A blank experiment with benzene and lead tetraacetate failed to give a precipitate with 2,4-dinitrophenylhydrazine.

Potassium Diacetone *d*-Galactonate.—Oxidation of 50 g. of α-diacetone dulcitol with alkaline permanganate as described for the preparation of the corresponding levo isomer gave 20 g. of a crystalline solid which after two recrystallizations from acetone–alcohol melted at 194–197°; yield 17 g.

Anal. Calcd. for $C_{12}H_{19}O_7K$: K, 12.42. Found: K, 12.42. Rotation: 0.4777 g. subst. in 5.0 g. H_2O; $l = 1$, $\alpha = -4.85$; $[\alpha]^{27}D$ -50.8.

Cadmium *d*-Galactonate.—Seventeen grams of potassium diacetone *d*-galactonate when treated as described for the levo isomer gave 7.0 g. of cadmium *d*-galactonate. Thrice recrystallized, it decomposed at 200–205°.

Anal. Calcd. for $(C_6H_{11}O_7)_2Cd \cdot H_2O$: C, 27.69; H, 4.61; Cd, 21.54. Found: C, 27.65; H, 4.82; Cd, 21.54.

***d*-Galactose.**—The *d*-galactose was prepared from the cadmium *d*-galactonate in the same manner as that described for the preparation of *l*-galactose. Seven grams of salt gave 1.4 g. of *d*-galactose which melted at 165–166° and had, in aqueous solution, a specific rotation of +80.3°.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.67. Found: C, 40.07; H, 6.74.

The gift of a generous supply of dulcitol from the Atlas Powder Company is gratefully acknowledged. The microanalyses were carried out in this Laboratory by Mr. J. F. Alicino.

Summary

The constitution of the two isomeric α- and β-diacetone dulcitols has been investigated. Both compounds are believed to be chemical entities. They are optically inactive and contain, as seen by lead tetraacetate oxidation, two adjacent free hydroxyl groups in terminal position.

β-Diacetone dulcitol can be transformed by alkaline oxidation into diacetone *l*-galactonic acid and subsequently on acid hydrolysis into *l*-galactono lactone, which reduced with sodium amalgam is converted into *l*-galactose.

α-Diacetone dulcitol oxidized in the identical manner gave rise to diacetone *d*-galactonic acid, *d*-galactono lactone and *d*-galactose.

In the light of these experimental findings the conclusion seems to be justified that β-diacetone dulcitol is 1,2-3,4-diacetone dulcitol and the α-isomer 3,4-5,6-diacetone dulcitol. The two forms therefore would be enantiomorphic. This conclusion, however, is obviously conflicting with the fact that in the carbohydrate group the separation of enantiomorphs by direct crystallization has so far been found impossible.

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[CONTRIBUTION FROM THE DIVISION OF PHARMACOLOGY, NATIONAL INSTITUTE OF HEALTH, UNITED STATES PUBLIC HEALTH SERVICE]

Organic Compounds in Chemotherapy. I. Derivatives of Sulfanilamide

BY HUGO BAUER

The following report deals with a series of derivatives of 4-aminobenzenesulfonamide (sulfanilamide) which were prepared in collaboration with Dr. S. M. Rosenthal for the purpose of chemotherapeutic studies. Comparative studies of sulfonamide compounds in experimental pneumococcus, streptococcus and meningococcus infections,[1] studies of the chemotherapy of choriomeningitis virus infection in mice with sulfonamide compounds,[2] and studies of some new sulfur compounds active against bacterial infections[3] have already been published.

Some of the compounds reported in this paper have been described also by other investigators working simultaneously in this field (see Table I). Their results in most cases agree with ours, although the methods of preparation differ in some respects.

Crossley, Northey and Hultquist[4] recently have suggested a system of terminology for these compounds. They have applied the name "sulfanilyl" to the radical $H_2NC_6H_4SO_2$-. We have adopted this terminology and have accordingly discarded the name "di-sulfanilamide" previously used by us for the compound $H_2NC_6H_4SO_2NHC_6H_4SO_2NH_2$. The name of this compound therefore becomes sulfanilyl sulfanilamide.

