alanine, this error was of the order of 1%. The *p*H measurements were reproducible to within 0.02 of a *p*H unit and in the case of the base titration correction was made for the presence of sodium ion. The titration curves are given in Fig. 1 and the apparent dissociation constants, K_A, K_{B_1}, and K_{B_2}, in Table I.

TABLE I

APPARENT DISSOCIATION CONSTANTS

Amino acid	$K_{B_1} \times 10^{-10}$	$K_{B_2} \times 10^{-13}$	$K_A \times 10^{-10}$
dl-β-(2-Pyridyl)-alanine	0.89 ± 0.05	2 ± 1	6 ± 1
dl-β-(3-Pyridyl)-alanine	3.7 ± .5	5 ± 1	8 ± 1
dl-β-(4-Pyridyl)-alanine	6 ± 1		

Summary

1. The three isomeric *dl*-β-pyridylalanines have been synthesized and their apparent acid and basic dissociation constants determined. The effect of structure on acid and base strength is discussed.

2. Picolinaldehyde and nicotinaldehyde have been synthesized by the McFadyen–Stevens reaction and the applicability of this reaction to the pyridine series is discussed.

PASADENA, CALIFORNIA RECEIVED APRIL 21, 1942

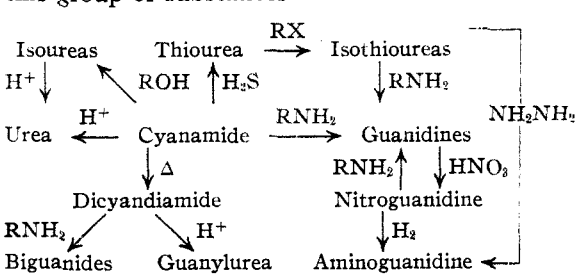
[CONTRIBUTION FROM THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. V. Sulfanilylcyanamide and Related Compounds[1]

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

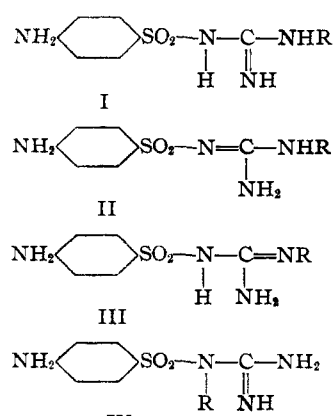
Shortly after sulfanilylguanidine was described in a preceding paper of this series,[2] Marshall and co-workers[3] reported the compound independently. On the basis of a comprehensive bacteriological and pharmacological study, they suggested its use for the treatment of intestinal infections.

Guanidine is one of a large group of compounds which may be prepared from cyanamide. Because of the somewhat unusual characteristics of sulfaguanidine, it appeared to be of interest to investigate the sulfanilyl derivatives of cyanamide and a number of related compounds including a series of substituted guanidines. The following diagram illustrates some of the inter-relationships among this group of substances



Many of the same relationships have been found to exist among the sulfanilyl derivatives of these compounds (Table I). Thus, in addition to the more obvious method, sulfanilylguanidines were prepared from the sulfanilyl derivatives of cyanamide, nitroguanidine and isothioureas. Similarly, sulfanilylcyanamide was converted to the urea or isourea compounds. *p*-Nitrobenzenesulfonyl chloride and isoureas also led to the formation of sulfanilylisoureas, which in turn could be hydrolyzed to the urea derivative.[4] Other similar reactions such as the conversion of sulfanilyldicyandiamide to guanylurea and biguanide derivatives were also investigated.

Because of the alkali insolubility of many of the substituted sulfanilylguanidines (Table I), it was not possible to establish their structure directly when they were prepared through acetylsulfanilyl chloride and the substituted guanidines. For example, the sulfanilylalkylguanidines might have any of the following structures



(1) Presented in part before the Division of Medicinal Chemistry, Memphis meeting of the American Chemical Society, April 22, 1942.

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

(3) Marshall, Bratton, White and Litchfield, *Bull. Johns Hopkins Hosp.*, **67**, 163 (1940).

(4) *Cf.* Cox and Raymond, THIS JOURNAL, **63**, 300 (1941).

