using zinc dust and decolorizing carbon to remove color. In several cases it was necessary to carry out the recrystallizations in an atmosphere of nitrogen or hydrogen in order to obtain colorless products.

2 - *p* - Hydroxylaminobenzenesulfonamido - 3 - ethoxypyridine.—An attempt to reduce 2-*p*-nitrobenzenesulfonamido-3-ethoxypyridine catalytically to the corresponding amino compound resulted in only partial reduction with the formation of the analogous hydroxylamine derivative. 3.2 grams (0.01 mole) of 2-*p*-nitrobenzenesulfonamido-3-ethoxypyridine was partially dissolved in 200 cc. of 95% ethanol at 50°. The suspension was reduced catalytically using 1.0 g. of $Pd(OH)_2$ on calcium carbonate as the catalyst and a pressure of 3 to 4 atmospheres. Fresh 0.5-g. portions of catalyst were added twice during the course of the reduction. After recrystallization from alcohol–water, the product melted with decomposition at 189–190° (cor.). It gave a silver mirror test with ammoniacal silver nitrate, which is characteristic of hydroxylamines. The compound was identified as 2-*p*-hydroxylaminobenzenesulfonamido-3-ethoxypyridine by this test and by the analytical figures which are recorded in Table II.

TABLE II

	Calculated for			Found	
	Nitro compound	Amino compound	Hydroxylamino compound		
C	48.30	53.24	50.49	50.4	50.6
H	4.02	5.12	4.86	4.8	4.6
N	13.00	14.32	13.59	13.5	13.5

Summary

A number of substituted sulfanilamidopyridines have been synthesized. Several of these compounds showed marked chemotherapeutic activity against experimental streptococcal and pneumococcal infections in preliminary studies in mice.

In the cases where two isomeric substituted sulfanilamidopyridines were compared, one was found to be effective while the other was not. The 5-sulfanilamido-2-halogen substituted pyridines were active, but these same isomers were inactive when the substituent was an amino, hydroxy or ethoxy group.

Evidence is presented to show that the differences in the chemotherapeutic activity of the isomeric substituted sulfanilamidopyridines cannot be attributed to differences in solubility or the establishment and maintenance of adequate blood concentrations, but must be due to some inherent difference in the compounds themselves.

STAMFORD, CONN. RECEIVED APRIL 25, 1940

[CONTRIBUTION FROM THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID COMPANY]

Chemotherapy. II. Some Sulfanilamido Heterocycles[1]

BY RICHARD O. ROBLIN, JR., JAMES H. WILLIAMS, PHILIP S. WINNEK AND JACKSON P. ENGLISH

Of the many types of sulfanilamide derivatives synthesized in attempts to improve and expand the chemotherapeutic activity of the parent substance, sulfanilamido heterocycles have shown the greatest promise. This report describes the preparation of a number of new heterocyclic derivatives of sulfanilamide. Since the initiation of this investigation several of the compounds listed in Tables I and II have been described by Fosbinder and Walter,[2] Lott and Bergeim[3] and others.

Two of the new substances, namely, 2-sulfanilamidopyrimidine and 2-sulfanilamido-4-methylpyrimidine, have shown considerable promise as chemotherapeutic agents in preliminary animal studies.[4] In order to avoid possible confusion between sulfapyridine and sulfapyrimidine, the term sulfadiazines is suggested for these compounds. As the pyrimidine is a diazine ring, this name seems to be a logical choice.

The preparation of the intermediate amino heterocycles followed, in general, methods already described in the literature. The methods of Büttner, Wheeler, Johnson and co-workers[5] were used for the first synthesis of the two isomeric aminopyrimidines. In order to prepare larger quantities of sulfadiazine, a simplified synthesis of 2-aminopyrimidine was devised. Guanidine sulfate was condensed with formylacetic acid in fuming sulfuric acid. Malic acid was the starting product, from which formylacetic acid was generated in a manner similar to that described by Davidson and Baudisch.[6] The isocytosine produced in this reaction was treated with phosphorus oxychloride using a modification of the method described by

(1) Presented in part before the Division of Medicinal Chemistry, Cincinnati meeting of the American Chemical Society, April 11, 1940.

(2) Fosbinder and Walter, THIS JOURNAL, **61**, 2032 (1939).

