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May 10 1939

I hereby recommend that the thesis prepared under my supervision by William A. Zobel, Jr.

entitled Some Derivatives of Sulfonilamide

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy.

Approved by:

Hoke S. Greene

W. M. Burgess, Chairman

Form 668—G. S. and Ed.—1M—7-31

SOME DERIVATIVES OF SULFANILAMIDE

A dissertation submitted to the

Graduate School

of the University of Cincinnati

in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

1939

by

William Andrew Zobel, Jr.
B.S. University of Nebraska 1936
M.A. University of Cincinnati 1938

Cincinnati, Ohio
May 10, 1939

Graduate Faculty
Graduate College
University of Cincinnati
Cincinnati, Ohio

Gentlemen:

I herewith submit a dissertation entitled "Some Derivatives of Sulfanilamide" in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The purpose of this investigation was to synthesize compounds, (I) which have high anti-streptococcal action in vivo, (II) which are water soluble, (III) and stable in aqueous solution.

The following compounds were synthesized: N^4-acetyl sulfanilyl semicarbazide, sulfanilyl semicarbazide, N^4-acetyl sulfanilyl phenyl hydrazine, N^4-acetyl sulfanilyl hydrazine, sulfanilyl hydrazine, N^4-acetyl sulfanilyl hydroxylamine, sulfanilyl hydroxylamine, N^4-acetyl sulfanilyl glycine, sulfanilyl glycine, tyrosine derivative, and leucine derivative.

Of all the compounds prepared, the sulfanilyl hydroxylamine is comparable to sulfanilamide therapeutically. All compounds have not been fully tested as yet, but the experiments are being carried on at the present time. These results will be published in some Pharmacology Journal.

Yours very truly,

William A. Zobel, Jr

8 Ag '39

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr. H. S. Greene the Director of Research for his cordial cooperation and encouragement.

Thanks are also due to Dr. L. H. Schmidt of the College of Medicine, University of Cincinnati, for testing the chemotherapeutic effect of the prepared compounds.

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SOME DERIVATIVES OF SULFANILAMIDE

I. A: HISTORY[1][2]

Sulfanilamide was first prepared by Gelmo[3], a German dabbler in theoretical chemistry, in 1909. Just before the World War, Horlein, Dressel, and Kothe[4] produced the first azo dyes from Gelmo's little known compound which dyed textiles a brick-red color and which were very fast to light and washing.

Sulfanilamide is a drug used for the treatment of streptococcal and other infections. The accident by which the power of sulfanilamide was discovered will probably remain forever a mystery. The action of sulfanilamide would have been known twenty years sooner had the two American scientists, Heidelberger and Jacobs[5] in 1919 gone a little further. They combined a quinine molecule with sulfanilamide and reported successful bactericidal properties of some of their azo dye compounds in vitro. Later studies showed these compounds also had therapeutic action in vivo.

Investigations were renewed in 1925 at the Elberfeld laboratories in Germany. The first paper was read on the fiftieth anniversary of the great scientist Koch, who discovered the tubercle bacillus. This work was done with

the red dye prontosil.

Dust collected on this work and it was not until 1935 that a patent was issued to Mietzsch and Klarer[6][7] who worked with Professor Domagk, the director of the Elberfeld pathological laboratory of the great dye factory in Elberfeld, Germany. The results of the action of this and similar compounds seemed too good to be true; before publishing any paper on this work, several years were devoted to intensive clinical and experimental work. Thus, the chemotherapeutic action of prontosil in experimental streptococcal infections in mice and rabbits was not published until February, 1935[8]. These results were at this time checked by workers in England, France and soon thereafter in America.

At about this time Mr. and Mrs. J. Tréfouël[9] at the Pasteur Institute in Paris reported that the azo-linkage (in prontosil) was not essential, and that sulfanilamide was just as effective. This equal efficacy was explained by the assumption that the prontosil and sulfanilamide were about equally distributed in the human organism. Consequently the sulfanilamide group in the para position was assumed to be the active group. Other French workers (Fourneau and his coworkers)[10] demonstrated that by shifting and substituting other groups for the sulfonamide group, the activity of sulfanilamide was either reduced or destroyed.

In England Colebrook and his investigators[11] specialized in the treatment of puerperal sepsis with prontosil and later with sulfanilamide. Buttle and his coworkers showed that sulfanilamide would give protection against different types of streptococci and some protection against meningococcal infections, but not against staphylococci or pneumococci. It was also found by the latter that sulfanilyl aniline was as effective as sulfanilamide.

In 1935 the Royal Academy of Medicine in London became the exchange place of sulfanilamide progress. Two Americans, Drs. Perrin Long and Eleanor Bliss, were at this gathering. Their presence was made possible through a generous fund established by Francis P. Garvan in memory of his lovely daughter, who had fallen prey to the deadly streptococci infection. Francis P. Garvan, the father, dedicated his finances to finding a cure for this dreaded disease. Drs. Long and Bliss had the honor of reporting the first human treated and saved in 1936.

Also at this time three other Americans, Drs. Mellon, Gross, and Cooper[12] began their work with sulfanilamide at the Western Pennsylvania Hospital. One of these men owes his life to this chemical and is a very faithful worker with sulfanilamide compounds to this day.