TABLE I

PROPERTIES OF SULFANILYL COMPOUNDS

Compound, sulfanilyl-	M. p.,[a] °C. (cor.)	Water[b] soly. 37°	Alk.[c] soly.	Max. blood[b] level[d]	*In vitro* activity[e]	Method of prepn.	Ref. to inter-med.	Formula	Analyses,[f] % Calcd. C	H	N	Found C	H	N
Cyanamide	292–5	384	sol.	3.7	Less	A	*i*	$C_7H_7O_2N_3S$	42.6	3.6	21.3	42.5	3.7	21.2
Urea	140–4	811	sol.	7.4	Equal	B, C	*j*	$C_7H_9O_3N_3S$	39.1	4.2	19.5	39.1	4.1	19.2
Methylisourea	172–3	157	sol.	22.6	Equal	D, E	*j*	$C_8H_{11}O_3N_3S$	41.9	4.8	18.3	42.1	4.8	18.3
Ethylisourea	126–7	199	sol.	16.0	Equal	D	*j*	$C_9H_{13}O_3N_3S$	44.4	5.4	17.3	44.7	5.4	17.2
Methylisothiourea	184–5	33	sol.	8.6	Equal	F	*k*	$C_8H_{11}O_2N_3S_2$	39.2	4.5	17.1	39.2	4.5	17.2
Ethylisothiourea	154–5	30	sol.	6.4	Equal	F	*l*	$C_9H_{13}O_2N_3S_2$	41.7	5.0	16.2	41.8	5.1	16.2
Guanidine[g]	189–90	190	in.	2.6[h]	Standard									
Nitroguanidine	194–5	44	sol.	2.8	Less	G	*i*	$C_7H_9O_4N_6S$	32.4	3.5	27.0	32.5	3.6	27.0
Aminoguanidine	209–10	188	in.	2.8	Greater	H, m	*j*	$C_7H_{11}O_2N_6S$	36.7	4.8	30.6	36.7	4.7	30.8
Ethylguanidine	160–1	226	in.	4.2	Equal	G	*n*	$C_9H_{14}O_2N_4S$	44.6	5.8	23.1	44.4	5.7	23.5
Propylguanidine	147–8	429	in.	12.0	Sl. less	G	*o*	$C_{10}H_{16}O_2N_4S$	46.9	6.2	21.9	46.8	6.0	21.6
Butylguanidine	184–6	28	in.	6.9	Less	G, H	*p*	$C_{11}H_{18}O_2N_4S$	48.9	6.7	20.7	49.1	6.7	20.7
Phenylguanidine	231–3	24	in.	1.6	Equal	I, J	*j*	$C_{13}H_{14}O_2N_4S$	53.8	4.8	19.3	53.8	4.7	19.6
p-Aminophenylguanidine	200–1	205	in.	2.4	Equal	J	*j*	$C_{13}H_{15}O_2N_5S$	51.1	4.9	22.9	51.1	4.6	22.6
p-Carboxyphenylguanidine	234–5	19	sol.	0.8	Less	J	*j*	$C_{14}H_{14}O_4N_4S$	50.3	4.2	16.8	50.2	4.1	16.6
2-Pyridylguanidine	239–41	2.6	in.	3.4	Sl. less	J	*j*	$C_{12}H_{13}O_2N_5S$	49.5	4.5	24.0	49.5	4.3	23.6
Dicyandiamide	236–7	255	sol.	1.2	Less	G	*i*	$C_9H_9O_2N_6S$	40.2	3.8	29.3	40.3	3.8	29.2
Guanylurea	225–6	20	sol.	1.3	Sl. less	B, G	*q*	$C_8H_{11}O_3N_6S$	37.3	4.3	27.2	37.2	4.4	27.4
Biguanide	244–5	134	in.	1.3	Less	G	*r*	$C_8H_{13}O_2N_6S$	37.4	4.7	32.8	37.6	4.9	33.1
Butylbiguanide	214–5	5.4	in.	2.8	Less	G	*r*	$C_{12}H_{20}O_2N_6S$	46.1	6.4	26.9	46.4	6.4	26.6
Dimethylbiguanide	191–2	28	in.	3.0	Equal	G	*s*	$C_{10}H_{16}O_2N_6S$	42.2	5.6	29.6	42.2	5.7	29.5
o-Tolylbiguanide	214–6	8.0	in.	1.5	Less	G, l	*t*	$C_{16}H_{18}O_2N_6S$	52.0	5.2	24.3	52.5	5.1	24.6

