TABLE IV

HYDROLYSIS OF PIPERIDYLALKANOL ESTERS

Compound	Substrate	Non-enzymatic hydrolysis	Enzymatic hydrolysis: Horse serum	Enzymatic hydrolysis: Rabbit serum (I)	Enzymatic hydrolysis: Rabbit serum (II)
34	β-(2-Piperidyl)-ethyl benzoate·HCl	13	0	120	29
35	β-(2-Piperidyl)-ethyl *p*-aminobenzoate·HCl[a]	0	0	0	0
36	β-(2-Piperidyl)-ethyl *o*-aminobenzoate·HCl	3	0	0	0
37	β-(2-Piperidyl)-ethyl *p*-ethoxybenzoate·HCl	10	0	20	15
38	β-(2-Piperidyl)-ethyl cinnamate·HCl[a]	17	0	12	9
39	Metycaine	10	0	22	20
40	β-(N-Piperidyl)-ethyl *m*-thiobenzoate·HCl[a]	4	0	460	18
41	2-(N-Methylpiperidyl)-methyl acetate methiodide	52	352	52	50
42	Atropine sulfate	4	0	50	0
43	Vitamin B_6 triacetate	170	96	182	

[a] Insoluble, suspension used.

Further confirmation of the rule, that the presence of certain nitrogen groups in the acid component of an ester prevents hydrolytic enzyme action, was obtained.

Evidence was presented for the possible existence of an enzyme or enzymes distinct from any of the known azolesterases, with the exception perhaps of cocainesterase, that can hydrolyze certain esters of β-diethylaminoethanol and related compounds, as well as piperidylalkanol esters.

NEWARK, N. J. RECEIVED SEPTEMBER 19, 1941

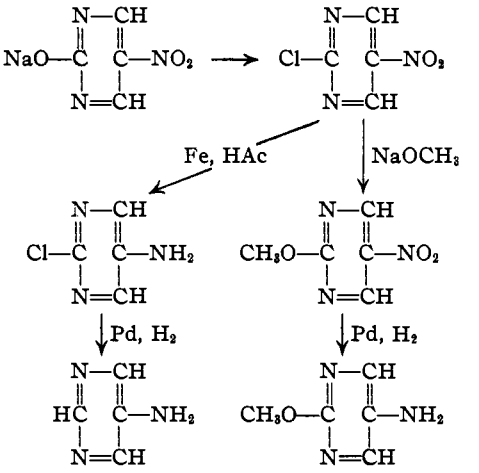
[CONTRIBUTION FROM THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. IV. Sulfanilamidopyrimidines[1]

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

In a previous paper[2] of this series, sulfadiazine and several other sulfanilamidopyrimidines were described. The successful application of sulfadiazine as a chemotherapeutic agent[3] made it seem desirable to carry out a more extensive investigation of the various possible pyrimidine derivatives. The present report describes the preparation and properties of 5-sulfanilamidopyrimidine and a number of alkyl, alkoxy, amino and halogen substituted compounds. The results are recorded in Table I. For comparison the derivatives of this series which have been reported previously are included in the table.

Since 5-aminopyrimidine and several of the other aminopyrimidines required were unknown, it was first necessary to devise a synthesis of these intermediates. The sodium salt of 2-hydroxy-5-nitropyrimidine[4] provided the starting point in the synthesis of 5-aminopyrimidine and several derivatives as outlined below.



An attempt to prepare 5-aminopyrimidine more directly by the condensation of sodium nitromalondialdehyde and formamidine followed by reduction was unsuccessful. No 5-nitropyrimidine could be isolated from the condensation reaction. A one step catalytic reduction of 2-

(1) Presented in part before the Divisions of Medicinal and Organic Chemistry, Atlantic City meeting of the American Chemical Society, September 10, 1941.

(2) Roblin, Williams, Winnek and English, THIS JOURNAL, **62**, 2002 (1940).

(3) Feinstone, Williams, Wolff, Huntington and Crossley, *Bull. Johns Hopkins Hospital*, **63**, 427 (1940); Plummer and Ensworth, *Proc. Soc. Exptl. Biol. Med.*, **45**, 734 (1940); Long, *Can. Med. Assoc. J.*, **44**, 217 (1941); Flippin, Rose, Schwartz, Dorn and Doak, *Am. J. Med. Sci.*, **201**, 585 (1941); Finland, Strauss and Peterson, *J. Am. Med. Assoc.*, **116**, 2641 (1941).

