A solution of the ene-diol in alcoholic ferric chloride produces a deep green-blue color which gradually fades to yellow on standing. Evaporation of this solution yields a light yellow solid, melting point and mixed melting point with the above 134° melting compound unchanged.

Summary

Herein are reported the preparation and properties of a new ene-diol having the open chain —C(OH)=C(OH)—C(=O)—, and some further evidences of the activating influence of the mesityl nucleus upon α-substituents together with its stabilizing effects on the resulting compound.

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[CONTRIBUTION FROM THE COLLEGE OF PHARMACY, UNIVERSITY OF ILLINOIS]

Derivatives of 4-Aminobenzenesulfonanilide. I

BY G. L. WEBSTER AND L. D. POWERS

Following the observation made by Trefouel, Trefouel, Nitti and Bovet[1] that 4-aminobenzenesulfonamide was a valuable therapeutic agent in the treatment of infections caused by the β-hemolytic streptococcus, a large number of derivatives and analogs of this compound were tested in the search for other compounds which would be effective against the same and other organisms. Biological tests have shown only a few substances which have comparable action against bacterial organisms of any type.

It has been shown by Buttle, Gray and Stephenson[2] and by Rosenthal, Bauer and Branham[3] that, when given in equal molecular doses, 4-aminobenzenesulfonanilide is just as effective against pneumococcic infections in mice as is 4-aminobenzenesulfonamide. It has also been shown[1,3] that the presence of an amino group para to the sulfonamide group is necessary for therapeutic action.

Only a few derivatives of 4-aminobenzenesulfonanilide, in which an amino or a nitro group has been substituted for a hydrogen atom of the anilide ring, have been reported in the literature. Whitby[4] has published results on the protective action of the tartrates of 4,4′-diaminobenzenesulfonanilide and 4,3′-diaminobenzenesulfonanilide against experimental infections in mice and Bauer and Rosenthal[5] have reported their results with the first of these two diamines and with 4-aminobenzenesulfon-4′-nitroanilide. The authors of these papers presented no syntheses or chemical characterization of these compounds.

We have prepared a series of derivatives of 4-aminobenzenesulfonanilide in which a hydrogen atom of the anilide ring has been substituted with a nitro, amino or hydroxyl group in the hope that biological tests might disclose active chemotherapeutic agents.

Preliminary reports to us have indicated that several of these compounds show some slight protective action against experimental streptococcal infections in mice and one, 4-acetaminobenzenesulfon-4′-aminoanilide, has been described as moderately effective.[6] A more detailed report will be published elsewhere by Dr. Long.

Nitro derivatives of 4-acetaminobenzenesulfonanilide were prepared by the action of 4-acetaminobenzenesulfonchloride on a hot solution of the nitroaniline in dimethylaniline.

The corresponding amino derivatives were prepared by the ferrous hydroxide reduction method of Jacobs and Heidelberger.[7]

The hydroxyl derivatives of 4-acetaminobenzenesulfonanilide were prepared by the action of 4-acetaminobenzenesulfonchloride on a hot aqueous solution of the corresponding aminophenol and also by the method used for preparing the nitro derivatives.

Preparation of derivatives of 4-aminobenzenesulfonanilide was accomplished by boiling the acetamino compounds with an alcoholic solution of hydrochloric acid.

Diazotization of 4-acetaminobenzenesulfon-3′-aminoanilide and heating the resulting solution yielded the corresponding 3′-hydroxy derivative. Diazotization of 4-acetaminobenzenesulfon-4′-aminoanilide and boiling the diazonium solution

(1) Trefouel, Trefouel, Nitti and Bovet, *Compt. rend. soc. biol.*, **120**, 756 (1935).

(2) Buttle, Gray and Stephenson, *Lancet*, I, 1286 (1936).

(3) Rosenthal, Bauer and Branham, *U. S. Pub. Health Repts.*, **52**, 662 (1937).

(4) Whitby, *Lancet*, I, 1517 (1937).

(5) Bauer and Rosenthal, *U. S. Pub. Health Repts.*, **53**, 40 (1938).

(6) Private communication from Dr. Perrin H. Long, The Johns Hopkins Hospital.