B. TYPES OF COMPOUNDS PREPARED

In the last three years several thousand derivatives of sulfanilamide have been synthesized, and their therapeutic action investigated. Many of these compounds have been reported in literature, while many others have not, especially those synthesized in private industry. Due to the large number of derivatives prepared, it will be impossible to list all in this work, but the derivatives can be classified into types[13][14][15][16][17]. Consequently, only the types will be listed and their therapeutic effect briefly stated.

1. Position of amino to sulfonamide group.

Substituting the groups into the other possible positions in the benzene nucleus reduces the therapeutic action (para > meta > ortho). The rule also holds if a radical is substituted on the hydrogen of the amino group.

2. Replacement of hydrogen of the amide group ($-SO_2NHR$)

(a) Aliphatic

The activity of this type of compound decreases. The decrease is proportional to the molecular weight of the radical. The mono- and di- substituted derivatives have approximately the same effect as sulfanilamide, i.e. if R is a small aliphatic radical as C_2H_5 or CH_3.

(b) Aromatic

When R is an aromatic or pyridine ring, the therapeutic action is as good or better than sulfanilamide.

Substitution of a carboxyl or sulfonic group in second phenyl radical changes the action noticeably (ortho,> meta,> para)[15]. When R is a phenyl p-sulfonamide group, the action is increased. Uniting as many as seven phenyl p-sulfonamide radicals has been done, but the poly sulfanilamides become so insoluble that its action decreases with such a large molecule.

3. Replacement of amino group by another group e.g. hydroxyl.

The therapeutic action decreases.

4. Replacement of sulfonamide group by other groups.

All such compounds have no noticeable therapeutic action.

5. Substitution derivatives of amino group as -NHR.

The therapeutic action is sharply lowered in this type of compound. Derivatives where R was an acetyl group, the toxicity increased in every known compound.

6. Azo Derivatives.

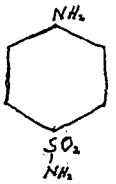
When sulfanilamide is diazotized and coupled with aromatic amines or sulfonic naphthols, the therapeutic action is approximately the same as sulfanilamide. If other compounds such as phenols or phenolic ethers are coupled, the action is decreased.

7. Addition of a third group into benzene ring of sulfanilamide.

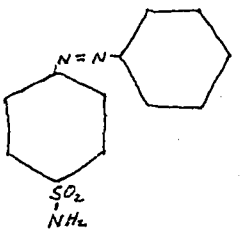
The therapeutic action is sharply lowered, and in many cases totally lost.

Many other derivatives have been prepared but they fall into one of these types by changing the meaning of the R's. All of these are inactive or slightly active.

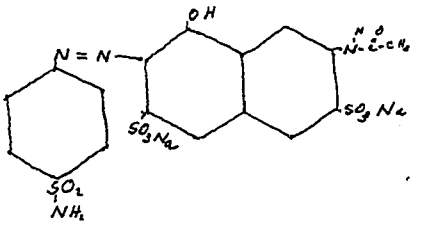
The derivatives which have now the widest use are: sulfanilamide, prontosil, and prontosil soluble.



Sulfanilamide



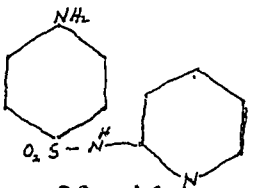
Prontosil



Prontosil Soluble

Sulfanilamide has such trade names as, Prontylin, Streptocide, Zeamine, Sulfamidyl, etc. Sulfanilamide is 1% soluble in water at body temperature. It has no perceptible odor and possesses a bitter taste.

Another compound which only this year has come into prominence is ∝-sulfanilamidopyridine, whose structure is



It is reported that this compound is very effective against all thirty-two types of pneumococci, but it is too early to draw definite conclusions. It is nevertheless safe to say that it is more effective than sulfanilamide against pneumococcal infections.

The therapeutic action is determined with mice infected with a virile strain of beta hemolytic streptococci.

The infected mice are given the chemotherapeutic agent either subcutaneously in a suitable solvent, or orally as a paste made up with gum acacia and water. The time elapsing between the subcutaneous injection with streptococci and the initial dose of the therapeutic agent was two hours. A similar dose was then given every eight hours over a period of seventy-two hours. The mice surviving were compared in number and physical condition with those that had been similarly infected and then given sulfanilamide as the protective agent. Thus a relationship between the effectiveness of the new substance and that of sulfanilamide could be obtained.

From the foregoing it can be seen that most compounds which are derivatives of the amido substituted nitrogen have anti-streptococcal activity. All other compounds prepared, except the diazo, sulfone, and sulfide compounds have not. Therefore, in the present work, derivatives of amido substituted nitrogen were prepared. This generalization was proven definitely a year later by Crossley, Northey and Hultquist[17]. They proved it with the following compounds: N^3- sulfanilyl metanilamide and N^4- metanilyl sulfanilamide. The first compound is behaving as a sulfanilamide substituted on the amido nitrogen while the second is behaving as a sulfanilamide substituted on the amino nitrogen. The action of the two compounds was as expected, in that the first had high activity while the second had none. Due to this proof, it can be

assumed without doubt that, if new derivatives are synthesized having high activity, they will be substituted on the amido nitrogen.

