - 1.01\ \overline{\Delta S^2} \qquad (1)$$

in which

$$\Delta S = 1 - d^{25}_{25} = 1 - \frac{n^{25}_4}{0.99707_4}$$

This equation is due to Lewis and Luten[1] and modified by Baker and La Mer.[2] It seemed desirable therefore to redetermine the densities of these mixtures as a function of the mole fraction, N_{D_2O}, of deuterium oxide.

The starting material was a sample of heavy water having a specific gravity of 1.10700. Accepting the value of 1.10790[3] as the specific gravity of pure D_2O the value of N_{D_2O} for this sample may be taken tentatively as 0.10700/0.10790 = 0.9916$_6$. Weighed quantities of this and ordinary water were mixed and the density of the resulting liquid was determined. This process was repeated until N_{D_2O} had been decreased to 0.2 in steps of 0.2.

The density measurements were made with the aid of a pycnometer similar to that described by Smith and Wojciechowski.[4] The volume of the pycnometer was 8.5 ml. and the capillary neck, marked with a single graduation, had an internal diameter of 1.4 mm. The pycnometer was filled with the aid of a hollow stainless steel needle. The pycnometer was then placed in a thermostat at 25.00° and the position of the meniscus relative to the graduation was observed to 0.001 cm. with a traveling microscope. Weighings were then made with a duplicate pycnometer as counterpoise. An air density of 0.0012 was used in the reduction of the weights to vacuum.

The results are given in Table I. This table also includes the density of D_2O-free water and the value of N_{D_2O} for natural water from the work of Johnston[5] and Tronstad, Nordhagen and Brun.[6] The values of ΔS, column 4, are, in contrast to those of equation (1), referred to the density of *D_2O-free water*. The atomic weights used were O = 16.0000, H = 1.00756, D = 2.01309, as given by Urey and Rittenberg.[7] The oxygen isotope ratio was assumed to be normal in all H_2O–D_2O mixtures. It will be noted that the atomic weight of hydrogen has been given a correction for the deuterium normally present.

TABLE I

THE DENSITIES AND MOLAL VOLUMES OF MIXTURES OF H_2O AND D_2O

1	2	3	4	5	6
Material	N_{D_2O}	d^{25}_4	ΔS	ΔS calcd.	$V - V_a$, ml.
D_2O[8]	1.00000	1.10466	0.10792	0.10790	0.0000
Starting material	0.99166	1.10376	.10702	.10701	.0001
Dilution 1	.82358	1.08570	.08891	.08892	.0005
Dilution 2	.61023	1.06279	.06593	.06593	.0003
Dilution 3	.40243	1.04044	.04351	.04351	.0001
Dilution 4	.20192	1.01884	.02185	.02185	.0000
Natural water[5]	.00017$_8$	0.99707$_4$	.00001$_9$	.00001$_9$	.0000
H_2O[5,8]	.00000	.99705$_8$	.00000		.0000

It is of considerable interest that H_2O–D_2O mixtures form a perfect solution, almost within the experimental error, as tested by the absence of a volume change on mixing. Identifying H_2O and D_2O with the subscripts 1 and 2, respectively, a perfect solution conforms to the relation[8]

$$V_a = N_1 v_1 + N_2 v_2 \qquad (2)$$

in which V_a is the volume of a mole of solution

(1) Lewis and Luten, THIS JOURNAL, **55**, 5061 (1933).
(2) Baker and La Mer, *J. Chem. Phys.*, **3**, 406 (1935).
(3) Selwood, Taylor, Hipple and Bleakney, THIS JOURNAL, **57**, 642 (1935).
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(6) Tronstad, Nordhagen and Brun, *Nature*, **136**, 515 (1935).
(7) Urey and Rittenberg, *J. Chem. Phys.*, **1**, 137 (1933).
(8) J. H. Hildebrand, "Solubility of Non-Electrolytes," Reinhold Publishing Corp., New York, 1936, 2d edition, p. 58.