(7) Jacobs and Heidelberger, THIS JOURNAL, **39**, 1435 (1917).

TABLE I

Name 4-Acetaminobenzenesulfon-	M. p., °C.	Formula	Nitrogen, %[a] Calcd.	Found	Sulfur, %[b] Calcd.	Found
2′-Nitroanilide	200–201	$C_{14}H_{13}O_5N_3S$	12.53	12.22[c]	9.56	9.50
3′-Nitroanilide	236–237	$C_{14}H_{13}O_5N_3S$	12.53	12.53[c]	9.56	9.55
4′-Nitroanilide	237–238	$C_{14}H_{13}O_5N_3S$	12.53	12.22[d]	9.56	9.65
2′-Aminoanilide	222–223	$C_{14}H_{15}O_3N_3S$	13.77	13.48	10.50	10.47
3′-Aminoanilide	217–218	$C_{14}H_{15}O_3N_3S$	13.77	13.53	10.50	10.28
4′-Aminoanilide	232	$C_{14}H_{15}O_3N_3S$	13.77	13.68	10.50	10.60
2′-Hydroxyanilide	216–217	$C_{14}H_{14}O_4N_2S$	9.15	9.00	10.47	10.43
3′-Hydroxyanilide	217–218	$C_{14}H_{14}O_4N_2S$	9.15	9.07	10.47	10.29
4′-Hydroxyanilide	.....[e]	$C_{14}H_{14}O_4N_2S$	9.15	9.01	10.47	10.43
4′-Acetaminoanilide	.....[e]	$C_{16}H_{17}O_4N_3S$	11.76	11.66	8.97	8.80

[a] Unless otherwise indicated nitrogen was determined by the Kjeldahl method. [b] Sulfur was determined as barium sulfate after oxidation of the compound with a mixture of nitric and perchloric acids. [c] Dumas method. [d] Modified Kjeldahl method, Assoc. Official Agr. Chem., Tentative and Official Methods of Analysis, 1930, p. 11. [e] No m. p. below 260°.

TABLE II*

Name 4-Aminobenzenesulfon-	M. p., °C.	Formula	Nitrogen, %[a] Calcd.	Found	Sulfur, %[b] Calcd.	Found
3′-Nitroanilide	171–172	$C_{12}H_{11}O_4N_3S$	14.33	14.11[d]	10.94	10.72
4′-Nitroanilide	165–166	$C_{12}H_{11}O_4N_3S$	14.33	14.11[d]	10.94	10.85
2′-Aminoanilide	201–202	$C_{12}H_{13}O_2N_3S$	15.97	15.72	12.18	12.00
3′-Aminoanilide	176–177	$C_{12}H_{13}O_2N_3S$	15.97	15.85	12.18	12.03
4′-Aminoanilide	155–156	$C_{12}H_{13}O_2N_3S$	15.97	15.78	12.18	12.01
2′-Hydroxyanilide	182–183	$C_{12}H_{12}O_3N_2S$	10.60	10.55	12.14	12.06
3′-Hydroxyanilide	195–196	$C_{12}H_{12}O_3N_2S$	10.60	10.58	12.14	12.04
4′-Hydroxyanilide	196–197	$C_{12}H_{12}O_3N_2S$	10.60	10.58	12.14	12.07

* The significance of the notations in this table is the same as in Table I.

yielded 4-aminobenzenesulfon-4′-hydroxyanilide. When 4-acetaminobenzenesulfon-2′-aminoanilide in dilute sulfuric acid solution was treated with nitrous acid the *o*-diazoimide of 4-acetaminobenzenesulfonanilide was formed.

Experimental Part

4-Acetaminobenzenesulfonnitroanilides.—A solution of 13.8 g. (0.1 mole) of 2-(3- or 4)-nitroaniline in 50 cc. of dry, hot dimethylaniline was treated with 23.3 g. (0.1 mole) of crude 4-acetaminobenzenesulfonchloride[8] added in several portions.[9] The mixture was heated for one hour, on a steam-bath, cooled and the dimethylaniline removed by treating the mixture with cold 1:1 hydrochloric acid. The 2′- and 4′-nitroanilides were obtained first as gums which crystallized after repeated washings with the dilute acid. The 3′-nitroanilide was obtained as a granular mass immediately. The yield of the 2′-nitroanilide was 10 g.; 3′-nitroanilide, 30 g.; 4′-nitroanilide, 15 g.