II. PURPOSE OF PRESENT INVESTIGATION

Sulfanilamide is unstable, a precipitate forming in an aqueous solution on standing 5-7 days. Because of lack of equipment, it is also difficult for general medical practitioners to make up their solutions when needed. Its general use is therefore greatly curtailed. Furthermore, sulfanilamide is only 1% soluble in water. Therefore, this investigation had the following purposes:

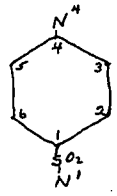
1. To synthesize a stable compound.
2. To synthesize a water soluble compound.
3. To synthesize a compound whose therapeutic action is as effective as sulfanilamide.

III. GENERAL INTRODUCTION

A. Nomenclature

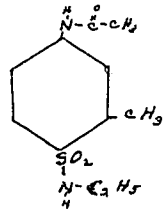
The nomenclature used in literature for sulfanilamide derivatives is very difficult to comprehend. The system of nomenclature suggested in The American Chemical Society(15) simplifies the naming of complex derivatives of sulfanilamide. The systems suggested are the following:

(a) Naming as substituted sulfanilamides



In sulfanilamide the sulfonamide group occupies the 1-position since it is the principal functional group. The nitrogens are differentiated by giving them subscripts corresponding to the carbon in the benzene ring on which the nitrogen substituents are found.

The following illustrates the method.

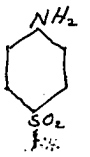


2-methyl N^4-acetyl N'-ethyl sulfanilamide

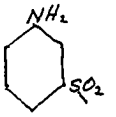
(b) Radical names

Simple derivatives can be named very easily by the use of the above method. For compounds with complex substituents, the above method of naming as substituted

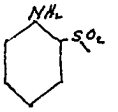
sulfanilamides becomes very unwieldy. For this reason the following radicals will be used throughout this paper:



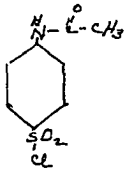
Sulfanilyl-



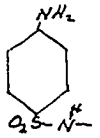
Metanilyl-



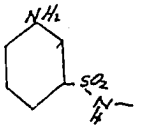
Orthonilyl-

The important intermediate  is called N^4-acetyl sulfanilyl chloride, instead of p-acetamido benzene sulfonyl chloride.

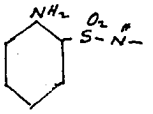
The other radicals which are useful are the following:



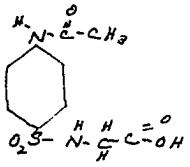
Sulfanilamido



Metanilamido



Orthonilamido

As an example the compound  is called N^4acetyl sulfanilamido acetic acid.

B. Reagents and Analytical Methods

1. Reagents

A very important intermediate in the synthesis of all derivatives was N^4-acetyl sulfanilyl chloride. This was prepared by the reaction of acetanilid (1 mol) with chlorosulfonic acid (2-1/2 mols). The mixture was heated for 2-1/2 hours at 60°C. when it was cooled, slowly poured on ice (2 Kg.) with stirring and then filtered. The crude paste was used directly in some synthesis and in others the N^4-acetyl sulfanilyl chloride was purified. Purification was accomplished by drying the paste at room temperature, and then recrystallizing from benzene (solubility 1.5-2g. per 100 cc.). The purified N^4-acetyl sulfanilyl chloride could be kept indefinitely in a glass stoppered bottle.

Since the desired free amino compound could not be synthesized directly without protecting the amino group, the acetyl derivative was first prepared for all compounds and then the acetyl group was hydrolyzed in 2N acid or base, depending on the type of compound. One and one-half mols of acid or base were used for each acidic or basic group produced in the hydrolysis. The actual hydrolysis will be described in the procedure for each compound.

2. Analytical Method

The sulfur was determined with a Parr bomb. Usually 0.2g. of sample was added to a weighed Parr bomb which contained 2-3g. of KNO_3-sugar mixture as oxidizing agent. The

KNO_3-sugar ratio was 3 to 1 by weight. At first $KClO_4$ was used as accelerator, but this did not oxidize all of the compounds. Therefore the KNO_3-sugar mixture was substituted. The KNO_3-sugar was added with the aid of a small calibrated test tube. To the KNO_3-sugar-sample mixture 15g. of Na_2O_2 were added. The bomb was sealed and ignited by heating over a Bunsen burner. Ignition took place in 30-60 seconds. The bomb was cooled with water, opened, and placed in a 400cc. beaker to which water was added. After digestion the bomb was washed. The solution was allowed to cool and con HCl added until just acid. The iron was reduced by adding aluminum filings and heating until colorless. The solution was filtered, diluted to 300cc., and heated to its boiling point. Normal $BaCl_2$ (5cc.) was added and then allowed to stand for ten hours after which the $BaSO_4$ was filtered into weighed sintered glass crucibles.

C. Remarks

All melting points are uncorrected.