(4) Hale and Brill, THIS JOURNAL, **34**, 82 (1912).

TABLE I

PROPERTIES OF SULFANILAMIDOPYRIMIDINES

Pyrimidine derivative	M. p., °C. (cor.)	Water[d] soly. 37°	Max.[d] blood[e] level	Chemo-[e] therapeutic activity[f]	Ref. to interm.	Formula	Analyses,[g] % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
2-S[a,b]	255–256	12.3	26	Active								
2-S-4-Methyl[b]	235–236	31.8	30	Active								
2-S-4-Methoxy	241–242	18.2	9.4	Active	*j*	$C_{11}H_{12}O_3N_4S$	47.1	4.3	20.0	47.2	4.4	19.9
2-S-4-Ethoxy	255–256	5.3	6.5	Sl. active	*k*	$C_{12}H_{14}O_3N_4S$	49.0	4.8	19.0	48.6	4.7	19.4
2-S-4,6-Dimethyl[c]	198–199[h]	75[i]	31	Active	*l*	$C_{12}H_{14}O_2N_4S$	51.8	5.0	20.1	52.0	5.1	20.0
2-S-5-Chloro	246–247	1.8	27	Sl. active	*k*	$C_{10}H_9O_2N_4SCl$	42.2	3.2	19.7	42.2	3.3	19.6
4-S[b]	231–232	354	18	Inactive								
4-S-2-Methyl	207–208	623	30	Sl. active	*m*	$C_{11}H_{12}O_2N_4S$	50.0	4.6	21.2	50.2	4.6	21.4
5-S	260–261	9.8	11	Active	*k*	$C_{10}H_{10}O_2N_4S$	48.0	4.0	22.4	48.1	4.2	22.5
5-S-2-Methoxy	232–234	9.2	7.6	Active	*k*	$C_{11}H_{12}O_3N_4S$	47.1	4.3	20.0	47.3	4.0	20.1
5-S-2-Chloro	206–207	32.1	53	Sl. active	*k*	$C_{10}H_9O_2N_4SCl$	42.2	3.2	19.7	42.3	3.2	19.8
5-S-2-Amino	293–298	8.3	1.7	Sl. active	*k*	$C_{10}H_{11}O_2N_5S$	45.3	4.1	26.4	45.3	4.0	26.2
5-S-Uracil[b]	277–279	48.6	1.8	Inactive								
2,5-DiS	231–232	2.2	0.5	Inactive	*k*	$C_{16}H_{16}O_4N_6S_2$	45.6	3.8	20.1	45.4	3.9	20.1

[a] S = sulfanilamido. [b] Roblin, Williams, Winnek and English, Ref. 2. [c] Caldwell, Kornfeld and Donnell, THIS JOURNAL, **63**, 2189 (1941), report m. p. 178–180° (cor.), solubility 150 mg./100 cc., and acetyl derivative m. p. 246.8–247.4° (cor.). [d] Mg./100 cc. Water solubility determinations were made by Mr. H. E. Faith in these Laboratories. [e] White mice, dosage 0.5 g./kg. body weight. [f] Against experimental streptococcal or pneumococcal infections or both. [g] Microanalyses were carried out in these Laboratories under the direction of Mrs. Thelma Kirk. [h] Acetyl derivative, m. p. 249–250° (cor.). [i] Compound tends to remain supersaturated indefinitely in the presence of excess solid. Value represents approximate equilibrium when approached from low side. [j] Hilbert and Johnson, THIS JOURNAL, **52**, 1152 (1930). [k] See Experimental. [l] Combes and Combes, *Bull. soc. chim.*, (3) **7**, 791 (1900). [m] Gabriel, *Ber.*, **37**, 3641 (1904).

chloro-5-nitropyrimidine likewise did not yield the desired 5-amino derivative. However, the above syntheses gave satisfactory results and completed the preparation of all possible unsubstituted monoaminopyrimidines. The methods employed for the preparation of the corresponding sulfanilamido derivatives have been described previously.[5]

Many of the sulfanilamidopyrimidines showed some degree of activity,[6] but only the 4,6-dimethyl derivative was as effective as sulfadiazine and its monomethyl analog against experimental streptococcal and pneumococcal infections. Because of the unfavorable influence of a methyl group on toxicity as evidenced by sulfamethylthiazole, an intensive investigation of the toxic properties of the dimethyl compound will be required before any evaluation of its potential utility can be given.