4-Acetaminobenzenesulfonaminoanilides.—A solution of 200 g. of technical ferrous sulfate in 500 cc. of water was made alkaline with a 10% solution of sodium hydroxide (about 700 cc.). A solution of 33.5 g. (0.1 mole) of the 4-acetaminobenzenenitroanilide in 250 cc. of 10% sodium hydroxide solution was added and the mixture well stirred for fifteen minutes. The mixture was filtered through a large suction filter and the ferric hydroxide residue extracted again with about 300 cc. of water. The combined filtrates were acidified to litmus paper with acetic acid and the precipitated aminoanilide recovered by filtration.

4 - Acetaminobenzenesulfonhydroxyanilides.—These compounds were made (a) by the method described above for the preparation of the nitro derivatives. The yields of hydroxyanilides were smaller than those obtained by the following method. (b) A solution of 21.8 g. (0.2 mole) of 4-aminophenol in 1800 cc. of water at 75° containing 17 g. of anhydrous sodium acetate was treated with 58 g. of partially dried crude 4-acetaminobenzenesulfonchloride, estimated to be 80% pure, with mechanical stirring. Stirring was continued for thirty minutes, the mixture cooled and filtered. The yield of crude, air-dried product was 42 g. The isomeric hydroxyanilides were prepared in like yield.

The derivatives of 4-acetaminobenzenesulfonanilide described above are insoluble in water, ether and benzene; soluble in alcohol, acetone, dioxane and acetic acid. They may be crystallized from alcohol or from diluted alcohol of suitable concentration (50–20%). They dissolve in dilute (10% or less) sodium hydroxide solutions from which the sodium salt is precipitated by the addition of more concentrated alkali. The amino derivatives dissolve in dilute solutions of mineral acids.

4,4′ - Diacetaminobenzenesulfonanilide.—This compound was prepared from 4-acetaminobenzenesulfon-4′-aminoanilide by warming with an excess of acetic anhydride. It is insoluble in water and most of the usual organic solvents. It can be crystallized from a large volume of acetic acid.

Hydrolysis of the Acetyl Group.—A mixture of 10 g. of the 4-acetaminobenzenesulfonanilide in 200 cc. of alcohol and 15 cc. of concd. hydrochloric acid was refluxed on a

(8) *Org. Syntheses*, **5**, 3 (1925).

(9) Ullmann and Gross, *Ber.*, **43**, 2694 (1910), prepared toluenesulfontoluide by this method using diethylaniline as a solvent.

steam-bath for two hours. The alcohol was distilled off and the residue dissolved or suspended in 75 cc. of water. The aqueous mixture was cooled and made alkaline with ammonium hydroxide and then slightly acid with acetic acid. The desired 4-aminobenzenesulfonanilide was obtained by filtration.

The derivatives of 4-aminobenzenesulfonanilide prepared as described above are insoluble in ether and benzene; slightly soluble in cold water but appreciably soluble in boiling water; soluble in alcohol, acetone, dioxane and acetic acid. They may be crystallized from diluted alcohol (50% for the nitro derivatives, 35–20% for the amino derivatives) or from water (hydroxy derivatives). Amino and hydroxy derivatives were crystallized in an atmosphere of carbon dioxide. All of these compounds are soluble in dilute sodium hydroxide solutions and in dilute mineral acids.

4,4'-Diaminobenzenesulfonanilide Dihydrochloride.—To a solution of 1.2 g. of the pure base in a mixture of 10 cc. of alcohol and 1 cc. of concd. hydrochloric acid an additional 5 cc. of concd. hydrochloric acid was added. The precipitated dihydrochloride was filtered and dried in a desiccator over calcium chloride and sodium hydroxide. The white crystals begin to decompose at 200°. A solution (1:1000) in water has *p*H 2.5. *Anal.*[10] Calcd. for $C_{12}H_{15}O_2N_3Cl_2S$: Cl: 21.10; Found: Cl, 21.27.

