asparagine to give a methylene derivative. One might expect that the second mole of formaldehyde reacts with the amide group forming a methylol derivative. In support of this view it may be said that many methylol compounds of acid amides and formaldehyde have been reported in the literature, and in common with our second asparagine compound all are unstable and give off formaldehyde. Further work is in progress in this Laboratory to ascertain the formaldehyde binding of l(+)-aspartic and l(+)-glutamic acids, in an effort to elucidate the point of attachment of the second mole of formaldehyde.

In 6.64% aqueous solution $[\alpha]^{20}D$ is $-116.04°$ for the sodium salt of methylene-l(−)-asparagine and $-23.06°$ for the sodium salt of the methylene-l(−)-asparagine compound with one additional mole of formaldehyde, the latter concentration being 9.04% and dissolved in 14.4% formaldehyde solution. For sodium l(−)-asparaginate $[\alpha]^{20}D$ is $-7.28°$ for a 6.16% solution in water. Clough[7] records for the latter a value of $-7.53°$ for a 14.51% solution at 25° and Becker[8] a value of -7.42 for a 11.68% solution at 20°. Clough notes that the rotation becomes more negative as the temperature is raised and hence our value compares well with the older data.

Summary

1. The reaction between solutions of (l−)-asparagine containing an equivalent of sodium hydroxide and various amounts of formaldehyde was followed by polariscopic and hydrogenion measurements, and estimation of unreacted aldehyde.

2. l(−)-Asparagine reacts with formaldehyde, mole per mole, to form methylene-l(−)-asparagine. The latter reacts further with one additional mole of formaldehyde to form an unstable compound of unestablished constitution which readily loses aldehyde.

(7) G. W. Clough, *J. Chem. Soc.*, **107**, 1509 (1915).
(8) A. Becker, *Ber.*, **14**, 1028 (1881).

GENEVA, N. Y. RECEIVED AUGUST 17, 1942

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

Studies in Chemotherapy. VI. Sulfanilamido Heterocycles[1]

BY GEORGE W. ANDERSON, H. ELDRIDGE FAITH, HARRY W. MARSON, PHILIP S. WINNEK AND RICHARD O. ROBLIN, JR.

On the basis of our present knowledge, N^1-heterocyclic substituted sulfanilamide derivatives still appear to offer the greatest possibilities for therapeutically effective sulfanilamide type compounds. Continuing our investigations in this field,[2] we have prepared a number of new derivatives of this type. These compounds, together with pertinent data concerning them, are listed in Table I. Several of the amino heterocycles required as intermediates have not been reported previously. The syntheses and properties of these substances are described in the Experimental part.

Most of the sulfanilamide derivatives were prepared by the standard procedure. In a few cases, such as the conversion of 2-aminoöxazole to the corresponding sulfanilamido compound, it was necessary to use *p*-nitrobenzenesulfonyl chloride. Even this method was hardly satisfactory, since in this particular case, the over-all yield, including the synthesis of the amino heterocycle, was only about 0.2%. Numerous attempts to prepare an unsubstituted sulfanilamido triazine from 2-amino-1,3,5-triazine were unsuccessful. Under all of the conditions employed, this intermediate appeared to be unstable in the presence of sulfonyl chlorides. Both sulfanilamide and sulfaguanidine were isolated as final products of the various reactions.

Some of the sulfanilamido derivatives are of interest because of their chemical relationship to well-known sulfonamides. For example, 2-sulfanilamidoöxazole is the oxygen analog of sulfathiazole, and 3-sulfanilamidopyridazine is an isomer of sulfadiazine and sulfapyrazine. The imidazole derivative corresponds to sulfadiazine in the five-membered ring series. 4-Sulfanilamido-1,2,4-triazole represents a somewhat different type of heterocyclic derivative, in that the sulfanilamido group is joined to the ring through one of the hetero-atoms rather than through a carbon atom.

(1) Presented in part before the Division of Medicinal Chemistry, Buffalo meeting of the American Chemical Society, Sept. 9, 1942.
(2) Roblin, Williams, Winnek and English, THIS JOURNAL, **62**, 2002 (1940).