IV. EXPERIMENTAL WORK

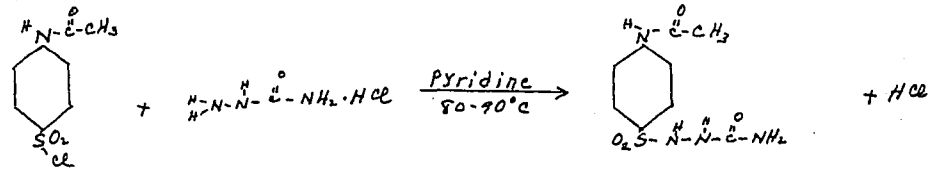
1. N^4- Acetyl Sulfanilyl Semicarbazide

A. Nomenclature

1. N^4Acetyl Sulfanilyl Semicarbazide
2. N^4Acetyl Sulfanilamido Urea

B. Preparation

1. Equation



2. Reactants

	Grams	Mols	Melting Point
N^4Acetyl Sulfanilyl Chloride	2.33	0.01	
Semicarbazide Hydrochloride	1.12	0.01	
Pyridine	1.6	0.02	

3. Yield

Theoretical	2.72	0.01	
Actual	1.0-1.2		232° (decomposed)

4. Procedure

The semicarbazide hydrochloride was added to the pyridine. The specified amounts of pyridine must be taken, as otherwise lower yields result. The hydrochloride forms a semi-solid with the pyridine which dissolved when the purified N^4 acetyl sulfanilyl chloride was slowly added. The viscous material which resulted was heated on a steam bath for one hour, then allowed to cool and 30 cc. of water added

on which the N^4-acetyl sulfanilyl semicarbazide precipitated out, as the viscous material dissolved. The mixture was filtered, and the precipitate washed with water, followed by ethyl alcohol (95%) and then ethyl ether.

Crude yield = 2.1g.

C. Purification

The impure product was added to a boiling 50% ethyl alcohol-water mixture (solubility 1 g. per 100 ccs.), decolorized with activated charcoal, filtered, and then chilled. The white precipitate was filtered, washed with water, ethyl alcohol (95%) and then with ethyl ether,

y = 1.0-1.2g. Melting Point = 232°C.(decomposes)

The following methods of purification were also tried. The impure product was dissolved in an aqueous solution of sodium hydroxide or sodium carbonate, and reprecipitated by the addition of hydrochloric acid. The N^4-acetyl sulfanilyl semicarbazide so purified does not become white as does the pure product obtained by the first method. It retains a slight pinkish color.

Since the solubility of the compound is a function of the acidity and precipitation occurs very quickly by the addition of acid, gaseous carbon dioxide was bubbled through the alkaline solution, so that occlusion would not take place. The precipitation was thus accomplished more slowly, allowing the crystals to grow. The compound so obtained also had a slight coloration.

N^4-acetyl sulfanilyl semicarbazide is soluble in hot glacial acetic acid and can be reprecipitated by the addition of water. When this was tried the compound had a slight pink color, but the physical characteristics were the same as those of the pure product obtained by recrystallizing from 50% ethyl alcohol-water solution.

D. Analysis

To prove that the obtained product was the desired one, the sulfur content was checked, and found to be 11.68%. The theoretical is 11.77%. This was done by the Parr bomb method using 1 g. of potassium perchlorate as accelerator.

The nitrogen content by the Kjeldahl method was also investigated. The results were inconsistent, and could not be taken as reliable proof for the structure. The reason for the inconsistent results is unknown.

The N^4-acetyl sulfanilyl semicarbazide gave a silver mirror test with Tollen's reagent. Since hydrazine reduces ammoniacal silver oxide, this positive test indicated a hydrazine linkage.

Since N^4-acetyl sulfanilyl semicarbazide is not soluble in benzene, camphor or water, the molecular weight could not be determined. A weighed sample however was titrated with standard sodium hydroxide using a Beckman pH meter. By plotting the volume of added base against pH, the quantity of base necessary to neutralize the compound could be determined. If

it is assumed that the N^4-acetyl sulfanilyl semicarbazide is mono-acidic, the molecular weight can be calculated.

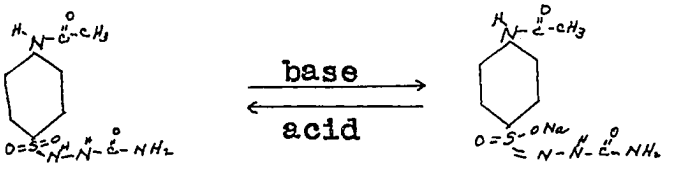
$$\text{Molecular Weight} = 1000 \frac{\text{Weight of Sample}}{\text{N x ccs.}}$$

Molecular weight found = 284g.
Molecular weight calculated = 272.28g.

E. Properties

1. The compound is not soluble in aqueous acid solutions, benzene, carbon disulfide, ethyl ether and acetone.

2. The compound is soluble in bases. Sodium carbonate will dissolve it with evolution of carbon dioxide. The solubility in alkaline solution is explained by the probable keto-enol isomerism.



(insoluble) (soluble)

3. N^4-acetyl sulfanilyl semicarbazide gives a silver mirror test with Tollen's reagent.

F. Other Possible Methods of Preparation

1. Recrystallized N^4-acetyl sulfanilyl chloride (0.1M) was added to the pyridine (0.3M). The N^4-acetyl sulfanilyl chloride dissolved readily in pyridine. The semicarbazide hydrochloride (0.1M) was added slowly with constant stirring. The mixture was held at 35-40°C. by external cooling. After

all the semicarbazide hydrochloride was added, stirring was continued for 30 minutes at room temperature. The mixture was then transferred to a distilling flask and the excess pyridine distilled off under reduced pressure (water pump) at 40°C. Distillation was continued until no more pyridine distilled over and there remained a viscous material. To this 150-200 ccs. of water were added. Since the viscous material dissolved very slowly, the flask was shaken. The reddish precipitate was filtered, washed with water, ethyl alcohol (95%) and ethyl ether.

Yield = 10-11 g.

