indicate that while in the parent ring highest antistreptococcal activity is found in the para derivative, this is reversed completely in the second ring, where compounds in which the acid function is ortho to the nitrogen have the highest activity.

Addition of $-CH_3$, $-OH$, $-OR$ or $-Cl$ to the second ring greatly lowers or destroys the activity.

Sodium 2,4-*bis*-sulfanilamidobenzenesulfonate and 2,5-*bis*-sulfanilamidobenzenesulfonic acid appear to have activity against influenza virus in mice, as well as to have high antistreptococcal powers.

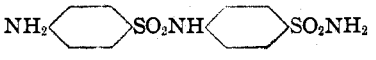
BOUND BROOK, N. J. RECEIVED APRIL 27, 1938

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CALCO CHEMICAL COMPANY, INC.]

Sulfanilamide Derivatives. II. Disulfanilamides and Related Compounds[1]

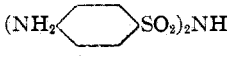
BY M. L. CROSSLEY, E. H. NORTHEY AND MARTIN E. HULTQUIST

The compound called by Rosenthal[2] "Disulfanilamide"



is incorrectly named. We suggest that it be called N^4-sulfanilylsulfanilamide.

We have synthesized true disulfanilamide



and have prepared a number of derivatives having the disulfonamide linkage, $-SO_2NHSO_2-$. These compounds as a class behave as strong acids, forming neutral sodium salts of the type $-SO_2NNaSO_2-$. The salts have high water solubility and are very stable to heat, decomposing at temperatures above 300°. However, if the hydrogen of the disulfonamide is replaced by alkyl, the compound becomes water and alkali insoluble and is broken down more readily by strong acids or bases.

Disulfanilamide was synthesized by treating more than two moles of N-acetylsulfanilyl chloride with one mole of ammonia, keeping the *p*H at 10–11 by addition of sodium hydroxide solution and holding the temperature below 45°. It was also made starting with N-acetylsulfanilyl chloride and N^4-acetylsulfanilamide. The resulting N^1-sodium-N^4,$N^{4'}$-diacetyldisulfanilamide was hydrolyzed by boiling with sodium hydroxide and was purified by recrystallization as the sodium salt.

Alkylation was accomplished by boiling the N^1-sodium-N^4,$N^{4'}$-diacetyldisulfanilamide with excess dimethyl sulfate in xylene. The resulting N^1-methyl-N^4,$N^{4'}$-diacetyldisulfanilamide was hydrolyzed to N^1-methyldisulfanilamide by adding the theoretical amount of 36% hydrochloric acid, dropwise, to a boiling alcoholic solution so that the acidity at no time was sufficient to give a green spot on methyl violet paper. Boiling aqueous 20% hydrochloric acid caused rapid breakdown at the amide linkage giving sulfanilic acid and N^1-methylsulfanilamide. The same type of decomposition occurred during hydrolysis with sodium hydroxide but at a much slower rate.

Dimetanilamide was made by treating an excess of *m*-nitrobenzenesulfonyl chloride with *m*-nitrobenzenesulfonamide at 50–60° in aqueous solution and at *p*H 10–11. The *p*H was maintained by addition of sodium hydroxide as necessary. The resulting 3,3′-di-*m*-nitrodibenzenesulfonamide was reduced to dimetanilamide with ammonia and hydrogen sulfide.

The compounds synthesized and their relative effectiveness as indicated by preliminary studies on β-hemolytic streptococcal infections in mice are shown in the table, where sulfanilamide = ++.[3,4]

The inferences drawn from the results are:

1. Acetylation of the amino groups lowered the therapeutic effect. This was in agreement with all other work on sulfanilamide derivatives.

2. Alkylation of the amide nitrogen had no apparent effect on the therapeutic efficiency although the compound had lost its water solubility almost completely.

3. Metanilyl derivatives had a lower effectiveness than the corresponding sulfanilyl derivatives. However, dimetanilamide had about the same activity as sulfanilamide.

In addition to the sodium and magnesium salts

(1) Presented in part before the Division of Medicinal Chemistry, A. C. S., April 20, 1938.

