[CONTRIBUTION FROM THE CHEMICAL LABORATORY, THE UNIVERSITY OF VERMONT, BURLINGTON, VERMONT]

GUANIDINE STRUCTURE AND HYPOGLYCEMIA: SOME SULFUR-CONTAINING DIGUANIDINES

CHARLES E. BRAUN* AND BERNARD J. LUDWIG†

Received October 27, 1937; revised April 4, 1938

INTRODUCTION

In 1935 Ackermann and Heinsen[1] reported that a marked fall in the blood-sugar level of dogs followed the administration of diguanylcystamine, $H_2N—C(:NH)—NH—CH_2CH_2—S—S—CH_2CH_2—NH—C(:NH)—NH_2$, a diguanidine containing the dithio linkage. This observation was especially interesting, as the dithio linkages appear to be essential for the physiological activity of the insulin molecule. With the possibility in mind of a relationship between guanidine structure, dithio linkages and insulin-like properties, we undertook to prepare and study physiologically the following new compounds: β,β'-dithiobis (α-guanidopropionic acid) dihydrochloride (I)‡, 4,4′-diguanidodiphenyldisulfide (II) and 4,4′-diguanidodiphenylsulfide (III), and thereby to extend the work on sulfur-containing diguanidines and their physiological behavior.

Only a few diguanidines containing dithio linkages appear to have been reported in the literature to date. Kapfhammer and Müller[2] prepared β,β'-dithiobis (α-guanidopropionic acid) (IV). Greenstein[3], in 1935, synthesized and described 5,5′-(dithiodimethylene) diglyco-

* On leave: now at Department of Chemistry, The Massachusetts Institute of Technology, Cambridge, Massachusetts.

† Eli Lilly Fellow in Organic Chemistry 1935–1936. Present address, Department of Chemistry, Columbia University, New York City.

[1] ACKERMANN AND HEINSEN, *Z. physiol. Chem.*, **235**, 115 (1935).

‡ The nomenclature in this field is unfortunately complicated by lack of uniformity. For example, Kapfhammer and Müller[2] reported β,β'-dithiobis(α-guanidopropionic acid) (IV) as α-diguanido-β-dithiodilactic acid. Greenstein[3,4] designated the anhydro form of this compound as anhydro α,α'-diguanido-di-(β-thiopropionic acid) and also as cystine cyamidene, and described α,α'-dithiobis(ϵ-guanidocaproic acid) as ϵ,ϵ'-diguanido-di-(α-thio-n-caproic acid). In discussing compounds of this type we have applied the rules for nomenclature adopted by *Chemical Abstracts* as an authoritative basis of nomenclature and have used it throughout this paper.

[2] KAPFHAMMER AND MÜLLER, *ibid.*, **225**, 1 (1934).

[3] GREENSTEIN, *J. Biol. Chem.*, **112**, 35 (1935).

cyamidine (V) and its dihydrochloride (VI), and also α,α'-dithiobis (ϵ-guanidocaproic acid)[4]. Ackermann and Heinsen (1) reported the synthesis and physiological properties of diguanylcystamine and tetramethyldiguanylcystamine.

As the low solubility in water of β,β'-dithiobis (α-guanidopropionic acid) (IV) renders it unsatisfactory for physiological studies, and also, since 5,5′-(dithiodimethylene) diglycocyamidine (V) is not a diguanidine but is a meta diazine, we prepared the dihydrochloride of β,β'-dithiobis (α-guanidopropionic acid) (I) which was readily soluble in cold water and therefore suitable for animal experimentation. It is significant to note here that this dihydrochloride (I) is the uncommon reaction product of hydrochloric acid reacting with β,β'-dithiobis (α-guanidopropionic acid) (IV), and is not merely another salt of a known base. The major product is the dihydrochloride (VI)§ of 5,5′-(dithiodimethylene) diglycocyamidine (V), (the meta diazine mentioned above), which was prepared and described by Greenstein.[3]

