obtained from the hydrochloride by treating an aqueous solution with dilute sodium hydroxide. It formed elongated prisms from absolute methanol and melted at 81–82°, the melting point given by Straus and Rohrbacher for the free base. *Anal.* Calcd. for $C_{22}H_{25}O_2N$: N, 4.18. Found: N, 4.10, 4.11.

Phenyl Urethans.—These were prepared by refluxing equimolecular quantities of the amino alcohol and phenyl isocyanate in dry benzene. They were crystallized from 95% ethyl alcohol. The hydrochlorides were purified by crystallization from ether–ethyl alcohol or ether–acetone.

1 - Diethylamino - 2 - hydroxy - 1,2,3,4 - tetrahydronaphthalene phenyl urethan was obtained in 90% yield; m. p. 104–104.5°. *Anal.* Calcd. for $C_{21}H_{26}O_2N_2$: N, 8.28. Found: N, 8.31, 8.27.

Hydrochloride, m. p. 206–206.5°. *Anal.* Calcd. for $C_{21}H_{27}O_2N_2Cl$: Cl, 9.48. Found: Cl, 9.39, 9.41.

1 - Piperidino - 2 - hydroxy - 1,2,3,4 - tetrahydronaphthalene phenyl urethan was obtained in 90% yield; m. p. 145–146°. *Anal.* Calcd. for $C_{22}H_{26}O_2N_2$: N, 8.00. Found: N, 7.89, 7.93.

Hydrochloride, m. p. 203–204°. *Anal.* Calcd. for $C_{22}H_{27}O_2N_2Cl$: Cl, 9.18. Found: Cl, 9.07, 9.10.

1 - Piperidino - 2 - hydroxy - 1,2,3,4 - tetrahydronaphthalene - *p* - nitrobenzoate Hydrochloride.—Equimolecular quantities of the amino alcohol and *p*-nitrobenzoyl chloride were heated together on a steam-bath for two hours after which dry benzene was added and refluxed for six hours. After removal of the benzene the solid was crystallized from ethyl acetate, giving small brownish plates, m. p. 238.5–239.5° (mixed m. p. with *p*-nitrobenzoic acid, 160–170°). *Anal.* Calcd. for $C_{22}H_{25}O_4N_2Cl$: N, 6.72. Found: N, 6.56, 6.60.

Summary

Several esters of 1-dialkylamino-2-hydroxy-1,2,3,4-tetrahydronaphthalenes have been prepared. These compounds possess local anesthetic properties.

NEW HAVEN, CONN. RECEIVED MAY 24, 1940

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

Chemotherapy. I. Substituted Sulfanilamidopyridines[1]

BY RICHARD O. ROBLIN, JR., AND PHILIP S. WINNEK

The successful application of sulfapyridine[2] as a chemotherapeutic agent for the treatment of pneumococcal and other bacterial infections has led us to the preparation of a number of substituted sulfanilamidopyridines.[3] The properties of these compounds are summarized in Table I. Several interesting and unexpected effects on chemotherapeutic activity were observed when the substituents on the pyridine ring were varied in this series of closely related sulfanilamide derivatives. Since the inception of this work, two of the compounds, namely, 3-sulfanilamidopyridine and 5-sulfanilamido-2-aminopyridine, have been reported by Winterbottom.[4] However, they are included along with sulfapyridine for purposes of comparison, and because their chemotherapeutic activity has not been reported previously.

It was felt that the solubilities[5] should be investigated in order to furnish a comparison with the blood level data. Accordingly, the water solubility of all the compounds was determined at 37°. These data were obtained by heating and stirring an excess of the compound in question with water on a steam-bath for one-half hour. The suspension was then agitated in a thermostat for twenty-four hours at 37°. A sample of the saturated solution was withdrawn through a sintered glass filter into a bottle held at the same temperature. An aliquot of the saturated solution was then diluted and analyzed by means of the Marshall[6] method. A General Electric recording spectrophotometer was used in comparing the colors developed with those of the standards. While separate standards for each compound were prepared, it was soon found that by taking into account the differences in molecular weights, any one compound could serve as a standard for all.