Evaporation of the filtrate under reduced pressure at 40-45°C., or if mineral acid or sodium chloride were added to it, no additional yield was obtained.

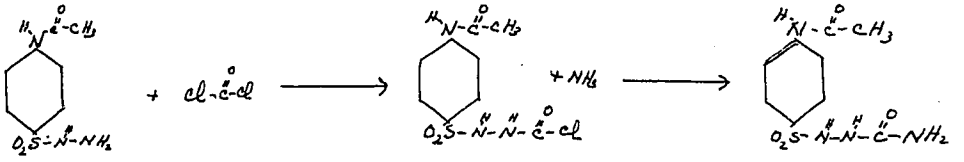
2. Semicarbazide hydrochloride was added to ethyl alcohol (95%) and the N^4-acetyl sulfanilyl chloride at room temperature. N^4-acetyl sulfanilyl etholate was formed. Negative results.

3. The semicarbazide hydrochloride and N^4-acetyl sulfanilyl chloride were added to a 50% ethyl alcohol-water solution. N^4acetyl sulfanilic acid was formed. Negative results.

4. Semicarbazide hydrochloride was added to water and the acid neutralized with sodium carbonate. The N^4-acetyl sulfanilyl chloride was then added and the mixture heated to 40-45°C. If the temperature of the reaction was changed, all trials gave negative results.

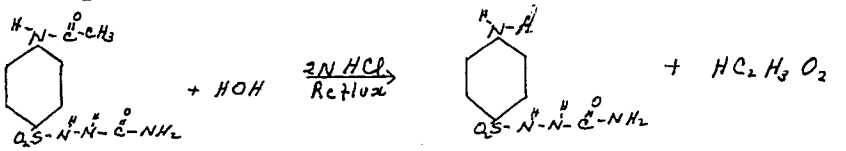
5. When procedure (3) was repeated but instead sodium bicarbonate was used as neutralizing agent, negative results were also obtained.

6. A suggestion is here offered, that it probably is possible to prepare the compound by the use of phosgene.



G. Hydrolysis to Sulfanilyl Semicarbazide

1. Equation



2. Reactants

	Grams	Mols	Melting Point
N^4-acetyl sulfanilyl semicarbazide	2.72	0.01	
HCl		0.02	

3. Yield

Theoretical	2.30	0.01	
Actual	1.5	65%	232°C. (decomposes)

4. Procedure

Crude N^4-acetyl sulfanilyl semicarbazide was placed in a round bottom flask and 2N HCl (2 mols acid for every mol) were added and the mixture refluxed for 30 minutes after the precipitate disappeared. The mixture was cooled and

anhydrous sodium carbonate added until alkaline at which time the sulfanilyl semicarbazide separated. The precipitate was filtered, washed with water, ethyl alcohol (95%) and then with ethyl ether.

Crude yield = 2.2g.

5. Purification

Sulfanilyl semicarbazide was added to a boiling 50% ethyl alcohol-water solution (solubility 2g. per 120cc.) and decolorized with activated charcoal. The hot solution was filtered and the filtrate chilled, at which time beautiful white crystals formed.

Yield = 1.5g. (60-65%) Melting Point = 232°C. (decomposes)

6. Analysis.

(a) The sulfur was determined by the Parr bomb method using 2.5g. of potassium nitrate-sugar mixture with a 0.2g. sample.

Sulfur found = 13.80%
Sulfur calculated = 13.92%

(b) The compound was diazotized and coupled to beta-naphthol on which an orange color formed. This positive result indicated a free amino group.

7. Properties

(a) Melting point = 232°C. (decomposes)

(b) White needle crystals

(c) Soluble in acids

(d) Insoluble in aqueous carbonate solution.

2. N^4-Acetyl Sulfanilyl Phenyl Hydrazine

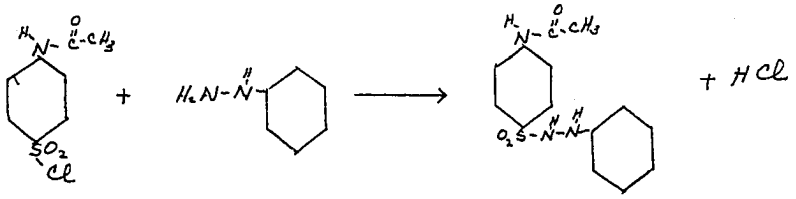
A. Nomenclature

N^4-acetyl sulfanilyl phenyl hydrazine

N^4-acetyl sulfanilamido anilid

B. Preparation

1. Equation



2. Reactants

	Grams	Mols	Melting Point
N^4-acetyl sulfanilyl chloride	2.3	0.01	
Phenyl-hydrazine	3.2	0.03	

3. Yield

Theoretical	3.05	0.01	
Actual	1.00	.003	161°C.

4. Procedure

Impure N^4-sulfanilyl chloride paste (.01M) was added slowly to .03 mols of phenyl-hydrazine at room temperature. When the reaction became too violent, the mixture was cooled externally. Charring resulted if the reaction became too fast. The semi-solid mass was occasionally stirred and when the reaction became slower the mixture was heated to 70° for one minute and then allowed to cool. This procedure was repeated two times. The excess phenyl-hydrazine was removed by adding 20 ccs. of water to the solid mass and strongly

acidifying with concentrated hydrochloric acid. The semi-solid mass was filtered and thoroughly flushed with water. This procedure was repeated. The precipitate was washed with ethyl alcohol (95%) and then with ethyl ether until the filtrate became clear. The ether removed the remaining phenyl-hydrazine.