TABLE I

PROPERTIES OF SULFANILAMIDO HETEROCYCLES

Compound[a]	M. p., °C. (cor.)[b]	Water soly.[c] 37°	Max.[c] blood[d] level	Chemotherapeutic activity in vivo[e]	in vitro[f]	Ref. to intermed.	Formula	Analyses,[g] % Calcd. C	H	N	Found C	H	N
2-S-imidazole[h]	262	178	2.2	Inactive	+	*j*	$C_9H_{10}O_2N_4S$	45.4	4.2	23.5	45.8	4.6	23.7
3-S-1,2,4-triazole	195–96	60	11.4	Inactive	±	*k*	$C_8H_9O_2N_5S$	40.2	3.8	29.3	40.6	3.8	29.1
4-S-1,2,4-triazole[i]	237	216	0.9	Inactive	±	*l*	$C_8H_9O_2N_5S$	40.2	3.8	29.3	40.1	3.8	29.4
2-S-oxazole	175–76	282	20.6	Inactive	+++	*m*	$C_9H_9O_3N_3S$	45.2	3.8	17.6	45.0	3.9	17.6
5-S-3-methylisoxazole	169–70	104	7.4	Sl. active	++	*n*	$C_{10}H_{11}O_3N_3S$	47.4	4.4	16.6	47.4	4.2	16.5
3-S-4-methylfurazan	148–50	180	8.1	Inactive	++	*o*	$C_9H_{10}O_3N_4S$	42.5	3.9	22.0	42.3	4.4	22.0
3-S-5-methyl-1,2,4-oxadiazole	211–13	113	5.5	Sl. active	++	*m*	$C_9H_{10}O_3N_4S$	42.5	3.9	22.0	42.7	3.8	22.2
2-S-5-amino-1,3,4-thiadiazole	259	36.3	2.1	Inactive	++	*p*	$C_8H_9O_2N_5S_2$	35.4	3.3	25.8	35.3	3.5	25.5
3-S-pyridazine	189–90	221	50.9	Inactive	+++	*m*	$C_{10}H_{10}O_2N_4S$	48.0	4.0	22.4	47.7	4.0	22.8
2-S-4-aminopyrimidine	271–72	186	0.7	Inactive	+	*q*	$C_{10}H_{11}O_2N_5S$	45.3	4.2	26.4	45.7	4.6	26.5
2-S-4-diethylaminopyrimidine	>300	4.2	0.4	Inactive	±	*m*	$C_{14}H_{19}O_2N_5S$	52.3	5.9	21.8	52.5	5.8	21.7
2-S-4,6-diamino-1,3,5-triazine	290–95	728	1.7	Inactive	+	*r*	$C_9H_{11}N_7O_2S$	38.4	3.9	34.9	38.9	4.3	34.7

[a] S = Sulfanilamido. [b] With decomposition in most cases. [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] Approx. as follows: ± < sulfanilamide, + = sulfanilamide, ++ = sulfapyridine, +++ = sulfathiazole, against *E. coli* in a synthetic medium. [g] Microanalyses were carried out in these Laboratories by Mrs. Thelma Kirk and the Misses Helen Chubb, Margaret Oliver, Rebecca Teston and Lucy Vanderwort. [h] Ewins and Ashley, British Patent 521,821, reported m. p. 259°. [i] N^4-acetyl deriv. m. p. 237°; N, calcd. 29.3%; N, found 29.4%. [j] Fargher and Pyman, *J. Chem. Soc.*, **115**, 243 (1919). [k] Morgan and Reilly, *ibid.*, **109**, 159 (1916). [l] Ruhemann and Merriman, *ibid.*, **87**, 1772 (1905). [m] See experimental. [n] Burns, *J. prakt. Chem.* (2), **47**, 120 (1893). [o] Ponzio and Ruggeri, *Gazz. chim. ital.*, **52**, I, 289 (1922); **53**, 297 (1923); *Chem. Abst.*, **16**, 2676 (1922); **17**, 3873 (1923). [p] Fromm, *Ann.*, **433**, 8 (1923). [q] Johnson and Johns, *Am. Chem. J.*, **34**, 190 (1905). [r] American Cyanamid Company, New York, N. Y.