The general method of preparing the derivatives described herein consisted of condensation of amino compounds with acetanilide-4-sulfochloride, followed by deacetylation. The facility of the reaction is influenced by the more or less basic

(1) S. M. Rosenthal, H. Bauer and S. E. Branham, *Pub. Health Repts.*, **52**, 662 (May 21, 1937).

(2) S. M. Rosenthal, I. G. Wooley and H. Bauer, *ibid.*, **52**, 1211, Sept. 3, 1937.

(3) H. Bauer and S. M. Rosenthal, *ibid.*, **53**, 40 (Jan. 14, 1938).

(4) Crossley, Northey and Hultquist, THIS JOURNAL, **60**, 2217, 2222 (1938).

TABLE I

Compound	Formula	M. p., °C.	Analyses, %[k] Calculated N	Calculated S	Found N	Found S
Acetylsulfanilyl-						
-sulfanilamide[a,d,e]	$C_{14}H_{15}O_5N_3S_2$	274	11.40	17.36	11.41	
-sulfanilyl-sulfanilamide	$C_{20}H_{20}O_7N_4S_3$	268		18.34		18.44
-aminoethanol	$C_{10}H_{14}O_4N_2S$	156	10.85		10.74	
-sulfanilyl-aminoethanol	$C_{16}H_{19}O_6N_3S_2$	153		15.51		15.28
-glycine[h]	$C_{10}H_{12}O_5N_2S$	237		11.78		11.65
-sulfanilyl-glycine	$C_{16}H_{17}O_7N_3S_2$	247	9.84		9.61	
-4-carboxyaniline[h]	$C_{15}H_{14}O_5N_2S$	252	8.38		8.25	
4-nitroaniline[i]	$C_{14}H_{13}O_5N_3S$	264		9.57		9.49
Sulfanilyl-						
-sulfanilamide[a,b,c,e,f,g]	$C_{12}H_{13}O_4N_3S_2$	137	12.84	19.59	12.39	19.24
-sulfanilamide sodium[b]	$C_{12}H_{12}O_4N_3S_2Na$			Na, 6.59		Na, 6.72
-sulfanilyl-sulfanilamide[b,j]	$C_{18}H_{18}O_6N_4S_3$	209		19.94		20.27
-aminoethanol[b]	$C_8H_{12}O_3N_2S$	101	12.96		12.75	
-sulfanilyl-aminoethanol[b,j]	$C_{14}H_{17}O_5N_2S_2$	144		17.27		17.59
-glycine[b,h]	$C_8H_{10}O_4N_2S$	154	12.17		11.90	
-sulfanilyl-glycine[b,g]	$C_{14}H_{15}O_6N_3S_2$	188	10.91		10.62	
-4-carboxyaniline[b,h]	$C_{13}H_{12}O_4N_2S$	202		10.97		11.02
-4-carboxyaniline + C_2H_5OH	$C_{15}H_{18}O_5N_2S$[k]	196	8.28	9.48	8.45	9.42
-4-nitroaniline[b,i]	$C_{12}H_{11}O_4N_3S$	165	14.33	10.94	14.05	10.95
-4-aminoaniline[b,i]	$C_{12}H_{13}O_2N_3S$	138		12.18		11.99

[a] Ref. 1. [b] Ref. 3. [c] Ref. 2. [d] Fourneau, *et al.*, *Compt. rend. soc. biol.*, **122**, 258 (1936). [e] Gray, Buttle and Stephenson, *Biochem. J.*, **31**, 724 (1937). [f] Domagk, *Klin. Wochschr.*, **41**, 1412 (1937). [g] I. G. Farbenindustrie, French Pat. 817,034. [h] Kolloff, THIS JOURNAL, **60**, 950 (1938). [i] Webster and Powers, *ibid.*, **60**, 1553 (1938). [j] Crossley, *et al.*, *ibid.*, **60**, 2225 (1938). [k] Calcd.: C_2H_5OH, 13.62. Found: C_2H_5OH, 13.24.