Crude yield = 2.5 g.

C. Purification

Purification was accomplished by dissolving the product in a boiling 50% ethyl alcohol-water solution (solubility 1 g. per 30 ccs.) and decolorizing with charcoal. The N^4-acetyl sulfanilyl phenyl-hydrazine decomposes very easily, so that long heating must be avoided.

Yield = 1.0 g.; Melting Point = 161°C.

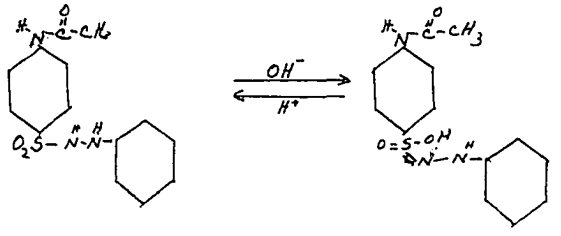
D. Analysis

Sulfur determined with Parr bomb

$S_{calcd.} = 10.50\%$ $S_{found} = 10.66\%$

E. Properties

Soluble in aqueous sodium hydroxide and sodium carbonate solution. The solubility is explained by the probable keto-enol isomerism:



F. Hydrolysis

It was impossible to hydrolyze the acetyl group with 2N hydrochloric acid, as decomposition occurred. Even at room temperature for a length of two months with 2N acid, no hydrolysis took place.

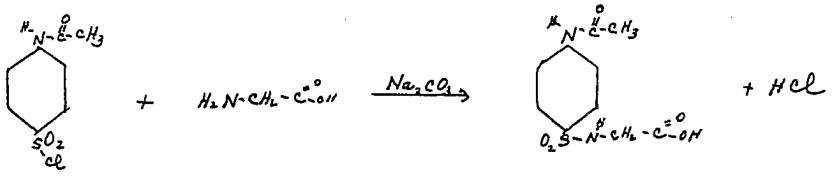
3. N^4-Acetyl Sulfanilyl Glycine

A. Nomenclature

N^4-acetyl sulfanilyl glycine(18)

B. Preparation

1. Equation



2. Reactants

	Grams	Mols	Melting Point
N^4-acetyl sulfanilyl chloride	3.5	0.015	
Glycine	0.75	0.01	
Sodium carbonate	3.1	0.03	

3. Yield

Theoretical	2.72	0.01	
Actual	1.80	—	235° dec.

4. Procedure for Preparation

The glycine (.01M), sodium carbonate (.03M) and water (6 ccs.) were heated to 40-45°C. with vigorous stirring. N^4-acetyl sulfanilyl chloride paste (.015M) were added slowly, keeping the temperature at 40-45°C. The precipitate dissolved, indicating complete reaction. The solution was filtered, strongly acidified with con. HCl, and on cooling the product precipitated. The precipitate was washed with water, followed by ethyl alcohol (95%) and then ethyl ether.

Crude yield = 2.2 g.

C. Purification

The product was purified by dissolving in hot water (solubility 1 g. per 30 ccs.) and decolorizing with charcoal. On cooling, white needles formed.

Yield = 1.8 g.; Melting Point = 235°C(decomposes)

D. Analysis

The sulfur was determined with a Parr bomb.

$S_{calcd.} = 11.78\%$ $S_{found} = 11.70\%$

E. Hydrolysis to sulfanilyl glycine(18)(19)

1. Crude N^4-acetyl sulfanilyl glycine (2.2g.) was refluxed with 10 ccs. of 2N HCl one half hour after the precipitate disappeared. This mixture was evaporated under reduced pressure (water pump) until dryness at 40-45°C.

2. Purification

The solid remaining was extracted with 40 ccs. of ethyl alcohol (95%) and decolorized with charcoal. The product was thrown out of solution by the addition of 200-250ccs. of ethyl ether. The ether was added in small quantities until cloudiness appeared. This was continued until no cloudiness appeared on the addition of ether. The pure compound has white needle like crystals.

Yield = 1.2 g.; Melting Point = 154°C.

Note: The sulfanilyl glycine is much more soluble in alcohol, but if more is dissolved per cc., it will precipitate out as an oily liquid on the addition of the ether. On

standing this oil turns to a solid, but has a slight coloration.

3. Analysis

(a) The sulfur was determined with a Parr bomb.

$S_{calcd.} = 13.93\%$ $S_{found} = 13.94\%$

(b) On diazotization and coupling with beta naphthol a color formed. This indicated a primary amino group on a benzene ring.

4. Properties

1. Soluble in water.
2. Soluble in acidic and basic solutions.

4. N^4-acetyl Sulfanilyl Hydroxamic Acid

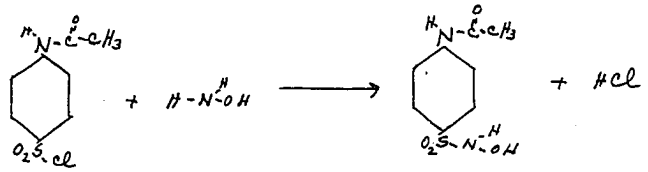
A. Nomenclature

N^4-acetyl sulfanilyl hydroxamic acid

N^4-acetyl sulfanilyl hydroxylamine

B. Preparation

1. Equation



2. Reactants

	Grams	Mols	ccs.	Melting Point
Hydroxylamine Hydrochloride	.69	.01		
N^4-acetyl sulfanilyl chloride	1.17	.005		
Methyl alcohol			3.5	
Ethyl alcohol			4.2	
Sodium	.21			

3. Yields

Theoretical	1.15	
Actual	.86	199°C.