Diazotization of 4-Acetaminobenzenesulfon-2'-aminoanilide.—A solution of 3.05 g. (0.1 mole) of 4-acetaminobenzenesulfon-2'-aminoanilide in a mixture of 250 cc. of water and 5 cc. of concentrated sulfuric acid was diazotized with 0.7 g. (0.1 mole) of sodium nitrite, dissolved in 14 cc. of water, at room temperature. The *o*-diazoimide of 4-acetaminobenzenesulfonanilide is precipitated from the reaction mixture immediately.

The product was filtered from the reaction mixture and washed with hot water to remove inorganic compounds and recrystallized from 50% alcohol. The yield was 2.5 g. This compound was stable at the boiling temperature of the mixture but, when recrystallized and dried, it decomposed at 138–140°. The reaction is analogous to that reported by Morgan and Micklethwait[11] in the diazotization of benzenesulfon-2'-aminoanilide. *Anal.* Calcd. for $C_{14}H_{12}O_3N_4S$: N, 17.72; S, 10.14. Found: N (Dumas), 17.82; S, 10.07.

Diazotization of 4-Acetaminobenzenesulfon-3'-aminoanilide.—A solution of 1.5 g. of the 4-acetaminobenzenesulfon-3'-aminoanilide in a mixture of 5 cc. of concd. sulfuric acid and 25 cc. of water was diazotized with 0.35 g. of sodium nitrite in 7 cc. of water without cooling. The solution was heated on a steam-bath for one hour, cooled and filtered. After recrystallization from 20% alcohol, the compound melted at 217–218°. The mixed melting point with the product obtained from coupling 3-aminophenol with 4-acetaminobenzenesulfonchloride was unchanged.

Diazotization of 4-Acetaminobenzenesulfon-4'-aminoanilide.—Three grams of 4-acetaminobenzenesulfon-4'-aminoanilide dissolved in a mixture of 10 cc. of concd. sulfuric acid and 50 cc. of water was diazotized, without cooling, with 0.7 g. of sodium nitrite in 14 cc. of water. A precipitate formed which dissolved when the temperature of the solution was raised to 75°. The solution was heated at 90–95° for three hours and then briefly boiled. After cooling, the solution was made alkaline with ammonium hydroxide and then acid with acetic acid. Recrystallized from water the product melted at 195–196°. The mixed melting point with 4-aminobenzenesulfon-4'-hydroxyanilide was unchanged, indicating that hydrolysis of the acetyl group had taken place.

Summary

The preparation of a number of new nitro, amino and hydroxy derivatives of 4-aminobenzenesulfonanilide and 4-acetaminobenzenesulfonanilide has been described. Work is in progress on other derivatives of these compounds.

CHICAGO, ILLINOIS RECEIVED APRIL 23, 1938

(10) Method of Thompson and Oakdale, THIS JOURNAL, **52**, 1195 (1930).

(11) Morgan and Micklethwait, *J. Chem. Soc.*, **87**, 73 (1905).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. XXXV. Carbinols from Stallions' Urine

BY RUSSELL E. MARKER, ELMER J. LAWSON, EWALD ROHRMANN AND EUGENE L. WITTLE

Although the presence of oestrone in stallions' urine has been reported by several investigators,[1] apparently no work has been done on the neutral fractions from this source.

As part of an extensive investigation of the steroid substances in various urines, we have made a preliminary study of stallions' urine. After hydrolysis of the urine, and removal of the phenolic and acidic substances with alkali, the neutral extract was treated with Girard's reagent. Only a very small amount of ketonic material, amounting to 100 mg. per 200 gallons (760 liters) was obtained. The non-ketonic fraction was treated with phthalic anhydride and pyridine to separate the carbinols. The carbinol fraction was treated with digitonin to separate the β-sterols. From the digitonide a sterol of m. p. 134° was obtained. This sterol does not decolorize bromine in acetic

(1) For the literature on this subject, see Fieser, "Chemistry of Natural Products Related to Phenanthrene," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1936, pp. 198–199.