TABLE II

TOXICITY AND THERAPEUTIC ACTIVITY OF SULFANILAMIDE DERIVATIVES COMPARED TO SULFANILAMIDE IN STREPTOCOCCAL INFECTIONS IN MICE

MTD = Maximum tolerated dose. MED = Minimum effective dose.

Compound	Toxicity in mice g. per kg.	Therapeutic activity g. per kg.
Sulfanilamide	MTD subcut. (oil) and orally 2.5	MED subcut. 0.5; oral 0.75
Sulfanilyl-		
-sulfanilamide	MTD subcut. (oil) and orally >8.0	MED subcut. 0.35; oral 1.6
-sulfanilamide sodium	Increased toxicity	Lowered activity
-sulfanilyl-sulfanilamide		Inferior activity
-aminoethanol		Inferior activity
-sulfanilyl-aminoethanol	Low toxicity	Slightly less active
-glycine		Inferior activity
-sulfanilyl-glycine	Low toxicity	Inferior activity
-4-carboxyaniline	Low toxicity	Little action
-4-nitroaniline	Twice as toxic as sulfanilamide	2 to 3 times as active
-4-aminoaniline	Same toxicity as sulfanilamide	2 times as active

character of the amino compound employed. When condensing strong bases, an excess of these may be used for neutralization of the hydrochloric acid which is formed by the reaction (Method I). When the compound is less basic in nature, the reaction occurs more easily in the presence of sodium bicarbonate (Method II) or sodium acetate, while the use of strong alkali as sodium hydroxide (Method III) or pyridine (Method IV) is required in condensing amino acids or nitro compounds. The addition of acetone, which dissolves the acetanilide-4-sulfochloride, sometimes accelerates the speed of the reaction.

The therapeutic activity of the compounds thus obtained is diminished by the presence of the acetyl group. The deacetylation is performed as originally described by P. Gelmo[5] by heating on a steam-bath with hydrochloric acid of specific gravity 1.05 (approximately 5 *N*) until solution occurs. Upon cooling a crystalline hydrochloride of the amino compound separates out in most

(5) Gelmo, *J. prakt. Chem.*, [2] **77**, 369 (1908).

cases. Sometimes a mixture of concentrated hydrochloric acid with alcohol is preferable for deacetylation.

Fractional precipitation was found to be useful in the purification of the acetylated as well as the deacetylated products.

Sodium hydrosulfite was found to be a convenient reagent for the reduction of nitro compounds.

Many of the derivatives of sulfanilamide do not show a sharp melting point, but soften before the melting point is reached. The melting point of those compounds which melt at high temperatures may be influenced by the rapidity of heating.

Table I shows a series of sulfanilyl and acetylsulfanilyl derivatives.

Table II gives a summary of the results obtained by Dr. S. M. Rosenthal in the treatment of beta hemolytic streptococcic infections in mice. Several of the compounds were found to possess a chemotherapeutic activity superior to that of sulfanilamide. The author wishes to thank Dr. E. Elvove and Dr. C. G. Remsburg for a part of the analyses reported.

Experimental

Acetylsulfanilyl-sulfanilamide. Method II.—Acetanilide-4-sulfochloride (25 g.) and sulfanilamide (17 g.) were dissolved in a mixture of acetone (150 cc.) and water (150 cc.). After addition of sodium bicarbonate (10 g.) the mixture was stirred. The bicarbonate dissolved with the evolution of carbon dioxide; after one hour the separation of the condensation product started and was completed by addition of water. The yield was 28 g. The product was purified by dissolving in sodium carbonate solution and precipitating with dilute hydrochloric acid. It was very sparingly soluble in water, soluble in alcohol, acetone and glacial acetic acid.

Acetylsulfanilyl-sulfanilyl-sulfanilamide. Method II.—Sulfanilyl-sulfanilamide and acetanilide-4-sulfochloride were condensed as described above. The reaction product was purified by fractional precipitation and finally by crystallization from acetone.