4. Procedure for Preparation

The hydroxylamine hydrochloride (.01M) was dissolved in 3.5 ccs. of boiling methyl alcohol with the aid of refluxing. To this hot solution 4.2 ccs. of ethyl alcohol, in which 0.21 g. of sodium had been previously dissolved, were added. The mixture was cooled and the sodium chloride filtered giving free hydroxylamine. N^4-acetyl sulfanilyl chloride (.01M) was added to the mixture. The product precipitated on

cooling and was filtered. The filtrate was evaporated on which additional crystals formed.

Crude yield = 1.4 g.

C. Purification

The product was purified by dissolving in 80 cc. of water and decolorizing with charcoal. On cooling, white crystalline plates formed.

Yield = 0.86 g. Melting Point = 199°C.

D. Analysis

The sulfur was determined with the Parr bomb.

$S_{calcd.} = 13.92\%$ $S_{found} = 13.97\%$

E. Properties

Slightly soluble in water.

F. Other Possible Method of Preparation

N^4-acetyl sulfanilyl hydroxamic acid was at first prepared by dissolving hydroxylamine hydrochloride in water, neutralizing with sodium carbonate, and adding N^4-acetyl sulfanilyl chloride. The mixture was heated to 40-45°C. The acid precipitated. When this procedure was repeated, no yields were often obtained. Therefore, the first time it must have been coincidence so the method was discarded. The yields were also low. This procedure may have possibilities as it was not fully investigated.

G. Hydrolysis to sulfanilyl hydroxamic acid

1. Procedure

Crude N^4-acetyl sulfanilyl hydroxamic acid (1.4g.) were refluxed with 7 cc. of 2N HCl until the precipitate disappeared. Boiling longer lowered the yield. On cooling the mixture was made alkaline with Na_2CO_3, at which time the product precipitated.

Yield = 0.7 g.

2. Purification

The product was recrystallized from 7 cc. of water.

Yield = 0.5 g. Melting Point = 172°C.

3. Analysis

The sulfur was determined with the Parr bomb.

$S_{calcd.}$ = 17.04% S_{found} = 16.89%

4. Properties

Slightly soluble in water, 1.5%.

Soluble in acid.

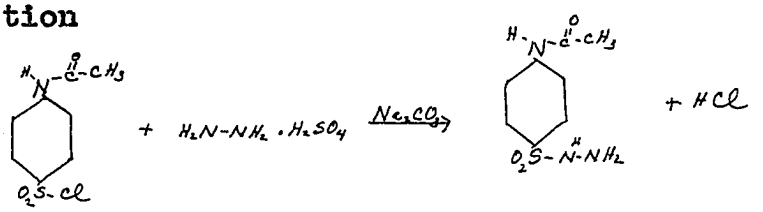
5. N^4-Acetyl Sulfanilyl Hydrazine

A. Nomenclature

N^4-acetyl sulfanilyl hydrazine

B. Preparation

1. Equation



2. Reactants

	Grams	Mols	Melting Point
Hydrazine sulfate	1.3	.01	
Na_2CO_3	3.1	.03	
N^4-acetyl sulfanilyl chloride	2.3	.01	

3. Yields

Theoretical	2.29	0.1	
Actual	1.6		183°C.

4. Procedure for Preparation

To the hydrazine sulfate (.01M), Na_2CO_3 (.03M) and 25 cc. of water was added N^4-acetyl sulfanilyl chloride paste (.01M) with vigorous agitation. The mixture was then heated for 1 hour at 40-45°C. The product precipitated and was filtered, washed with water, ethyl alcohol (95%) and ethyl ether.

Yield = 1.93 g.

C. Purification

Purification was effected by dissolving (1.9 g.) in

hot water (70 cc.) and decolorizing with charcoal. The product crystallized in long white needles.

Yield = 1.6 g. Melting Point = 183°C.

D. Analysis

The sulfur was checked with a Parr bomb.

$S_{calcd.} = 13.98\%$ $S_{found} = 14.20\%$

E. Hydrolysis to Sulfanilyl Hydrazine

1. Hydrolysis was accomplished by adding to the N^4-acetyl sulfanilyl hydrazine (34 g.) 140 cc. of 2N HCl and letting the mixture stand at room temperature for three weeks. The product was thrown out of solution by the addition of Na_2CO_3 until alkaline. Hydrolyzing in hot acid or basic solution reduces the compound to a sulfide.

Yield = 21.7 g.

2. Purification

The product was recrystallized from water (solubility 18 g. per 75 cc.). The product crystallized with three molecules of water.

Yield = 12.5g. M.P. = 92°C.

3. Analysis

(a) The sulfur was determined with a Parr bomb.

$S_{calcd.} = 17.13\%$ $S_{found} = 13.16\%$

When the product was dried in a 100°C. oven, it darkened, but on analysis, 14.50% sulfur was found. This indicated water of crystallization was present. On

calculating, the amount of water present is approximately three molecules. The melting point also increased to over 140°C., which also is a good indication that a hydrate formed.

b) Diazotization and coupling to beta naphthol gave a color, which indicates a free amino group. Thus, it was assumed that the desired product was obtained.