In vitro bacteriostatic tests against *E. coli* are included in Table I for comparison with the results in experimental animals.[3] None of the new sulfanilamido heterocycles showed much *in vivo* activity. All of them, however, showed some degree of bacteriostasis, including two compounds (2-sulfanilamidoöxazole and 3-sulfanilamidopyridazine) which were as active as sulfathiazole. In spite of high blood levels, these two derivatives were without activity in experimental mouse infections. This phenomenon has been encountered previously.[2,4] It was suggested that some of the discrepancies between *in vitro* and *in vivo* results, where lack of absorption could be ruled out, might be due to the formation of less active substances in the animal body.[4c] On the other hand, all cases of this type may not be explainable on the basis of a breakdown to less active products.

Davis[5] has suggested that the *in vivo* activity of sulfonamides may be influenced by the binding effect of serum proteins. Based on preliminary bacteriostatic experiments, he also suggested the possibility that the bound form might be inactive. Since the degree of binding appeared to be a function of the particular sulfonamide, it is possible that such a phenomenon may explain some of the differences between *in vitro* and *in vivo* activity. In any event, it is evident from the data in Table I that factors other than bacteriostasis, absorption and generally recognized variables are important to the chemotherapeutic activity of sulfonamides against experimental animal infections. These observations emphasize again the number of variables, both known and unknown, which may complicate any attempts to correlate *in vivo* activity with chemical structure.

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

(4) (a) Roblin and Winnek, THIS JOURNAL, **62**, 1999 (1940); (b) Roblin, Williams and Anderson, *ibid.*, **63**, 1930 (1941); (c) Roblin, Winnek, and English, *ibid.*, **64**, 567 (1942).

(5) Davis, *Science*, **95**, 77 (1942).

Experimental

Aminoheterocycles, in general, were prepared by methods which have been described in the literature (see references in Table I). Several of these intermediates have not been described previously, and the data for these compounds are recorded in Table II. The following is a description of the procedures employed for the synthesis of the new aminoheterocycles.

2-Aminoöxazole was prepared by one of the standard methods for the synthesis of the analogous thiazole derivative,[6] substituting urea for thiourea. In spite of the numerous modifications tried, the yields were much poorer when urea was used. 379 g. (2.65 moles) of α,β-dichlorodiethyl ether, 800 cc. of water and 318 g. (5.3 moles) of urea were refluxed gently, with stirring, for five and one-half hours. After standing overnight, the clear solution was

(6) v. Traumann, *Ann.*, **249**, 36 (1888).

TABLE II

AMINOHETEROCYCLES

Compound	M. p., °C. (cor.)	Yield,[a] %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
2-Aminoöxazole	96– 98	4.4	42.9	4.8	33.3	43.0	4.9	33.1
3-Amino-5-methyl-1,2,4-oxadiazole	117–119	9.8	36.4	5.1	42.4	36.4	5.3	42.2
3-Aminopyridazine	168– 70	58	50.5	5.3	44.2	50.5	5.1	44.5
2-Amino-4-diethylaminopyrimidine	86– 88	59	57.8	8.5	33.7	58.2	8.7	33.3

[a] Yield of material suitable for conversion to the corresponding sulfanilamido derivative. In several cases samples were further purified before analysis; the melting point is that of the analyzed sample.

extracted with 200 cc. of ether, in two portions, to remove any chloroacetaldehyde. A solution of 240 g. (6 moles) of sodium hydroxide in 320 cc. of water was added with cooling. The strongly basic solution was then extracted with 1.3 liters of ethyl ether in five portions. After drying over flake sodium hydroxide and distilling to dryness, a residue of 2-aminoöxazole remained. Recrystallization from octanes gave 7.6 g. of the compound, m. p. 93–96°; 2.8 g. more was obtained by continuous extraction of the mother liquor (4.4% total yield, based on the α,β-dichlorodiethyl ether used). This product was suitable for the coupling reaction. A sample recrystallized from heptane had a m. p. of 96°–98°. Variations in the proportions of urea used and the time of reflux did not improve the yield. The reaction of bromoacetaldehyde and urea in water[7] gave similar yields.

3-Amino-5-methyl-1,2,4-oxadiazole was obtained by a procedure analogous to the preparation of 3-amino-5-phenyloxadiazole by Wieland and Bauer.[8] To a solution of 50 g. (0.29 mole) of dioxyguanidine hydrobromide in 135 cc. of glacial acetic acid was added 65 g. (0.65 mole) of acetic anhydride with cooling so that the temperature did not rise above 25°. Then 23.5 g. (0.29 mole) of sodium acetate was added and the mixture stirred overnight at room temperature. The insoluble salts were filtered off and washed with glacial acetic acid. Vacuum distillation of the filtrate left a viscous residue containing diacetoxyguanidine. Addition of 40% sodium hydroxide with cooling caused an evolution of gas. The alkaline solution was heated to 70–80° for twenty minutes to cyclize the diacetyl derivative. After cooling, 2.4 g. of 3-amino-5-methyloxadiazole was obtained by repeated ether extractions. It was purified by recrystallization from toluene, using activated alumina.