In the previous results,[2] 4-sulfanilamidopyrimidine was described as inactive in experimental infections. Since it seemed somewhat unlikely that there would be such a marked distinction between the 2- and the 4-isomers, this result was reexamined. It was found that while the 4-isomer was inactive *in vivo*, it showed as great a bacteriostatic effect *in vitro* as sulfadiazine. Thus the difference between these two closely related derivatives appears to be due to some unknown factor operating in the animal body. One possibility might be a hydrolytic breakdown of 4-sulfanilamidopyrimidine *in vivo*. This suggestion is strengthened by the fact that it has not been possible to prepare this compound by hydrolysis of the acetyl derivative. The hydrolysis in this case probably results in a rupture of the sulfonamide linkage with the formation of sulfanilic acid, which has very little chemotherapeutic activity.

It is possible that other discrepancies between *in vitro* and *in vivo* results might be explained by the formation of less active substances in the body, although other factors may be of equal or greater importance. In any case, the results obtained with 4-sulfanilamidopyrimidine illustrate the pitfalls involved in the use of *in vivo* data as a basis for attempts to correlate chemical structure with chemotherapeutic activity.

5-Sulfanilamidopyrimidine, while it was active, did not show the same high degree of absorbability and therapeutic effectiveness as sulfadiazine. The 2-amino derivative is reported as only slightly active. However, administration of its sodium salt subcutaneously produced a much better therapeutic effect by increasing the blood levels. 2-Sulfanilamido-4-methoxypyrimidine was less active than the corresponding 4-methyl compound.

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

(6) The bacteriological and pharmacological studies were carried out in these Laboratories under the direction of Dr. W. H. Feinstone.

TABLE II

SUBSTITUTED PYRIMIDINES

Pyrimidine	M. p., °C. (cor.)	Yield, %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
2-Acetylamino-5-nitro[a]	187–188[c]	83			30.8			31.0
2-Chloro-5-nitro	110–111	65			26.3			25.9[d]
2-Chloro-5-amino	198–199[c]	58	37.1	3.1	32.4	37.6	3.4	32.6
5-Amino	170–171	62	50.5	5.3	44.2	50.1	5.4	44.5
2-Amino-5-chloro[b]	234–236	74	37.1	3.1	32.4	37.1	3.1	33.2
2-Amino-4-ethoxy	154–156	75	49.0	4.8	19.0	48.4	4.7	19.4

[a] Hale and Brill, ref. 4, reported 172°. [b] Prepared by Dr. M. E. Hultquist, m. p. in sealed tube. [c] With decomposition. [d] Chlorine, calcd. 22.3%; found 22.7%.

Here again the blood concentration was not as high and the analogous ethoxy derivative gave a still lower blood level.

It is interesting to note the effect of methyl groups on water solubility and absorption. The water solubility increases from sulfadiazine through its mono and dimethyl derivatives. There are corresponding slight increases in the blood concentrations. The same is true in the case of 4-sulfanilamidopyrimidine and the analogous monomethyl derivative. In general, however, there is very little correlation between water-solubility and maximum blood concentrations in this series of sulfanilamidopyrimidines. The least water-soluble derivative, 2-sulfanilamido-5-chloropyrimidine, gave one of the highest blood concentrations, while 5-sulfanilamidouracil was much less effectively absorbed than sulfadiazine and many other less water-soluble derivatives. Similarly, there appears to be very little correlation between chemical structure and *in vivo* activity. Nevertheless, the study of closely related series such as this appears to offer an approach to this problem, and eventually to a more rational basis for the synthesis of new chemotherapeutic agents.

Experimental

Substituted pyrimidines are listed in Table II, together with melting points and analyses. In addition three other new pyrimidines, 2-methoxy-5-amino, 2-acetylamino-5-amino and 2,5-diamino, which were isolated only as their sulfanilamido derivatives, were prepared.