(2) Rosenthal, *et al.*, *Pub. Health Repts., U. S. Treas. Dept.*, **52** 662 (1937); *ibid.*, **53**, 40 (1938).

(3) A complete report on the pharmacology will be presented by Dr. D. R. Climenko, elsewhere.

(4) Microanalyses were made under the direction of G. L. Royer.

TABLE I

Compound	Formula	Therapeutic effect	Melting range, °C.	Assay by nitrite, %	Analyses, % Calculated C	H	N	S	Found C	H	N	S
Disulfanilamide	$C_{12}H_{13}O_4N_3S_2$		260.5–261.0	99.8	44.0	4.01	12.86	19.6	43.6	4.2	12.8	19.1
N^1-Sodium-N^4,$N^{4'}$-diacetyldisulfanilamide	$C_{16}H_{16}O_6N_3S_2Na$	++					9.68	Na, 5.3			9.81	Na, 5.5
N^1-Methyldisulfanilamide	$C_{13}H_{15}O_4N_3S_2$	+++	180.0–181.0	99.3	45.7	4.45	12.3	18.75	46.1	4.6	12.1	18.6
N^1-Methyl-N^4,$N^{4'}$-diacetyldisulfanilamide	$C_{17}H_{19}O_6N_3S_2$	±	228.5–229–230		48.0	4.5	9.9	15.1	47.9	4.5	10.1	15.0
N^1-Ethyldisulfanilamide	$C_{14}H_{17}O_4N_3S_2$	+++	153.3–154.7	100.3	47.3	4.84	11.85	18.03	47.25	5.04	12.3	18.0
N^1-Ethyl-N^4,$N^{4'}$-diacetyldisulfanilamide	$C_{18}H_{21}O_6N_3S_2$		229.5–230.5		49.2	4.8	9.7	14.6	49.38	4.94	9.7	14.8
Dimetanilamide	$C_{12}H_{13}O_4N_3S_2$	++	>300 dec.	100.0	44.0	4.0	12.86	19.6	43.9	3.7	12.8	19.1
N^1-Magnesium disulfanilamide	$C_{24}H_{24}O_8N_6S_4Mg\cdot H_2O$	++		98.4			Mg, 3.40			Mg, 3.35		
N^1-Sodium disulfanilamide	$C_{12}H_{12}O_4N_3S_2Na\cdot H_2O$	+++	>340 dec.	100.2	Sodium		6.28		Sodium		6.35	

TABLE II

SALTS OF DISULFANILAMIDE

Salt	Method of preparation	Appearance	Water solubility Hot	Cold
Lithium	DSA + $LICO_3$	Small white flat rods or plates	v. s.	v. s.
Sodium	DSA + NaOH	White prisms	ext. s.	v. s.
Magnesium	Sod. DSA + $MgCl_2$	White rectangular plates	v. s.	mod. s.
Calcium	DSA + $Ca(OH)_2$	Rods or needles	ext. s.	v. s.
Barium	Sod. DSA + $BaCl_2$	Needles	mod. s.	sl. s.
Cupric	Sod. DSA + $CuSO_4$	Light green needles[a]	mod. s.	sl. s.
Nickel	Sod. DSA + $NiCl_2$	Pale green thin plates	ext. s.	v. s.
Silver	Sod. DSA + $AgNO_3$	White grains[b]	sl. s.	v. sl. s.
Plumbous	Sod. DSA + $Pb(NO_3)_2$	White fine needles	sl. s.	v. sl. s.
Mercuric	Sod. DSA + $HgCl_2$	White diamond shaped plates	sl. s.	v. sl. s.
Zinc	DSA + ZnO	Thin white plates	v. s.	s.
Ammonium	DSA + NH_4OH	White needles	v. s.	v. s.
Diethylammonium	DSA + $(C_2H_5)_2NH$	Rectangular prisms	v. s.	sl. s.
Monoamylammonium	DSA + $C_5H_{11}NH_2$	Needles, tetrahedra and spherical segments	s.	mod. s.
Diamylammonium	Sod. DSA + $(C_5H_{11})_2NH\cdot HCl$	Feathers	sl. s.	v. sl. s.
Triethanolammonium	DSA + $(HOCH_2CH_2)_3N$	Not readily crystallizable sirup	ext. s.	ext. s.