Blood level studies[7] were carried out on practically all of the compounds. We believe that this information is fundamental to any attempt to correlate chemotherapeutic activity with molecular structure. It is obviously impossible to declare a compound inactive on any theoretical

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

(2) Whitby, *Lancet*, **1**, 1210 (1938); Evans and Gaisford, *ibid.*, **2**, 14 (1938); Long and Bliss, "The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds," The Macmillan Company, N. Y., 1939, p. 228–239.

(3) Nomenclature according to Crossley, Northey and Hultquist, THIS JOURNAL, **60**, 2217 (1938).

(4) Winterbottom, *ibid.*, **62**, 160 (1940).

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

(6) Bratton and Marshall, *J. Pharmacol.*, **66**, 4 (1939). The preliminary trichloroacetic acid treatment employed in this procedure was eliminated, since only aqueous solutions were being studied.

(7) The pharmacological and bacteriological investigations were made in these Laboratories under the direction of Dr. W. H. Feinstone.

TABLE I

PROPERTIES OF SUBSTITUTED SULFANILAMIDOPYRIDINES

Pyridine derivative	M. p., °C. (cor.)	Water[d] sol., 37°	Max. blood[d] level[e]	Chemo-therapeutic activity[f]	Ref. to intermed.	Formula	Analyses,[q] % Calcd. C	Calcd. H	Found C	Found H
2-S[a,b,c]	190–191	49.5	14.3	Active						
3-S[c]	258–259 dec.	3.3	6.7	Active						
5-S-2-chloro	186–187	18.0	8.0	Active	*h*	$C_{11}H_{10}O_2N_3SCl$	46.6	3.5	46.6	3.4
5-S-2-bromo	196–197	12.2	3.8	Active	*i*	$C_{11}H_{10}O_2N_3SBr$	40.2	3.0	40.4	3.1
2-S-5-bromo	199–200	3.8	7.4	Inactive	*j*	$C_{11}H_{10}O_2N_3SBr$	40.2	3.0	40.2	3.0
2-S-5-iodo	220–221	1.3	1.7	Inactive	*k*	$C_{11}H_{10}O_2N_3SI$	35.2	2.7	35.2	2.5
2-S-5-nitro	220–221	3.7	14.3	Active	*l*	$C_{11}H_{10}O_4N_4S$	44.9	3.4	45.0	3.2
2-S-5-amino	157–158	418	14.3	Active	*m*	$C_{11}H_{12}O_2N_4S$	50.0	4.5	49.8	4.5
5-S-2-amino[c]	207–208	129	12.5	Inactive						
2,5-Di-S	215–216	49.5	..	Sl. active	*n*	$C_{17}H_{17}O_4N_5S_2$	48.7	4.1	48.8	4.1
5-S-2-hydroxy	243–244 dec.	258	2.6	Inactive	*o*	$C_{11}H_{11}O_3N_3S$	49.8	4.2	49.8	4.2
5-S-2-ethoxy	207–208	3.6	4.8	Inactive	*i*	$C_{13}H_{15}O_3N_3S$	53.2	5.1	53.2	5.1
2-S-3-ethoxy[g]	198–200	23.5	3.9	Sl. active	*p*	$C_{13}H_{15}O_3N_3S$	53.2	5.1	53.0	5.0

[a] S = Sulfanilamido. [b] Whitby, Ref. 2. [c] Winterbottom, Ref. 4. [d] Mg./100 cc. [e] White mice; dosage 0.5 g./kg. body weight. [f] Against experimental streptococcal or pneumococcal infections or both in white mice. [g] This compound was originally considered to be 2-sulfanilamido-5-ethoxypyridine, but our attention was recently drawn to the fact that the proof of structure given in the literature (ref. *p*) for 2-amino-5-ethoxypyridine is probably incorrect; *cf.* v. Schickh, Binz and Schulz, *Ber.*, **69B**, 2593 (1936), and Plazek and Rodewald, *Roczniki Chem.*, **16**, 502 (1936). [h] Pieroni and Haupt, *Atti accad. Lincei*, (6) **2**, 127 (1925). [i] Binz and v. Schickh, *Ber.*, **68B**, 320 (1935). [j] Tschitschibabin and Tjashelowa, *J. Russ. Phys.-Chem. Soc.*, **50**, 483 (1918). [k] v. Schickh, German Patent 473,213. [l] Tschitschibabin, *J. Russ. Phys.-Chem. Soc.*, **46**, 1236 (1914). [m] Prepared by iron reduction of the corresponding nitro compound. [n] Räth and Prange, *Ann.*, **467**, 1 (1928). [o] Maier-Bode and Altpeter, "Das Pyridin und Seine Derivate in Wissenschaft und Technik," Verlag Wilhelm Knapp, Halle, 1934, p. 156. [p] Koenigs, Gerdes and Sirot, *Ber.*, **61B**, 1025 (1928). [q] Microanalyses were carried out in these Laboratories by Miss Margaret Humm and Miss Thelma Bills.