The two aromatic sulfur-containing diguanidines were selected for investigation for several reasons. Like neosynthalin,[5] which lowers blood sugar as a result of toxic action, they both contain a skeleton of twelve carbon atoms. The arrangement of these carbon atoms into two benzene rings is especially advantageous in that it should provide data in support or refutation of the statement that, "the benzene nucleus is not productive of hypoglycemia."[6] In addition, since one of our diguanidines is a disulfide and the other a monosulfide with their substituent groups in exactly corresponding positions, a direct comparison between these two types of sulfur linkages may be obtained. This is highly desirable because of possible connection with investigations on the rôle of the disulfide groups in the physiological activity of insulin. Furthermore, the availability of these two sulfur-containing diguanidines permits direct comparative physiological studies with two similar diguanidine structures, namely, 4,4′-diguanidobiphenyl and 4,4′-diguanidodiphenylmethane. The latter compounds together with certain other guanidines will be described in a subsequent paper.

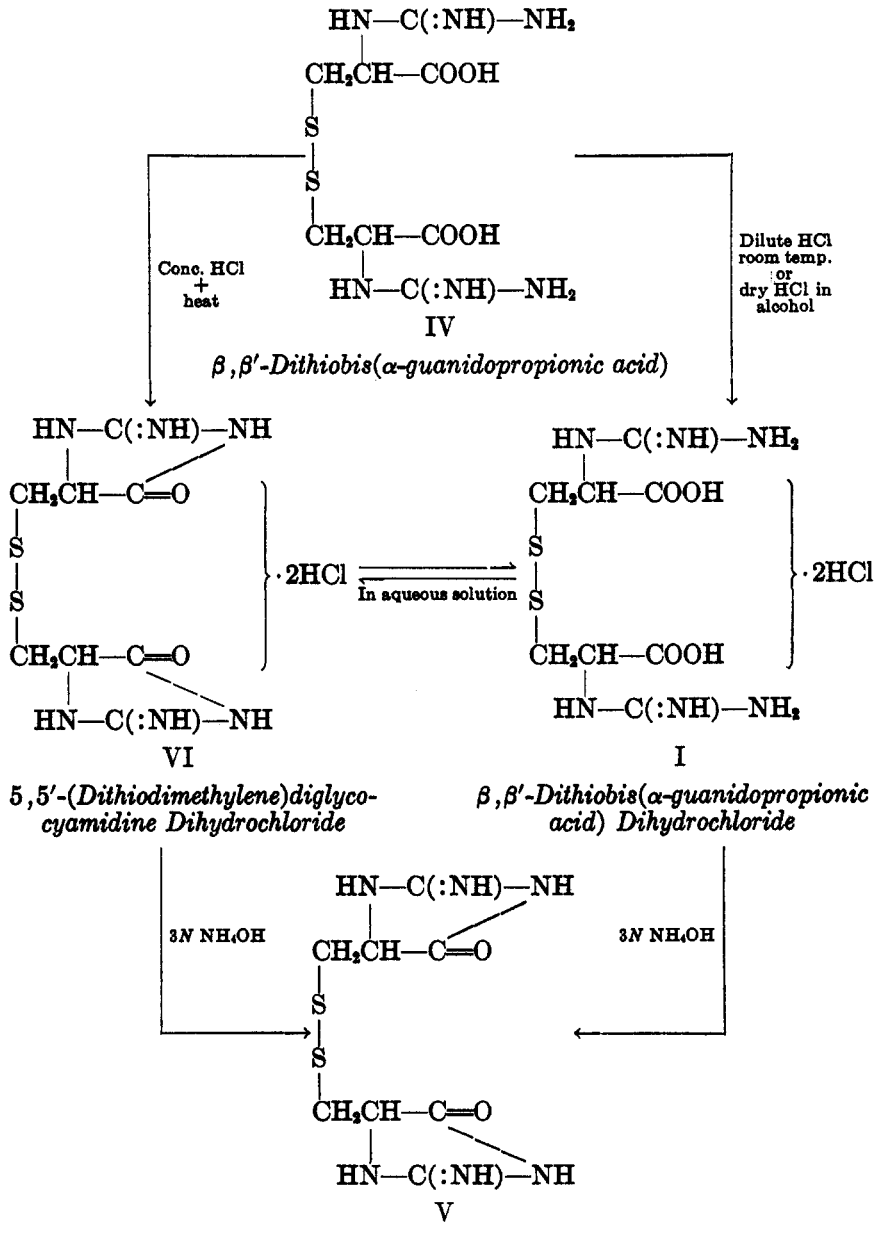
[4] GREENSTEIN, *ibid.*, **109**, 529 (1935).