Acetylsulfanilyl-aminoethanol. Method I.—To a mixture of ethanolamine (50 g.) with water (170 cc.), acetanilide-4-sulfochloride (75 g.) gradually was added. The mixture became hot. Upon cooling the condensation product separated in crystals which were washed with 2 *N* hydrochloric acid and with cold water. The product was purified by dissolving in sodium hydroxide and then by fractional precipitation with dilute hydrochloric acid. It was soluble in water, very easily soluble in alcohol or acetone, insoluble in ether.

Acetylsulfanilyl-sulfanilyl-aminoethanol. Method II.—The condensation product separated as an oil which solidified on standing. It was purified by crystallization from ethyl acetate and subsequently by fractional precipitation with dilute hydrochloric acid from a solution of sodium carbonate in which it was dissolved. The product was sparingly soluble in water, soluble in acetone, alcohol and ethyl acetate.

Acetylsulfanilyl-glycine. Method III.—A suspension of acetanilide-4-sulfochloride (47 g.) and of glycine (15 g.) in a 2 *N* sodium hydroxide solution (200 cc.) was stirred for thirty minutes. A solution was effected which was filtered and acidified with dilute hydrochloric acid; white crystals separated. The yield was 50 g. The product was purified by dissolving in sodium carbonate solution, precipitating with hydrochloric acid and finally by crystallization from alcohol.

Acetylsulfanilyl-sulfanilyl-glycine was prepared according to Method III. It was soluble in alcohol.

Acetylsulfanilyl-4-carboxyaniline. Method III.—By condensing 4-aminobenzoic acid (14 g.) and acetanilide-4-sulfochloride (25 g.) in a mixture of 2 *N* sodium hydroxide (110 cc.) and acetone (50 cc.), a yield of 26 g. was obtained. The product was purified by reprecipitation; it was sparingly soluble in water, soluble in alcohol.

Acetylsulfanilyl-4-nitroaniline. Method IV.—To a mixture of 4-nitroaniline (7 g.), acetanilide-4-sulfochloride (12 g.) and acetone (40 cc.), pyridine (8 cc.) was added. The mixture turned warm from the heat generated. After three hours water was added; the reaction product separated in crystals and was dissolved in 2 *N* sodium carbonate in order to remove some unchanged nitroaniline. Upon acidification with 2 *N* hydrochloric acid, the filtered solution yielded yellow crystals (13 g.) which turned almost white upon crystallization from alcohol.

Sulfanilyl-sulfanilamide.—The acetyl derivative (28 g.) was deacetylated by boiling with 5 *N* hydrochloric acid (100 cc.) for half an hour. Upon cooling the hydrochloride of the amino compound separated in needles. The free base was isolated by neutralizing the suspension with sodium bicarbonate. The yield was 25 g. By crystallization from hot water beautiful needles were obtained which were sparingly soluble in cold water. One hundred cc. of water at 25° dissolved 0.057 g. The compound was soluble in hot water, alcohol, acetone, glacial acetic acid, acetic ether and ethylene glycol, not soluble in ether and benzene.

The hydrochloride melts at 224°. It is hydrolyzed by water. Owing to the acid function of the hydrogen of the secondary imide group the substance is soluble in dilute sodium carbonate solution and in ammonia.

A sodium salt was prepared by dissolving sulfanilylsulfanilamide in 2 *N* sodium hydroxide and adding alcohol and ether. The sodium salt crystallized out and formed a white powder, readily soluble in water; the solution gave an alkaline reaction. It was soluble in hot alcohol.

Sulfanilyl-sulfanilyl-sulfanilamide.—The deacetylation of the corresponding acetyl derivative (10 g.) was performed by heating with a mixture of hydrochloric acid (sp. gr. 1.19, 40 cc.), alcohol (90 cc.) and water (20 cc.) until the acetyl derivative was transformed to glistening crystals of the hydrochloride of the deacetylated compound. The free amino compound was precipitated out of sodium carbonate solution with hydrochloric acid as an oil which solidified rapidly. It was purified by extraction with alcohol. It was soluble in solutions of sodium carbonate and sodium bicarbonate. With mineral acids crystalline salts were formed which were hydrolyzed by water.