basis unless it is known to be present in the blood stream in sufficient concentration and for sufficient time so that any potential activity may become apparent. Maximum blood level is only one factor, but it may be stated that all blood levels were sufficiently well maintained to demonstrate any inherent chemotherapeutic activity.

It is interesting to compare the solubilities at 37° with the maximum blood levels. Only in the case of the most insoluble compounds did the blood levels exceed the solubilities and then the difference was only about twofold. An exception to this was 2-sulfanilamido-5-nitropyridine. However, it seemed likely in view of many other similar cases that the nitro group was reduced in the animal body to an amino group. This was borne out by the identical maximum blood levels of this compound and the corresponding amino derivative. Furthermore, both compounds showed about the same degree of chemotherapeutic activity, although the nitro derivative seemed to be slightly more active, perhaps due to the fact that the blood level was maintained for a somewhat longer period.

All the compounds were tested against both streptococcal and pneumococcal infections in white mice. By this method none of the compounds with the exception of 3-sulfanilamidopyridine appeared to be as active against pneumococcal infections as sulfapyridine, although two, namely, 5-sulfanilamido-2-chloropyridine and 2-sulfanilamido-5-nitropyridine, seemed to be somewhat more active than either sulfanilamide or sulfapyridine against streptococcic infections. The term "active" merely indicates chemotherapeutic activity about equivalent to sulfanilamide or sulfapyridine against either streptococci or pneumococci or both. "Slightly active" may or may not be significant.

Taking into account the fact that both isomeric unsubstituted sulfanilamidopyridines show about the same degree of chemotherapeutic activity, it is surprising to find that when the same group is substituted in the same relative position, only one of the resulting isomers is active. This is particularly striking in the case of the two bromo-substituted and the two amino-substituted sulfanilamidopyridines, where a direct comparison can be made. It will be noted that the same isomer was not always the active one. 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. The results were consistent in that where

one halogen-substituted sulfanilamidopyridine was active, substitution of a different halogen retained the activity while all the isomeric halogen substituted sulfapyridines studied were inactive.

As illustrated in Table I, these differences cannot be explained by inadequate blood levels. In the case of the bromo-substituted derivatives, for example, the maximum blood level was higher for the inactive compound and both were equally well maintained. With the amino-substituted sulfapyridines both blood levels were ample for the demonstration of any chemotherapeutic activity.

Thus it is indicated that the differences in chemotherapeutic activity in this series of substituted sulfapyridines cannot be attributed to differences in the establishment and maintenance of adequate blood levels, but must be due to some inherent difference in the compounds themselves. The question remains as to why the introduction of a substituent in the pyridine ring of two equally active sulfanilamidopyridines should lead to one active and one inactive isomer. The orientation in the benzene ring has not been altered, and yet the introduction of a substituent in the same relative position in the pyridine ring has wrought some subtle change in the properties of one of the isomers, leaving the other unaltered in this respect. Whatever the nature of this change may be, it has a profound effect on the therapeutic properties of the resulting substituted sulfanilamidopyridines. Since these compounds are so closely related structurally, it is felt that a further study of the effect of substitution in the pyridine ring may hold the key to a clearer understanding of the molecular relationships involved in chemotherapeutic activity.