(3) Lott and Bergeim, *ibid.*, **61**, 3593 (1939).

(4) The pharmacological and bacteriological investigations were made in these Laboratories under the direction of Dr. W. H. Feinstone and will be reported in detail elsewhere.

(5) See Johnson and Hahn, *Chem. Rev.*, **13**, 193–303 (1933).

(6) Davidson and Baudisch, THIS JOURNAL, **48**, 2379 (1926).

TABLE I

PROPERTIES OF SULFANILAMIDO HETEROCYCLES

Compound	M. p., °C. (cor.)	Water soly., 37°C.[c]	Max. blood level[d]	Chemotherapeutic activity[e]	Ref. to intermed.	Formula	Analyses,[p] % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
2-Sulfanilamidothiazole[a,b]	201–202	94	12	Active								
2-Sulfanilamido-4-methylthiazole[a,b]	237–238	28.9	10.7	Active								
2-Sulfanilamido-4-*p*-diphenylthiazole	216–217	0.1	0.9	Inactive	*g*	$C_{21}H_{17}O_2N_3S_2$	61.9	4.2	10.3	62.1	4.7	10.1
2-Sulfanilamidobenzothiazole	304–305[f]	0.1	0.6	Inactive	*g*	$C_{13}H_{11}O_2N_3S_2$	51.1	3.6	13.8	51.1	3.9	13.6
2-Sulfanilamido-1,3,4-thiadiazole	216–218[f]	73	4.0	Active	*h*	$C_8H_8O_2N_4S_2$	37.5	3.1	21.8	38.1	2.9	21.0
1-Sulfanilyl-3-methyl-5-pyrazolone	166–167	45.9	1.2	Sl. active	*i*	$C_{10}H_{11}O_3N_3S$	47.4	4.4	16.6	47.5	4.4	16.6
4-Sulfanilamido-1-phenyl-2,3-dimethyl-5-pyrazolone	260–261[f]	15.6	2.5	Inactive	*j*	$C_{17}H_{18}O_3N_4S$	57.0	5.0	15.6	57.5	5.1	16.1
5-*p*-Nitrobenzenesulfonamidotetrazole	185–186[f]	0.8	4.5	Inactive	*k*	$C_7H_6O_4N_6S$	31.1	2.2	31.1	31.2	2.3	30.6
Sulfanilylguanidine	189–190[f]	190	4.7	Sl. active	*l*	$C_7H_{10}O_2N_4S$	39.3	4.7	26.2	39.2	4.6	25.6
2-Sulfanilamidopyrimidine	255–256[f]	12.3	26	Active	*m*	$C_{10}H_{10}O_2N_4S$	48.0	4.0	22.4	48.1	4.0	21.7
2-N[4]-Acetylsulfanilamidopyrimidine	258–259	15.0			*m*	$C_{12}H_{12}O_3N_4S$	49.4	4.1	19.2	49.2	4.1	19.2
2-Sulfanilamido-4-methylpyrimidine	235–236[f]	31.8	30	Active	*n*	$C_{11}H_{12}O_2N_4S$	50.0	4.6	21.2	49.6	4.4	21.1
2-N[4]-Acetylsulfanilamido-4-methylpyrimidine	248–249	28.0			*n*	$C_{13}H_{14}O_3N_4S$	51.0	4.6	18.3	51.0	4.8	17.9
4-Sulfanilamidopyrimidine	231–232[f]	354	18	Inactive	*o*	$C_{10}H_{10}O_2N_4S$	48.0	4.0	22.4	48.0	3.9	22.4
5-Sulfanilamidouracil	277–279[f]	48.6	1.8	Inactive	*g*	$C_{10}H_{10}O_4N_4S$	42.6	3.5	19.9	42.8	3.4	20.2

[a] Fosbinder and Walter, ref. 2. [b] Lott and Bergeim, ref. 3. [c] Mg./100 cc. [d] White mice; dosage 0.5 g./kg. body weight. [e] Against experimental streptococcal or pneumococcal infections or both in white mice. [f] With decomposition. [g] Eastman Kodak Company, Rochester, N. Y. [h] Freund and Meinecke, *Ber.*, **29**, 2514 (1896). [i] Knorr, *ibid.*, **29**, 253 (1896). [j] Knorr and Stolz, *Ann.*, **293**, 58 (1896); U. S. Patent 1,877,166. [k] Thiele, *Ann.*, **270**, 55 (1892). [l] American Cyanamid Company, New York, N. Y [m] See Experimental. [n] Ref. 8. [o] See Ref. 5. [p] Microanalyses were carried out in these laboratories by Miss Margaret Humm and Miss Thelma Bills.