A sodium salt was prepared by dissolving in sodium hydroxide solution and precipitating with alcohol.

Sulfanilyl-aminoethanol.—The corresponding acetyl compound was deacetylated by boiling with the calculated amount of 5 *N* hydrochloric acid for half an hour. The free amino compound was purified by repeated crystallization from ethyl acetate and water. It was readily soluble in water, alcohol, acetone, moderately soluble in ethyl acetate, insoluble in ether.

Sulfanilyl-sulfanilyl-aminoethanol.—This compound was prepared and purified as described for sulfanilyl-aminoethanol. It was soluble in hot water, alcohol, acetone, ethyl acetate, insoluble in ether.

Sulfanilyl-glycine.—This compound was prepared as described for sulfanilyl-aminoethanol, and crystallized from water, then from alcohol. It formed colorless needles which were soluble, with an acid reaction, in water.

Sulfanilyl-sulfanilyl-glycine.—The deacetylation was performed with 5 *N* hydrochloric acid and yielded a crystalline hydrochloride. The free amino compound formed colorless needles which were soluble in alcohol, sodium carbonate and dilute hydrochloric acid.

Sulfanilyl-4-carboxyaniline.—By heating the acetyl derivative (13 g.) with 5 *N* hydrochloric acid (50 cc.) no solution was formed, the acetyl compound changing to a suspension of the hydrochloride of the deacetylated compound. The free amino compound crystallized from water in needles; when crystallized from alcohol, the compound contained one molecule of alcohol of crystallization.

Sulfanilyl-4-nitroaniline.—The deacetylation was made with a mixture of hydrochloric acid (sp. gr. 1.19) and twice its volume of alcohol, and yielded a crystalline hydrochloride. The free amino compound crystallized from 50% alcohol in yellowish needles.

Sulfanilyl-4-aminoaniline.—The sulfanilyl-4-nitroaniline was dissolved in 2 *N* sodium carbonate and was reduced by adding sodium hydrosulfite. The reduced product separated in colorless crystalline flakes which crystallized from water in long needles of silky luster. It was easily soluble in hot water and in alcohol. The hydrochloride was soluble in water, sparingly soluble in concentrated hydrochloric acid.

Discussion

The discovery of the chemotherapeutic action of sulfanilamide[6] followed closely after the original work of G. Domagk[7] upon "Prontosil." The simple structure of sulfanilamide raised the hope that a study of this compound and its derivatives would help to clarify our knowledge of the as yet unsolved problem of correlation of chemical constitution with chemotherapeutic activity.

While there can be no doubt that the sulfonamide group is of importance for chemotherapeutic action, it has been found that the effect is not restricted to this radical. Not only has it been shown that derivatives of diphenyl sulfide are active, but also that some derivatives of diphenyl sulfone[8,9] and diphenyl sulfoxide[10] possess a higher activity than sulfanilamide itself.

On the basis of the numerous compounds which have been studied up to the present time, it is not possible to generalize upon the essential nature of certain chemical groups. All that we can say is that with a given fundamental atom or radical, substituents of a different kind produce certain changes in the chemical and physical properties which enable the resulting molecule in its entirety to exert characteristic and specific chemotherapeutic effects.

Summary

A series of derivatives of sulfanilamide and their methods of preparation have been described. Several of these compounds show a chemotherapeutic activity superior to sulfanilamide. A summary of the results obtained in beta hemolytic streptococcal infections in mice is given.

WASHINGTON, D. C. RECEIVED DECEMBER 28, 1938

(6) M. and Mme. J. Tréfouel, F. Nitti and D. Bovet, *Compt. rend. soc. biol.*, **120**, 756 (1935).

(7) Domagk, *Deut. med. Wochschr.*, **61**, 250 (1935).

(8) G. A. H. Buttle, *et al.*, *Lancet*, **222**, 1331 (1937).

(9) E. Fourneau, *Compt. rend. acad. sci.*, **204**, 1763 (1937).

(10) C. Levaditi, *et al.*, *ibid.*, **205**, 1018 (1937).