§ Repetition of Greenstein's preparation of 5,5′-(dithiodimethylene)diglycocyamidine dihydrochloride (VI) gave a product, which after two recrystallizations from a methyl-ethyl alcohol mixture, melted at 154–155° (uncorr.). This is slightly higher than the melting point (150°) reported by Greenstein.

[5] FRANK, *Deutch. med. Woch.*, **53**, 1845 (1927); FRANK, NOTHMANN, AND WAGNER, *Klin. Woch.*, **7**, 1996 (1928).

[6] BISCHOFF, SAHYUN, AND LONG, *J. Biol. Chem.*, **81**, 325 (1929).

The structural relationships between the compounds involved in this work are shown in the following chart.



IV

β,β′-Dithiobis(α-guanidopropionic acid)

VI

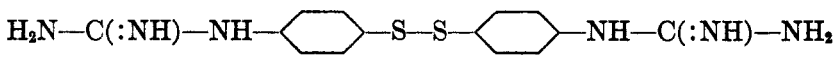
5,5′-(Dithiodimethylene)diglycocyamidine Dihydrochloride

I

β,β′-Dithiobis(α-guanidopropionic acid) Dihydrochloride

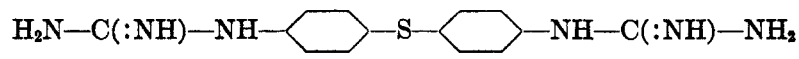
V

5,5′-(Dithiodimethylene)diglycocyamidine



II

4,4′-*Diguanidodiphenyldisulfide*



III

4,4′-*Diguanidodiphenylsulfide*

EXPERIMENTAL

Synthetic Part

Preparation of β,β′-dithiobis(α-guanidopropionic acid)dihydrochloride(I).—The starting compound for the preparation of β,β′-dithiobis(α-guanidopropionic acid) dihydrochloride (I) was β,β′-dithiobis (α-guanidopropionic acid) (IV) which was obtained in 57.4% yield (purified crystalline compound) from cystine by the method of Kapfhammer and Müller[2] excepting that *S*-methylisothiourea hydroiodide was substituted for *S*-ethylisothiourea hydrobromide.

Anal. Calc'd for $C_8H_{16}N_6O_4S_2$: N, 25.92. Found: N, 25.96 (micro-Kjeldahl).

A. β,β′-Dithiobis(α-guanidopropionic acid) (IV) with a molecular quantity of dilute hydrochloric acid.—β,β′-Dithiobis(α-guanidopropionic acid) (IV) heated on the steam bath with the stoichiometrical quantity of 0.1*N* hydrochloric acid (ratio 1:2) underwent decomposition accompanied by rupture of the dithio linkages as proved by the formation of free sulfur in appreciable quantity. Repetition of this experiment consistently gave the same result.

B. β,β′-Dithiobis(α-guanidopropionic acid) (IV) with an excess of dilute hydrochloric acid.—β,β′-Dithiobis(α-guanidopropionic acid) (IV) gave β,β′-dithiobis-(α-guanidopropionic acid) dihydrochloride (I) when treated with a large excess of dilute hydrochloric acid either at atmospheric pressure on a steam bath or at room temperature *in vacuo*. The latter procedure, which gave better yields, is described below.