The starting point in the synthesis of 5-aminopyrimidine and the substituted 5-amino derivatives was the sodium salt of 2-hydroxy-5-nitropyrimidine described by Hale and Brill.[4] These authors obtained this compound by the action of sodium hydroxide on 2-amino-5-nitropyrimidine, the product of the condensation of sodium nitromalondialdehyde and guanidine. They also prepared the same sodium salt by the direct condensation of urea and sodium nitromalondialdehyde. However, the yield by this method was reported as much lower than the over-all yield of the two-step process.

Several improvements in the preparations of Hale and Brill[4] were effected. For example, it was more satisfactory to purify 2-amino-5-nitropyrimidine by continuous extraction with benzene than by recrystallization from alcohol. The acetylation of this compound was found to proceed better in boiling acetic anhydride than in acetic anhydride with sodium acetate on a steam-bath. This was evidenced by the fourteen degree higher melting point for the present preparation of 2-acetylamino-5-nitropyrimidine, and by the fact that 2,5-disulfanilamidopyrimidine was obtained from the product of the catalytic reduction of this material when prepared according to the directions of Hale and Brill.

Pure 2-acetylamino-5-nitropyrimidine was reduced easily with palladium charcoal in methanol to give 2-acetylamino-5-aminopyrimidine. After evaporation of the solvent under reduced pressure in an atmosphere of nitrogen, the product was converted directly to the corresponding sulfanilamide derivative without further purification.

The sodium salt of 2-hydroxy-5-nitropyrimidine[4] was obtained in 55% yield by the action of dilute alcoholic sodium hydroxide on 2-amino-5-nitropyrimidine under an atmosphere of nitrogen. The heating period (three hours) and the temperature (70–75°) appeared to be critical. The product could be recrystallized from absolute ethanol. It was anhydrous when freshly prepared, but on standing in the air two moles of water were absorbed. From this sodium salt 2-chloro-5-nitropyrimidine was prepared according to the following procedure:

To 125 cc. (1.19 moles) of phosphorus oxychloride cooled in an ice-bath was added slowly, with stirring, 25 g. (0.153 mole) of the sodium salt of 2-hydroxy-5-nitropyrimidine. The resulting suspension was warmed slowly and finally refluxed gently for twenty minutes. Excess phosphorus oxychloride was then removed by vacuum distillation and the residue was hydrolyzed below 30° with 200 cc. of water. The yellow solid was filtered off and dried at 60°. (The wet material was very lachrymatory.) The filtrate was extracted with three 75-cc. portions of carbon tetrachloride which were then combined, dried over calcium sulfate and evaporated to dryness. The residue (1.7 g.) was combined with the yellow precipitate (18.6 g.). After recrystallization from heptane 15.9 g. of colorless crystals was obtained.

2-Methoxy-5-nitropyrimidine[4] was obtained by adding 16 g. (0.1 mole) of 2-chloro-5-nitropyrimidine gradually to 50 cc. of an absolute methanol solution containing 5.4 g. (0.1 mole) of sodium methylate. The reaction mixture was then refluxed for one and one-half hours. After filtering hot, the product (8.5 g.) separated from the filtrate as a light yellow crystalline material on cooling. 2-Meth-

oxy-5-aminopyrimidine was prepared from this nitro derivative by catalytic reduction in 200 cc. of methanol using palladium–charcoal and hydrogen pressures of 50 lb. The theoretical amount of hydrogen was taken up in three hours. After removal of the solvent, the product was used without further purification.

The 2-chloro-5-aminopyrimidine was prepared by the reduction of 2-chloro-5-nitropyrimidine. To 200 cc. of a 1.5% aqueous acetic acid solution was added 70 g. of iron dust. After the initial foaming had subsided, 20 g. of finely ground nitro derivative was added with vigorous stirring. The rate of addition was such that the temperature was maintained at approximately 80°. The mixture was then heated with stirring at 90° for three-quarters of an hour, 500 cc. of benzene was added to the mixture, and a water separator was placed under the reflux condenser. The flask was heated until all the water had been removed. After separation of the benzene from the iron residue by filtration, the residues were extracted with two 200-cc. portions of boiling benzene. The cooled benzene solutions were combined, the crystals filtered off, and the mother liquors evaporated to small volume to recover a second crop of crystals. In all 9.5 g. of 2-chloro-5-aminopyrimidine was obtained. It was purified further by recrystallization from water.