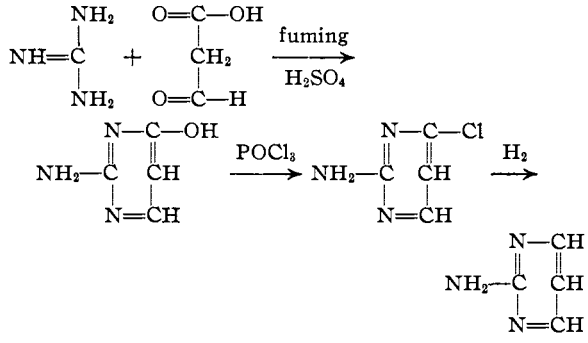
TABLE II

SOLUBILITY OF N[4]-ACETYLSULFANILAMIDO-HETEROCYCLES

Compound	Water solubility at 37°C.[d]
2-N[4]-Acetylsulfanilamidopyridine[a]	21.0
2-N[4]-Acetylsulfanilamidothiazole[b,c]	7.1
2-N[4]-Acetylsulfanilamido-4-methylthiazole[b]	5.5
2-N[4]-Acetylsulfanilamidopyrimidine	15.0
2-N[4]-Acetylsulfanilamido-4-methylpyrimidine	28.0

[a] Winterbottom, THIS JOURNAL, **62**, 160 (1940). [b] Ref. 2. [c] Ref. 3. [d] Mg./100 cc.

Gabriel and Colman.[7] The 2-amino-4-chloropyrimidine obtained in this manner was dehalogenated by catalytic reduction with palladium hydroxide on calcium carbonate. The following illustrates this synthesis of 2-aminopyrimidine



2-Amino-4-methylpyrimidine was prepared by Gabriel and Colman's procedure[8] except that catalytic reduction was again used to dehalogenate the intermediate 2-amino-4-methyl-6-chloropyrimidine.

The sulfanilamido derivatives were prepared by the usual method, employing either acetylsulfanilyl chloride or *p*-nitrobenzenesulfonyl chloride. In several cases temperatures lower than those previously employed were found to be essential in order to obtain good yields of the desired products.

Reduction of 5-*p*-nitrobenzenesulfonamidotetrazole led to a splitting of the tetrazole ring with the formation of sulfanilylguanidine. This result was confirmed by the synthesis of sulfanilylguanidine from guanidine itself. The reduction of the tetrazole ring occurred no matter what reducing agent was employed. Among the methods of reduction studied in this connection were iron in alcoholic solution with a small amount of hydrochloric or acetic acid; stannous chloride and dry hydrogen chloride in glacial acetic acid; catalytic reduction with palladium; ammonium sulfide and hydrogen sulfide in pyridine. In the last case mentioned, only the tetrazole ring appeared to be affected, the nitro group remaining intact. This would seem to indicate that the tetrazole ring is generally more susceptible to reduction than the nitro group. Attempts to prepare 5-sulfanilamidotetrazole through acetylsulfanilyl chloride were unsuccessful, the intermediate acetyl derivative being unstable toward hydrolysis.

The water solubilities[9] at 37° have been deter-

(7) Gabriel and Colman, *Ber.*, **36**, 3382 (1903).

(8) *Ibid.*, **32**, 2925 (1899).

(9) Solubility determinations were carried out by Mr. H. E. Faith in these Laboratories.

mined for all of the compounds studied. The method employed was similar to that described in the preceding paper.[10] The low water solubility of the diphenyl and benzothiazole derivatives may well account for their lack of chemotherapeutic activity. This hypothesis is supported by the low blood levels obtained with these two compounds, and by the fact that the more soluble monophenyl derivative has been reported[11] to be active. The solubilities of the acetyl derivatives of sulfapyridine, sulfathiazole and sulfamethylthiazole as well as the sulfadiazines have been determined (Table II) because of the kidney damage described by several investigators[12] as being due to the acetylated forms of the former substances. We do not believe that any conclusions regarding kidney damage can be drawn from these data, since crystalline form, rate of acetylation and solubility in the body fluids are obviously other equally important factors. Nevertheless it is interesting to compare the water solubility of the conjugated forms with the solubility of the corresponding free amino derivatives (Table I). This relationship for sulfadiazine and its methyl derivative would appear to be particularly favorable.