Experimental

Intermediate Substituted Aminopyridines.—References to the methods for the preparation of the substituted aminopyridines are given in the footnotes of Table I. Where the amino compounds were derived by reduction of the corresponding nitro compounds, more satisfactory results usually were obtained by catalytic reduction with platinum black. Temperatures of 25–30°, pressures of 3 to 4 atmospheres and platinum black as a catalyst were employed in the catalytic reduction of these nitropyridines. In several instances, notably 2-amino-3-ethoxypyridine which decomposed completely on standing for eighteen hours, no attempt was made to isolate the substituted aminopyridines because of their strong tendency to oxidize. In these cases the solvent was removed by vacuum evaporation under hydrogen, and the product redissolved immediately in dry pyridine.

Substituted Sulfanilamidopyridines.—The sulfanilamido derivatives were prepared from the aminopyridines in dry pyridine either with acetylsulfanilyl chloride followed by hydrolysis, or with *p*-nitrobenzenesulfonyl chloride followed by reduction. Dilute aqueous alkali (about 10%) was used in most cases for the hydrolysis. However, with the 2-sulfanilamido-5-bromo- and 5-iodopyridines it was necessary to use alcoholic potassium hydroxide to effect hydrolysis, probably because of their low water solubilities. The 2-chloro, 2-bromo, and 2-hydroxy and the two ethoxy sulfanilamidopyridines were prepared from *p*-nitrobenzenesulfonyl chloride. Acetylsulfanilyl chloride was used in the preparation of the other substituted sulfanilamidopyridines. In most cases the intermediate acetyl or nitro derivative was not isolated in a pure state but was hydrolyzed or reduced before final purification was carried out.

One example will serve to illustrate the method used to prepare the sulfanilamido derivatives of the substituted aminopyridines. 2-Sulfanilamido-5-nitropyridine was prepared as follows: 68 g. (0.49 mole) of 2-amino-5-nitropyridine was suspended in 125 cc. of dry pyridine; 119 g. (0.51 mole) of acetylsulfanilyl chloride was added gradually, with stirring, at such a rate that the temperature did not exceed 55°. The reaction mixture was then heated on a steam-bath for one hour. A solution of 22 g. (0.55 mole) of sodium hydroxide in 110 cc. of water was added slowly, and the heating was continued for a short time. The pyridine was removed by distillation under reduced pressure, water being added from time to time to maintain the volume. Crude 2-N^4-acetylsulfanilamido-5-nitropyridine separated as a yellow-brown solid. It was collected by filtration and crystallized from glacial acetic acid, yield, 66 g. (40%) of purified product. Both higher and lower yields were obtained in other cases depending on the character of the substituted aminopyridine employed.

Forty-five grams (0.134 mole) of 2-N^4-acetylsulfanilamido-5-nitropyridine was dissolved in 135 cc. of water containing 13.5 g. (0.34 mole) of sodium hydroxide. The solution was boiled under reflux for one hour. It was then cooled, filtered, diluted with 50 cc. of water and neutralized cold with dilute hydrochloric acid. The yellow precipitate was removed by filtration and washed with water. The crude 2-sulfanilamido-5-nitropyridine was purified by recrystallization from 60% acetic acid after treatment with decolorizing charcoal, yield, 39 g. before purification.

When *p*-nitrobenzenesulfonyl chloride was used, the procedure was much the same. In some cases the nitro or acetyl derivatives were isolated by the alternative method of diluting the reaction mixture with 5–10 volumes of water rather than by distilling off the pyridine.

Reduction of the *p*-nitrobenzenesulfonamidopyridines to the corresponding sulfanilamido derivatives was accomplished as follows: 0.1 mole of the *p*-nitrobenzenesulfonamidopyridine was added gradually to a hot suspension of 100 g. of iron dust in about 300 cc. of 95% ethanol containing 3 cc. of (1:1) hydrochloric acid. The reaction mixture was agitated and heated on a steam-bath for six to eight hours. It was then neutralized with dilute sodium hydroxide, filtered hot and, after cooling, the filtrate was diluted with 4–5 volumes of water. The crude product which separated was recrystallized from alcohol–water mixtures,

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			
	Nitro compound	Amino compound	Hydroxylamino compound	Found
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).