[a] With decomposition. [b] Mg./100 cc. [c] Sol. indicates greater solubility in alkali than in water; in., no greater than in water. [d] White mice; dosage 0.5 g./kg. body weight. [e] Relative bacteriostatic activity compared with sulfaguanidine against *B. coli* in a synthetic medium. [f] Microanalyses were carried out in these Laboratories by Mrs. Thelma Kirk and the Misses Helen Chubb, Margaret Oliver, Rebecca Teston and Lucy Vandervort. [g] Refs. 2 and 3. [h] Average of large number of determinations. [i] American Cyanamid Co., New York, N. Y. [j] This paper. [k] Arndt, *Ber.*, **54B**, 2236 (1921). [l] Taylor, *J. Chem. Soc.*, **111**, 656 (1917). [m] Also prepared by iron reduction of sulfanilylnitroguanidine; m. p. N^4-acetylsulfanilylaminoguanidine 256–7°. [n] Schenck and Kirchhof, *Z. physiol. Chem.*, **154**, 292 (1926). [o] Piovano, *Gazz. chim. ital.*, **58**, 245 (1928). [p] Davis and Elderfield, THIS JOURNAL, **54**, 1499 (1932). [q] Söll and Stutzer, *Ber.*, **42**, 4534 (1909). [r] Rackmann, *Ann.*, **376**, 169 (1910). [s] Slotta and Tscheschi, *Ber.*, **62**, 1400 (1929). [t] U. S. Patent 2,195,073.

However, the synthesis of a sample of sulfanilylbutylguanidine from N^4-acetylsulfanilylmethylisothiourea and butylamine[5] identical with the product obtained from acetyl sulfanilyl chloride and butylguanidine served to eliminate formula IV as a possibility. We have not attempted to differentiate conclusively among formulas I, II and III, because they are potentially tautomeric isomers. With the possible exception of sulfanilyl-*p*-carboxyphenylguanidine, alkali solubility would seem to favor I, since there appears to be a general tendency against the formation of III.[6]

The use of sulfaguanidine for intestinal infections is based on the fact that, while it has a reasonable degree of water solubility and therapeutic activity, it is poorly absorbed. These properties result in a high concentration in the intestinal tract without a correspondingly high level in the blood and tissues. The most important properties for compounds of this type then appear to be activity against the coliform group of organisms, degree of absorption and solubility. Given these data for a new substance, it should be possible to determine whether or not it merits further investigation. These properties for the compounds reported in this paper are recorded in Table I.[7]

It is interesting that in general the presence of a guanidyl radical resulted in low absorption. An exception to this were the alkyl guanidines, which were more completely absorbed. Other closely related compounds which lacked the guanidyl group were also absorbed.

Sulfanilylaminoguanidine was the only substance in this group which was more active *in vitro* against *B. coli* than sulfaguanidine. From the standpoint of absorption in mice and water solubility there appears to be little to choose between these two compounds. Since the preliminary results indicate that the amino derivative is bacteriostatic at higher dilutions than sulfaguanidine, it is possible that a further study of sulfanilylaminoguanidine may demonstrate it to be useful in the treatment of intestinal infections.

(5) *Cf.* Phillips and Clarke, THIS JOURNAL, **45**, 1755 (1923).

(6) Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 2nd ed., 1940, p. 213.

(7) The bacteriological and pharmacological studies were carried out in this Laboratory under the direction of Dr. W. H. Feinstone.

Experimental

A number of different procedures have been used in preparing the sulfanilyl derivatives reported in this paper. In most cases only one example of each method is described in detail. Other derivatives prepared by the same general procedure are designated in Table I. Whenever the same compound was prepared by more than one method, the identity of the different samples was established by mixed melting points as well as separate analyses.

(**Method A**) **Sulfanilylcyanamide.**—Commercial calcium cyanamide (220 g. of minimum hydrated) was stirred in 1300 cc. of water for three hours at 25–30°. To the filtrate from this mixture (about a 10% solution of $Ca(HNCN)_2$) was added with stirring, over a period of forty-five minutes, 200 g. (0.855 mole) of acetylsulfanilyl chloride at a temperature of 25–30°. The reaction mixture was kept alkaline by adding 40% sodium hydroxide solution as required. Stirring was continued for two hours, during which time a precipitate separated. After filtering the cooled mixture, the precipitate was washed with cold water and then with acetone. A yield of 162 g. (73%) of calcium acetylsulfanilyl cyanamide was obtained. Without further purification the product analyzed as follows: Ca (calcd.) 7.75%; Ca (found) 7.73%.

Thirteen grams (0.025 mole) of the acetyl derivative was refluxed with 60 cc. of 10% sodium hydroxide solution for thirty-five minutes and filtered while hot. The cooled filtrate was then made strongly acid with concentrated hydrochloric acid to separate the sulfanilyl cyanamide. It was purified by dissolving in alkali, treating with activated charcoal, filtering and precipitating with hydrochloric acid. The yield amounted to 9.8 g. (95%).