For the preparation of 3-aminopyridazine, 10.7 g. (0.093 mole) of 3-chloropyridazine[9] dissolved in 30 cc. of absolute alcohol and 30 cc. of anhydrous ammonia were heated at 175° in a steel autoclave for three hours, with shaking. The cooled reaction mixture was removed, heated to boiling while nitrogen was bubbled through, and filtered. The filtrate was evaporated to dryness under a reduced pressure of nitrogen. 3-Aminopyridazine was extracted from the residue with hot ethyl acetate, from which it crystallized as a light yellow solid; yield 5.1 g. It was then recrystallized from ethyl acetate.

2-Amino-4-diethylaminopyrimidine was prepared by heating 9 g. (0.07 mole) of 2-amino-4-chloropyrimidine[2] with 35 g. (0.49 mole) of diethylamine in a bomb-tube at 110°–120° for three hours. The product was dissolved in water, made alkaline with sodium hydroxide and extracted with ether. The ether was distilled off and the residue extracted with hot hexane. Cooling gave a crystalline precipitate of 9.8 g. of 2-amino-4-diethylaminopyrimidine. This was recrystallized from hexane.

Sulfanilamidoheterocycles (Table I) were prepared, in general, by the reaction of the aminoheterocycle with acetylsulfanilyl chloride in dry pyridine followed by hydrolysis.[4a] Dioxane was used as a reaction solvent for the preparation of 2-(N^4-acetylsulfanilyl)-4-aminopyrimidine and *t*-butanol for 2-(N^4-acetylsulfanilyl)-4-diethylaminopyrimidine. No rigid proof of structure for the former compound was attempted. The position of the sulfanilamido group on the pyrimidine ring was inferred from the instability of 4-(N^4-acetylsulfanilamido)-pyrimidine to hydrolysis.[2,4c] If the acetylsulfanilyl chloride reacted with the 4-amino group, it was assumed that the 4-(N^4-acetylsulfanilyl)-2-aminopyrimidine would have been decomposed in the subsequent hydrolysis.

2-(*p*-Nitrobenzenesulfonamido)-oxazole was prepared by refluxing 42.8 g. (0.19 mole) of *p*-nitrobenzenesulfonyl chloride with 16.2 g. (0.19 mole) of 2-aminoöxazole and 23.7 cc. (0.3 mole) of dry pyridine in 200 cc. of dry acetone for thirty minutes. The acetone was distilled off, and the gummy residue extracted with dilute ammonium hydroxide. Careful neutralization of the extracts with hydrochloric acid gave a first precipitate which was a sticky gum. This was removed and the desired compound was precipitated as a solid by further addition of acid. After recrystallization from water, 4.7 g. (9.0% of the theoretical) of *p*-nitrobenzenesulfonamidoöxazole was obtained. The m. p. of a further recrystallized sample was 175–177°.

Reduction by ferrous sulfate and ammonium hydroxide[10] gave 2-sulfanilamidoöxazole. Recrystallization from water did not improve the melting point. By dissolving in dilute hydrochloric acid, stirring with decolorizing carbon, filtering and precipitating by neutralization with ammonium hydroxide, colorless crystals of pure 2-sulfanilamidoöxazole were obtained. The yield of pure compound in the reduction step was 45% of the theoretical.

3-Sulfanilamido-1,2,4-triazole and 2-sulfanilamido-4,6-diamino-1,3,5-triazine were prepared by treating *p*-nitrobenzenesulfonyl chloride with the appropriate amine and then reducing with iron dust in dilute acetic acid.

Summary

The preparation and properties of a number of new sulfanilamido heterocycles are described.

(7) *Cf.* Leitch and Brickman, U. S. Patent 2,230,962.

(8) Wieland and Bauer, *Ber.*, **40**, 1689 (1907).

(9) Gabriel, *ibid.*, **42**, 655 (1909).