We believe that blood level data on new compounds should be reported wherever possible in conjunction with chemotherapeutic activity. Not only do they serve as a basis for any correlation with structure, but if the Marshall[13] method, which depends on the presence of a free amino group, is employed, these data will indicate whether or not any change in the original compound has occurred *in vivo* which might result in the formation of a free amino group. In general such changes may be expected when the original substituent is an acylamino, nitro, azo, hydroxylamino, alkyl, aralkylamino or Schiff base group.

It is interesting to note that by the usual Marshall method an equally high blood level was found with the very slightly water-soluble 5-*p*-nitrobenzenesulfonamidotetrazole as with sulfanilylguanidine, which was quite water-soluble. This suggests that in the animal body only the nitro group was reduced, since a maximum blood level with 5-*p*-nitrobenzenesulfonamidotetrazole was attained at about the eighth hour, while sulfanilylguanidine was practically entirely eliminated in the same interval. In spite of this, only sulfanilylguanidine showed any chemotherapeutic activity.

The term "active" for chemotherapeutic activity as recorded in Table I indicates a chemotherapeutic activity similar to or greater than sulfanilamide or sulfapyridine against streptococcal or pneumococcal infections or both in white mice. "Slightly active" may or may not be significant. The thiadiazole derivative, while showing activity against pneumococcic infections, was practically inactive against streptococci. The low degree of chemotherapeutic activity of the sulfanilamidopyrazolones studied may have been due to the poor absorption of these compounds.

The pyrimidines present an interesting comparison, since in this limited series only the 2-isomers appeared to be active against the infections studied. The high blood levels obtained with most of the sulfanilamidopyrimidines would seem to indicate very efficient absorption by the animal body. This effect may be attributed to a greater compatibility of these substances with the body fluids. The sodium salts of sulfadiazine and sulfamethyldiazine were prepared, and 10% aqueous solutions of these substances were found to have a *p*H of 9.6–9.7. A number of other sulfanilamidopyrimidines have been synthesized and will be reported later.

In the preliminary mouse tests to date, the results have shown sulfadiazine and sulfamethyldiazine to be considerably more active against experimental streptococcal, pneumococcal and staphylococcal infections than sulfanilamide, sulfapyridine or sulfathiazole. While the greater activity may be due primarily to higher blood levels, it is hoped that the work now in progress will show sulfadiazine and sulfamethyldiazine, because of their higher activity, to be effective also against infectious agents which are not now susceptible to chemotherapy. In any event, it should be emphasized that much further work will be required before an accurate evaluation of these promising new chemotherapeutic agents can be given.

Experimental

2-Aminopyrimidine was prepared by the following simplified procedure: 1600 cc. of 20% fuming sulfuric acid was cooled and 350 g. (2.61 moles) of malic acid was added with good agitation at such a rate that the temperature did not rise above 0°. After all the malic acid had been stirred in, 300 g. (1.33 moles) of guanidine sulfate hemi-

(10) Roblin and Winnek, THIS JOURNAL, **62**, 1999 (1940).

(11) Lawrence, *Proc. Soc. Exptl. Biol. Med.*, **43**, 92 (1940).

(12) Marshall, *Science*, **89**, 12 (1939); Marshall and Litchfield, *J. Pharmacol.*, **67**, 454 (1939); Antopol and Robinson, *Proc. Soc. Exptl. Biol. Med.*, **42**, 410 (1939).

(13) Bratton and Marshall, *J. Pharmacol.*, **66**, 4 (1939).

hydrate was added with continuous stirring. The temperature during this period was not allowed to rise above 0°. The mixture, after the addition of all the guanidine sulfate, was permitted to warm spontaneously to room temperature. It was then heated cautiously on a steam-bath with vigorous stirring for about one hour.