6. Leucine Derivative

A. Preparation

1. Reactants

	Grams	Mols	Melting Point
Leucine	1.3	.01	
N^4-acetyl sulfanilyl chloride	5.9	.025	
NaOH	0.4	.01	
Na_2CO_3	1.59	.015	

2. Yield

Theoretical	5.3	.01	
Actual	2.8		195-205°C.

3. Procedure for Preparation

The leucine (1.3 g.), N^4-acetyl sulfanilyl chloride (5.9 g.), NaOH (.4 g.) and Na_2CO_3 (1.59 g.) were added to 25 cc. of water and heated to 50-60°C. for 40 minutes. The solution was made acid with con. HCl on which the product precipitated in a viscous mass.

B. Purification

The product was purified by dissolving it in 15 cc. of ethyl alcohol (95%) and pouring this solution into 125 cc. of water. The product precipitated on standing 24 hours.

Yield = 2.8 Melting Point = 195-205°C.

C. Analysis

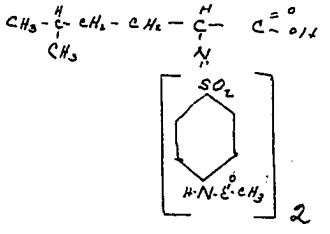
The sulfur was determined with a Parr bomb.

$S_{calcd.} = 12.58\%$ $S_{found} = 12.47\%$

D. Possible Structures

The sulfur analysis indicates that two molecules of sulfur are associated with one molecule of leucine. Therefore, two structures are possible.

1.



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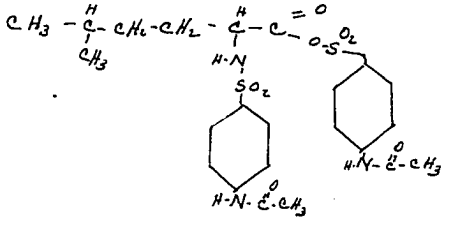


E. Hydrolysis of Acetyl Group

The product could be hydrolyzed by refluxing for 20 minutes with 2N NaOH. On the addition of HCl to its iso-electric point the product precipitated. No method of purification was found.

7. Tyrosine Derivative

A. Preparation

1. Reactants

	Grams	Mols	Melting Point
Tyrosine	1.8	.01	
N^4-acetyl sulfanilyl chloride	5.9	.025	
NaOH	0.4	.01	
Na_2CO_3	1.59	.015	

2. Yield

Theoretical	5.7	.01	
Actual	4.7		252°C.

3. Procedure for Preparation

The tyrosine (1.8 g.), N^4-acetyl sulfanilyl chloride (5.9 g.), NaOH (.4 g.) and Na_2CO_3 (1.59 g.) were added to 40 cc. of H_2O and heated for 30 minutes at 50-60°C. The solution was then acidified with HCl, on which the product precipitated.

B. Purification

Purification was accomplished by dissolving the product in 100 cc. of ethyl alcohol (95%) and pouring this solution into 300 cc. of H_2O. The product precipitated on standing 10 hours.

Yield = 4.7 Melting Point = 252°C.

C. Analysis

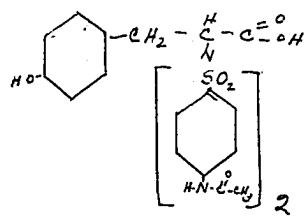
The sulfur was determined with a Parr bomb.

$S_{calcd.}$ = 11.14% S_{found} = 10.98%

D. Possible Structures

Since two molecules of sulfur are combined with one molecule of tyrosine as indicated by the sulfur analysis, four structures are possible.

1.



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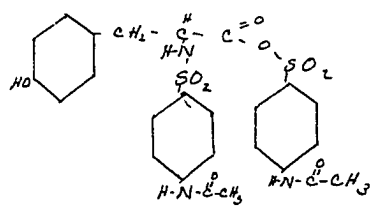

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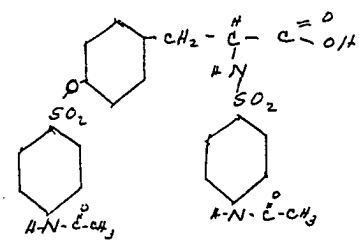

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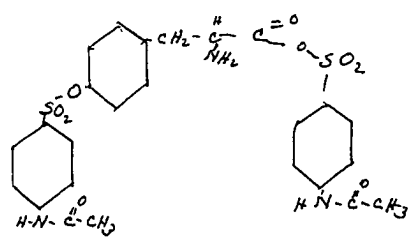

The structures 1 and 2 are most probable as in the leucine derivative the same type of compound is formed

in that 2 molecules of sulfur are associated with one molecule of leucine. Only these two structures are possible in the leucine derivative.

E. Hydrolysis of Acetyl Group

The product could be hydrolyzed by refluxing with 2N NaOH. When the solution was acidified with HCl to its iso-electric point, the product precipitated. No method of purification was found.

THERAPEUTIC EFFECT OF COMPOUNDS

Of all the amide derivatives that were prepared, only the sulfanilyl hydroxylamine derivative had chemo-therapeutic action. Its action is of the same magnitude as sulfanilamide to which it was compared, but it appeared that its toxicity was less than sulfanilamide.

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