5-Aminopyrimidine resulted from the catalytic dehalogenation of 2-chloro-5-aminopyrimidine; 6 g. of the chloro compound was dissolved in 200 cc. of methanol, and 3.5 g. of barium oxide was added. 0.5 gram of palladium hydroxide on calcium carbonate was added and the mixture was shaken with hydrogen at 60 lb. pressure and 25°. The theoretical amount of hydrogen was taken up in two hours. After filtration, the filtrate was evaporated to dryness under reduced pressure. The dry residue was leached with two 200-cc. portions of boiling benzene. The benzene solution was cooled to precipitate the 5-aminopyrimidine (2.8 g.), which was purified by recrystallization from 280 cc. of benzene. It was soluble in water and alcohol, slightly soluble in cold benzene and insoluble in hot petroleum ether. A dilute aqueous solution was neutral.

The 2-amino-5-chloropyrimidine was obtained through the kindness of Dr. Martin E. Hultquist, Calco Chemical Division, American Cyanamid Company, Bound Brook, N. J. It was prepared by him by condensing chloromalondialdehyde[7] with guanidine carbonate in fuming sulfuric acid.

A solution of chloromalondialdehyde, obtained from 90 g. (0.5 mole) of tetrachloropropene-2 and 125 cc. of 95% sulfuric acid, according to the method of Prins,[7] was mixed with 200 cc. of 20% fuming sulfuric acid. 50 g. (0.28 mole) of guanidine carbonate was then added with cooling and stirring below 50°. The mixture was heated to 90–95° for one and one-half hours. After cooling to 20°, the solution was drowned in ice, ammonia was added up to the start of precipitation (still a *p*H of 2 or less), keeping the temperature below 20° by the addition of ice. Decolorizing carbon was added, and the solution was clarified. Ammonia was then added to precipitate the product. After further treatment with decolorizing carbon in acid solution and recrystallization from cellosolve, the 2-amino-5-chloropyrimidine was obtained as colorless plates melting at 234–236° in a sealed tube. The material sublimed so rapidly that an open tube could not be used.

2-Amino-4-ethoxypyrimidine was prepared by refluxing 10 g. of 2-amino-4-chloropyrimidine[8] with 2.4 g. of sodium dissolved in 130 cc. of absolute ethanol for three hours. The reaction mixture was then filtered hot and evaporated to dryness. The residue was dissolved in dilute hydrochloric acid, filtered, and the base partially precipitated by the addition of concentrated sodium hydroxide solution. This mixture was extracted with ethyl acetate. The resulting ethyl acetate solution was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The product obtained by this procedure was colorless and required no further purification.

Sulfanilamidopyrimidines, with the exception of the two chloro derivatives and 4-sulfanilamido-2-methylpyrimidine, were prepared by the reaction of acetylsulfanilyl chloride with the requisite aminopyrimidine in dry pyridine. This method has been described previously.[5] 2-Amino-5-chloropyrimidine failed to react with acetylsulfanilyl chloride under these conditions. Consequently, *p*-nitrobenzenesulfonyl chloride was used in this case as well as for 5-amino-2-chloropyrimidine. The general procedure has been described[5] in this case also, although higher temperatures (130–140°) for fifteen minutes were required to bring about a reaction with 2-amino-5-chloropyrimidine. Since the closely related 4-sulfanilamidopyrimidine was found to be unstable to hydrolysis, 4-sulfanilamido-2-methylpyrimidine was also prepared via *p*-nitrobenzenesulfonyl chloride.

Summary

The preparation and properties of a series of sulfanilamidopyrimidines related to sulfadiazine is reported.

5-Aminopyrimidine and several other substituted pyrimidines required as intermediates have been synthesized for the first time.

With the exception of 2-sulfanilamido-4,6-dimethylpyrimidine, none of the compounds in this series showed the same high degree of absorption and chemotherapeutic activity as sulfadiazine.

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(7) Prins, *J. prakt. Chem.*, **89**, 421 (1914); German Patent 261,689, *Friedländer*, **11**, 1207.

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