(10) Jacobs and Heidelberger, THIS JOURNAL, **39**, 1435 (1917).

Several of these compounds are closely related chemically to well-known sulfanilamido derivatives of the same type.

All of the sulfonamides showed some degree of bacteriostatic activity, but very little effect on experimental animal infections. Two compounds, in particular, were highly active *in vitro* and well absorbed. Possible explanations for these discrepancies and their bearing on the relation of molecular structure to chemotherapeutic activity, are discussed.

STAMFORD, CONN. RECEIVED JULY 31, 1942

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

Studies in Chemotherapy. VII. A Theory of the Relation of Structure to Activity of Sulfanilamide Type Compounds[1]

BY PAUL H. BELL AND RICHARD O. ROBLIN, JR.

For the past three years we have been trying to find some relationship between the molecular structure and the chemotherapeutic activity of sulfanilamide type compounds. In spite of the many hundreds of derivatives which have been prepared and tested, no adequate explanation for the profound changes in therapeutic effect resulting from variations in structure has been proposed. Our approach to this problem has been through an attempt to utilize a fundamental physical property related to both structure and activity.[2] The present theory is based on the experimental observation that acid dissociation constants, which can be correlated with the structure of sulfanilamide derivatives, are also related to their bacteriostatic activity. The following is a description of this theory and a discussion of its implications.

I. Chemotherapeutic Activity and Mode of Action.—Before attempting to correlate the structure of sulfonamides with their chemotherapeutic activity, a reasonably accurate method for the determination of relative effectiveness is essential. Experimental animal tests for activity are frequently misleading because of the many factors, such as lack of absorption, rapid excretion, effect of diet and possible chemical changes in the compounds, as well as other less obvious variables,[3] which may affect the results. To a lesser degree, *in vitro* tests carried out in complex media are also somewhat confusing. In this investigation the term activity is used to indicate bacteriostatic activity against *E. coli* when the organisms are grown in a synthetic medium. This method of testing provides a more consistent and reproducible basis for the determination of relative activities by reducing the number of variables to a minimum. The relation of *in vitro* to *in vivo* results,[4] and the lack of any great degree of specificity among sulfanilamide derivatives,[5] appear to warrant this method of evaluation.

Another important prerequisite to this type of work is at least a partial understanding of the mechanism by which the compounds exert their bacteriostatic effects. None of the hypotheses advanced in recent years appeared to offer a very useful or convincing explanation, until Woods[6] demonstrated that *p*-aminobenzoic acid prevents the bacteriostatic action of sulfanilamide and sulfapyridine. This observation has since been extended by other investigators[7] to include sulfanilamide type compounds in general. Woods and Fildes[8] postulated that *p*-aminobenzoic acid is an essential metabolite associated with one or more of the enzymatic processes involved in bacterial growth. They pointed out the close structural relationship between the sulfonamides and this acid, and suggested that the former may act by blocking the enzyme system or systems with which *p*-aminobenzoic acid is involved and on which many bacteria depend for normal growth and development. Subsequent investigations have confirmed the essential nature of *p*-aminobenzoic acid,[9] and have shown experimentally

(1) Presented in part before the Divisions of Medicinal and Physical Chemistry, Buffalo Meeting of the American Chemical Society, September 9 and 10, 1942.

(2) Roblin and Bell, *Science*, **90**, 328 (1939).

(3) See, for example, Davis, *ibid.*, **95**, 78 (1942).

(4) White, Bratton, Litchfield and Marshall, *J. Pharmacol.*, **72**, 120 (1941).

(5) Wyss, Grubaugh and Schmelkes, *Proc. Soc. Exptl. Biol. Med.*, **49**, 618 (1942).

(6) Woods, *Brit. J. Expil. Path.*, **21**, 74 (1940).

(7) Landy and Wyeno, *Proc. Soc. Expil. Biol. Med.*, **46**, 59 (1941); Strauss, Lowell and Finland, *J. Clin. Investigation*, **20**, 189 (1941).

(8) Fildes, *Lancet*, **238**, I, 955 (1940).

(9) Rubbo and Gillespie, *Nature*, **146**, 838 (1940); Lampen and Peterson, THIS JOURNAL, **63**, 2283 (1941); Park and Wood, *Bull. Johns Hopkins Hosp.*, **70**, 19 (1942).