Sulfanilylcyanamide was also prepared by treating *p*-nitrobenzenesulfonyl chloride with free cyanamide in an aqueous medium in the presence of an excess of sodium hydroxide. The resulting sodium *p*-nitrobenzenesulfonylcyanamide was reduced to sulfanilylcyanamide with iron powder and 5% acetic acid.

(**Method B**) **Sulfanilylurea.**—Three grams (0.0058 mole) of calcium acetylsulfanilylcyanamide and 20 cc. of 4 *N* hydrochloric acid were warmed together on a steam-bath until solution was complete (fifteen to twenty minutes). When the solution was cooled a gummy solid separated. It was filtered off and dried. A yield of 2 g. (82%) of crude sulfanilylurea was obtained. The product was purified by repeated crystallization from water.

(**Method C**) **Sulfanilylurea** was also prepared from sulfanilylmethylisourea (see Methods D and E) by hydrolysis with concentrated hydrochloric acid on a steam-bath.[4]

(**Method D**) **Sulfanilylmethylisourea.**—14.9 g. (0.06 mole) of sodium *p*-nitrobenzenesulfonylcyanamide was added to 100 cc. of absolute methanol; most of the salt dissolved. Eight grams (0.22 mole) of hydrochloric acid gas was bubbled in from a cylinder in fifteen to twenty minutes with ice cooling. A white precipitate formed immediately. After one and one-half hours of standing in a stoppered flask at room temperature, the solid was filtered off and washed with methanol. It was then slurried with 25 cc. of water, made alkaline with ammonium hydroxide, filtered off and washed with 25 cc. of water, then with methanol. A yield of 14.4 g. (93%) of crude *p*-nitrobenzenesulfonylmethylisourea was obtained. It was purified by recrystallizing twice from methanol. Sulfanilylmethylisourea was produced in 81% yield by reduction of the purified nitro compound with iron powder in 5% acetic acid.

Sulfanilylmethylisourea was made more simply by the reaction of calcium acetylsulfanilylcyanamide with methanol and dry hydrochloric acid. Hydrolysis of the acetyl group apparently took place during the reaction, since the final compound was isolated directly from the reaction mixture.

(**Method E**) **Sulfanilylmethylisourea** was obtained also from *p*-nitrobenzenesulfonyl chloride and methylisourea hydrochloride following the general method of Cox and Raymond.[4] The nitro group was reduced as described under Method D.

(**Method F**) **Sulfanilylmethylisothiourea** was prepared from acetylsulfanilyl chloride and methylisothiourea sulfate by the general procedure described in a previous paper.[5] A mixture of 10 g. (0.035 mole) of acetylsulfanilylmethylisothiourea obtained by this procedure, 20 cc. of concentrated hydrochloric acid and 100 cc. of 95% ethanol was heated to boiling and the boiling continued for two minutes after all solid material had dissolved. The reaction mixture was then neutralized with 20% sodium hydroxide and cooled. The resulting precipitate of sulfanilylmethylisothiourea was purified by crystallization from water; yield 3.6 g. (42%).

(**Method G**) **Sulfanilylpropylguanidine.**—Fifteen grams (0.1 equiv.) of propylguanidine sulfate was suspended in 120 cc. of acetone and 10 g. (0.25 mole) of sodium hydroxide dissolved in 20 cc. of water was added. The mixture was cooled to 20°, and 25 g. (0.107 mole) of acetylsulfanilyl chloride was added gradually with stirring, while the temperature was maintained at 18–22°. After the reaction mixture had been stirred for four hours at room temperature, it was allowed to stand overnight. It was then diluted with 200 cc. of water and neutralized with acetic acid. Acetylsulfanilylpropylguanidine separated as a white solid, which was filtered off, washed with water and dried. The yield was 23 g. (78%) of crude product.

Nine grams (0.03 mole) of the crude acetyl compound was suspended in 21 cc. of 4 *N* hydrochloric acid and the mixture heated to boiling. All solid material was dissolved after five minutes and boiling was continued for three minutes. At this point a precipitate started to form. The mixture was at once diluted with two volumes of ice and the cold solution stirred for one hour with activated charcoal. The filtrate from this mixture was neutralized in the cold with 20% sodium hydroxide solution. Crude sulfanilylpropylguanidine separated as a gum which on stirring in the cold turned to a white solid. It was purified by crystallizing twice from hot water. A yield of 3 g. (40%) was obtained.