The reaction mixture was then cooled and poured onto 5 kg. of ice. The resulting aqueous solution was neutralized with approximately 5.25 liters of 28% ammonium hydroxide. After seeding and stirring for three to four hours, the crystalline precipitate of isocytosine which formed was filtered off and dried at about 60°. The yield was 200 g. or 69%, m. p. 268° (decompn.). The product was purified by conversion to the sulfate, using 2.5 parts of 10% sulfuric acid.

672 grams (2.1 moles) of isocytosine sulfate (m. p. 279–280°, decompn.) was suspended in 1670 cc. of phosphorus oxychloride. The reaction mixture was heated slowly until it refluxed gently, and was maintained at slow reflux for three and one-half hours. About 500 cc. of the phosphorus oxychloride was then removed by vacuum distillation. Enough excess oxychloride was left in the reaction mixture to ensure mobility. The concentrated solution was then poured onto 4 kg. of ice and water with vigorous stirring. (It is advisable to add a few drops of octanol at this stage to prevent foaming.) Twenty grams of decolorizing carbon was added, the mixture was stirred until it warmed to 25°, and then filtered.

The filtrate was made slightly alkaline (*p*H 8) with 28% ammonium hydroxide below 30°. The colorless precipitate was removed by filtration and washed well with water. It was then suspended in a liter of water to obtain more thorough washing, refiltered and rewashed. The product was dried at 60° and weighed 388 g., a yield of 71%. The 2-amino-4-chloropyrimidine obtained in this fashion darkened with sintering at 165–166° (cor.) and was sufficiently pure for the next step.

The catalytic dehalogenation of 2-amino-4-chloropyrimidine was carried out, using palladium hydroxide on calcium carbonate as the catalyst, at a temperature of 50° and pressures of 3–4 atmospheres of hydrogen. Methanol or ethanol was used as a solvent, and barium oxide was employed as a base to take up the hydrogen chloride. When the reaction was complete, the methanol was removed by vacuum distillation and the 2-aminopyrimidine recrystallized from benzene.

The source of the other intermediate heterocyclic compounds is indicated in Table I.

Sulfanilamido heterocycles were prepared by the procedures described in the previous paper.[10] The analytical results are recorded in Table I. Three of these compounds, namely, 1-sulfanilyl-3-methyl-5-pyrazolone, sulfanilylguanidine and 4-sulfanilamidopyrimidine, in addition to 5-*p*-nitrobenzenesulfonamidotetrazole, were prepared from *p*-nitrobenzenesulfonyl chloride. The others were all prepared through acetylsulfanilyl chloride. In many cases the intermediate nitro or acetylamino compounds were not isolated in pure form, but were converted into the final products by alkaline hydrolysis or iron reduction in alcohol solution as required.

When 2-aminopyrimidine and its methyl derivative were treated with acetylsulfanilyl chloride, temperatures not exceeding 60° were found to be more satisfactory. Higher temperatures favored the formation of colored by-products and complicated the purification of the final products.

Sodium salts of 2-sulfanilamidopyrimidine and 2-sulfanilamido-4-methylpyrimidine were prepared by dissolving 0.1 mole of the respective compounds in 12.5 cc. of water containing 0.1 mole of sodium hydroxide. To the resulting slurry was added 100 cc. of absolute alcohol, and the mixture was heated on the steam-bath for twenty minutes. After cooling, the sodium salt was removed by filtration, suspended in absolute alcohol, refiltered and washed with alcohol.

ANALYSIS OF SODIUM SALTS

Compound	Nitrogen, % Calcd.	Found	
Sodium-2-sulfanilamidopyrimidine	20.6	20.5	20.8
Sodium-2-sulfanilamido-4-methylpyrimidine	19.6	19.5	19.6

Summary

The preparation and properties of a number of sulfanilamido heterocycles, and a simplified synthesis of 2-aminopyrimidine from guanidine sulfate is described.

The water solubility and blood concentration, at a standard dosage, of all these compounds is reported. In several cases the water solubility of the acetylated forms also has been determined.

Two new heterocyclic derivatives of sulfanilamide, 2-sulfanilamidopyrimidine and 2-sulfanilamido-4-methylpyrimidine, which, in preliminary mouse tests, have shown greater chemotherapeutic activity than sulfapyridine or sulfathiazole, are reported.

In order to avoid possible confusion between sulfapyridine and sulfapyrimidine, the term sulfadiazines is suggested for these compounds.

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