[a] In cold solution was precipitated as bronze scales which spontaneously changed to the above. [b] Apparently light stable.

of disulfanilamide we have prepared (but have not analyzed) the other salts shown in Table II. The method of preparation for sparingly soluble salts was double decomposition between N^1-sodium disulfanilamide (sod. DSA) and a soluble salt of the metal. For soluble salts disulfanilamide (DSA) was neutralized with the corresponding hydroxide or carbonate of the metal.

The cupric salt is of interest since in the cold it was precipitated first as bronze scales which spontaneously changed over to light green needles. There was no evidence of the deep blue copper–ammonium coördination complex which might have been expected. The silver salt was white and apparently light stable. The monoamylammonium salt was peculiar in that it crystallized in unusual forms, one of which appeared to consist of spherical segments like the sections of an orange. The monoamylamine was obtained from the Sharples Solvent Corp. and was a mixture of isomers.

Of this series, N^1-sodium disulfanilamide and N^1-methyldisulfanilamide appeared very interesting and their pharmacology is being studied extensively. We have tested disulfanilamides of this type, successfully, in certain of the virus diseases. N^1-Methyl- and N^1-ethyldisulfanilamide are most outstanding, since they have shown promising results on infections caused by the Francis strain of influenza in mice.

Experimental

N^1-Sodium-N^4,$N^{4'}$-diacetyldisulfanilamide.—To one mole of ammonia dissolved in 150 cc. of water at 10° there was added over one hour with vigorous agitation, 2.5 moles of freshly prepared and analyzed N-acetylsulfanilyl chloride. Sufficient 50% sodium hydroxide solution was added to maintain the *p*H between 10 and 12. Ice was added to hold the temperature at 35–40°. After all of the N-acetylsulfanilyl chloride was added, stirring was continued for about an hour. The mixture was cooled to 10° and filtered. The crude product was recrystallized twice from concentrated aqueous solutions with use of activated

charcoal, after adjusting the *p*H to 6–7. Under these conditions, N^4-acetylsulfanilamide was very slightly soluble while N^1-sodium-N^4,$N^{4'}$-diacetyldisulfanilamide was very soluble, so that a separation was obtained readily. N^4,$N^{4'}$-Diacetyldisulfanilamide was recovered from the mother liquors by acidifying to *p*H 1–2. It was sparingly soluble in cold water. Anhydrous N^1-sodium-N^4,$N^{4'}$-diacetyldisulfanilamide was obtained by drying the hydrated salt at 130° *in vacuo*.

The same product was obtained starting with 1 mole of N^4-acetylsulfanilamide and 1 mole of N-acetylsulfanilyl chloride under similar conditions.

N^1-Sodium Disulfanilamide.—The filter cake of crude N^1-sodium-N^4,$N^{4'}$-diacetyldisulfanilamide prepared as above was hydrolyzed by boiling with 200 g. of sodium hydroxide and 200 cc. of water until there was no further increase in diazotizable amine (one to two hours). The mixture was then cooled to 10° and crude N^1-sodium disulfanilamide filtered. This was purified by recrystallization from concentrated aqueous solutions at *p*H 6–7 with use of activated charcoal. Solubility of N^1-sodium disulfanilamide in water was 9.6 g. per 100 cc. at 10° and 20 g. per 100 cc. at 37°. The anhydrous salt was obtained by drying at 100°.

Crude disulfanilamide was recovered from the mother liquors by acidification to *p*H 2–3 where it had a minimum solubility. Additional hydrochloric acid formed the more soluble hydrochloride or dihydrochloride. Disulfanilamide was only moderately soluble in boiling water. Disulfanilamide dihydrochloride was readily tetrazotized and this reaction served as a means of analyzing both disulfanilamide and its salts.