(**Method H**) **Sulfanilylbutylguanidine.**—4.1 g. (0.014 mole) of *p*-acetylsulfanilylmethylisothiourea was suspended in 20 cc. of 50% ethanol in a 3-necked flask fitted with a mercury-sealed stirrer and a reflux condenser leading through wash-bottles containing dilute hydrochloric

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

acid and sodium hydroxide solution; 0.86 g. (0.012 mole) of butylamine was added and the mixture warmed slowly with stirring on a steam-bath. There was a slow evolution of gas and after two hours the solid had dissolved. Heating was continued for one hour and the reaction mixture was then diluted with water and the acetylsulfanilylbutylguanidine separated as a solid; yield 3.5 g. (94%). Hydrolysis to sulfanilylbutylguanidine was accomplished by the method described under Method G except that 50% ethanol was employed as the medium.

(**Method I**) **Sulfanilylphenylguanidine.**—21.1 grams (0.07 mole) of acetylsulfanilylnitroguanidine, 13 g. (0.14 mole) of aniline, and 60 cc. of dioxane were refluxed for seven hours. All the solids had dissolved after the first half hour and the dark solution was allowed to stand overnight. Dilution with 200 cc. of water gave a sticky precipitate. The mixture was made slightly alkaline with ammonium hydroxide to dissolve any unreacted acetylsulfanilylnitroguanidine. After standing in the refrigerator for several hours, the oily material completely solidified. This was filtered off, washed with very dilute ammonia, and dried. The yield was 12.6 g. (54%). It was purified by crystallization from dilute alcohol. Deacetylation was carried out as described under Method G.

(**Method J**) **Sulfanilyl-*p*-aminophenylguanidine.**—7.5 grams (0.05 mole) of *p*-aminoacetanilide (Eastman Kodak Co., Rochester, N. Y.) was suspended in 50 cc. of water and 4 cc. of concentrated hydrochloric acid and 13 g. (0.025 mole) of calcium acetylsulfanilylcyanamide was added. A thick slurry resulted, which on heating to boiling dissolved. The mixture was refluxed for one-half hour and during the last fifteen minutes a light yellow precipitate separated. After cooling the product was filtered off, washed with water and dried. The crude acetylsulfanilyl-*p*-acetylaminophenylguanidine obtained in this manner amounted to 13 g. (67%). It was hydrolyzed as described under Method G in 54% yield.

Sulfanilyl-2-pyridylguanidine was obtained by a modification of the above method. Equivalent quantities of 2-aminopyridine hydrochloride and calcium acetylsulfanilylcyanamide were heated together at 200° for fifteen minutes. The resulting acetylsulfanilyl-2-pyridylguanidine was deacetylated by refluxing with 4 *N* hydrochloric acid. The yield of purified product was about 10%.

Sulfanilyldicyandiamide.—The acetyl derivative of this compound was obtained through the courtesy of Dr. D. W. Kaiser of this Laboratory. It was prepared by him using Method G. Sulfanilyldicyandiamide resulted from the alkaline hydrolysis of the intermediate acetyl derivative by the procedure described under Method A.

Sulfanilylguanylurea was prepared both by Method G and from the acid hydrolysis of acetylsulfanilyldicyandiamide employing the procedure described in Method B for the conversion of the cyanamide to the urea derivative. The product obtained by the latter method was somewhat difficult to purify. Only after fractional precipitation from alkaline solution was the melting point as high as that of the product obtained by Method G. Since only one product can be obtained from the dicyandiamide derivative, this method of synthesis in conjunction with the alkali solubility served to indicate the structure of sulfanilylguanylurea.

Sulfanilylbiguanides were produced by the same process that was employed for a number of the guanidines (Method G). In addition, sulfanilyl-*o*-tolylbiguanide was obtained by the reaction of acetylsulfanilyldicyandiamide and *o*-toluidine followed by hydrolysis. The conditions were the same as those described under Method I. This method helped to establish the structure of the *o*-tolyl derivative and by analogy the structure of the other sulfanilylbiguanides.

Summary

A group of sulfanilyl derivatives of cyanamide and related compounds has been prepared. The interrelationships among this group of compounds have made it possible to obtain many of them by more than one method. On the basis of this and other evidence the structure of the sulfanilyl derivatives is discussed.

The absorption, water solubility and bacteriostatic activity of these substances compared with sulfaguanidine are reported. These preliminary data suggest that sulfanilylaminoguanidine may be worth further investigation.

STAMFORD, CONNECTICUT RECEIVED APRIL 9, 1942