Dimetanilamide.—One mole of *m*-nitrobenzenesulfonamide was dissolved in 1600 cc. of water containing 16 g. of anhydrous sodium carbonate and 30 g. of sodium hydroxide at 45°; 1.3 moles of *m*-nitrobenzenesulfonyl chloride was then added with vigorous agitation at 45–60° over one-half hour while maintaining a *p*H of 10–11 by addition of 50% sodium hydroxide solution as required. After stirring for an hour the reaction mixture was cooled to 10° and the crude product filtered. Sodium di-(*m*-nitrobenzenesulfon)-amide was obtained by recrystallization from water.

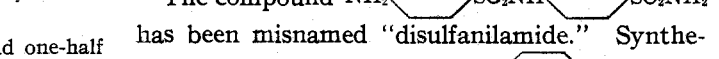
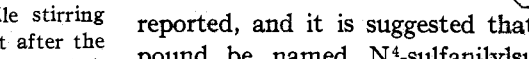
The nitro compound was reduced in two and one-half times its weight of 28% ammonia by passing a stream of hydrogen sulfide through the suspension while stirring vigorously. Cooling was necessary at first but after the reaction moderated, the mixture was held at a slow boil under a reflux condenser with constant passage of hydrogen sulfide for one hour. A rapid stream of air was then passed through the solution at the boil, to remove excess ammonia and oxidize sulfides to sulfur. The mixture was made alkaline to phenolphthalein with sodium hydroxide, and sulfur removed by filtration. The solution was acidified with hydrochloric acid to *p*H 4.5 to 5 and crude dimetanilamide obtained. This was purified by dissolving as the sodium salt, treating with activated charcoal and reprecipitating. Dimetanilamide melts to a characteristic blue-green liquid above 330°.

N^1-Methyl-N^4,$N^{4'}$-diacetyldisulfanilamide.—One mole of anhydrous N^1-diacetyldisulfanilamide was suspended in 2 liters of dry xylene containing two moles of dimethyl sulfate. The mixture was heated with constant agitation under a reflux condenser for two hours. The solid material was removed by filtration and the filtrate discarded. The solid was suspended in 3 liters of water and warmed with addition of sodium hydroxide solution until it remained alkaline to phenolphthalein. The insoluble material was filtered off and twice retreated with warm dilute sodium hydroxide. The final residue was the crude methylated product. It was purified by dissolving in glacial acetic acid, treating with activated charcoal and diluting with water. The yield of N^1-methyl-N^4,$N^{4'}$-diacetyldisulfanilamide was about 50%.

N^1-Methyldisulfanilamide.—One-half mole of N^1-methyl-N^4,$N^{4'}$-diacetyldisulfanilamide was suspended in 1200 cc. of 95% alcohol; 1.05 moles of 36% hydrochloric acid was added dropwise over one and one-half hours to the boiling, well agitated suspension. The liquid was spot tested on methyl violet paper frequently. Insufficient hydrochloric acid was present at any time to give a green spot. This was important since excess acid would have caused cleavage at the sulfonamide linkage. At the end a complete solution was obtained, which was exactly neutralized with 50% sodium hydroxide solution. After removal of salt the filtrate was evaporated to low volume and gradually diluted with water under agitation, so as to induce crystallization and avoid precipitation of the product as an amorphous mass. The crystals were suspended in 700 cc. of water at 35°, treated with excess sodium hydroxide and filtered, to remove any N^1-methylsulfanilamide. The crude product was then recrystallized several times from 80% alcohol (in which it was considerably more soluble than in 95%) using activated charcoal. N^1-Methyldisulfanilamide was water insoluble but readily dissolved in strong hydrochloric acid, in which it could be tetrazotized quantitatively. Yield in the hydrolysis was about 60%.

N^1-Ethyldisulfanilamide.—This was made by the same procedure using diethyl sulfate instead of dimethyl sulfate. Yields were somewhat lower.

Summary

The compound  has been misnamed "disulfanilamide." Synthesis of true disulfanilamide,  , is reported, and it is suggested that the first compound be named N^4-sulfanilylsulfanilamide to avoid confusion.

Disulfanilamide reacts with many bases to form a series of neutral salts, most of which are highly water soluble.

N^1-Alkyl derivatives have been made which are water insoluble and which do not form salts with bases.

These compounds appear from preliminary experiments in mice to have high antistreptococcal activity, and in the case of the N^1-alkyl derivatives, viruscidal activity as well.

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