Six grams (0.0185 mole) of β,β′-dithiobis(α-guanidopropionic acid) (IV) was dissolved in a solution of 120 cc. of concentrated hydrochloric acid diluted to 600 cc. with water. (This is exactly the same weight ratio as was used by Greenstein but differs in concentration of the acid.) The solution was evaporated to dryness *in vacuo* at room temperature. The residue (6.5 g.) was treated with boiling absolute ethyl alcohol, and the alcohol-insoluble fraction was separated by filtration and dried. It weighed 5 g. and melted at 144° (uncorr.), which agrees exactly with the melting point reported by Greenstein[3] for impure 5,5′-(dithiodimethylene)diglycocyamidine dihydrochloride (VI).

Anal. Calc'd for $C_8H_{14}Cl_2N_6O_2S_2$: N, 23.20. Found: N, 22.51 (micro-Kjeldahl).

Several volumes of cold dry acetone was added to the filtrate, whereupon a white amorphous precipitate formed at once. After standing over might in an ice chest, the acetone-alcohol supernatant liquid was decanted, and the precipitate was *rapidly* washed with anhydrous ether and dried at room temperature *in vacuo* over calcium chloride. The dried material was twice purified by dissolving it in a very small volume of boiling absolute ethyl alcohol, reprecipitating with dry acetone, washing with anhydrous ether and drying as described above. The purified compound did

not melt sharply but softened at about 94°, started to evolve a gas at 96°, the evolution of gas becoming very noticeable at 111° and continuing until 146° (uncorr.), when the substance finally decomposed. The final decomposition temperature is interesting in view of the melting point of impure 5,5′-(dithiodimethylene)diglycocyamidine dihydrochloride (144°). β,β′-Dithiobis(α-guanidopropionic acid) dihydrochloride (I) was a white, very hygroscopic powder whose aqueous solutions gave a positive Sakaguchi[7] reaction for guanidine residues. With alkaline lead acetate solution it gave a light-brown colored precipitate which did not turn black even after standing for one-half hour. The average yield from two experiments was 26.3%.

Anal. Calc'd for $C_8H_{18}Cl_2N_6O_4S_2$: C, 24.18. Found: C, 24.94
H, 4.57. Found: H, 4.99 (Semi-micro.)
N, 21.16. Found: N, 21.07 (Average of five analyses; micro-Kjeldahl.)

From the yields it appears that, even when a large excess of dilute acid is used, 5,5′-(dithiodimethylene)diglycocyamidine dihydrochloride (VI) predominates.

C. β,β′-Dithiobis(α-guanidopropionic acid) (IV) with dry hydrogen chloride.—Dry hydrogen chloride slowly bubbled into an ice-cold suspension of β,β′-dithiobis-(α-guanidopropionic acid) (IV) in either absolute methyl or ethyl alcohol followed by removal of the solvent *in vacuo* at 40-60° yielded β,β′-dithiobis(α-guanidopropionic acid)dihydrochloride (I). However, although this method gave higher yields and was less time consuming than the procedure described in Section B, it had the disadvantage of yielding a product which was difficult to purify and probably was always slightly impure as judged by the analytical data.

Anal. Calc'd for $C_8H_{18}Cl_2N_6O_4S_2$: N, 21.16. Found: N, 20.44 (micro-Kjeldahl).

Action of ammonia upon β,β′-dithiobis(α-guanidopropionic acid) dihydrochloride (I).—Three-gram samples of β,β′-dithiobis(α-guanidopropionic acid) dihydrochloride (I) prepared by three different procedures [*B-1*, by evaporation to dryness upon the steam bath of a solution of β,β′-dithiobis(α-guanidopropionic acid) (IV) with an excess of dilute hydrochloric acid; *B-2*, by evaporation to dryness at room temperature *in vacuo* of a solution of (IV) with an excess of dilute hydrochloric acid; *C-1*, by reaction between (IV) and dry hydrogen chloride in anhydrous alcoholic media], were dissolved in cold water, and each was treated with a slight excess of an ice cold solution of 3*N* ammonium hydroxide. In every case a white precipitate formed almost immediately. The precipitates were collected by filtration, washed with ice water, absolute ethyl alcohol, and ether, dried at room temperature *in vacuo*, and analysed.

Anal. Calc'd for $C_8H_{16}N_6O_4S_2$: N, 25.92
[β,β′-dithiobis(α-guanidopropionic acid) (IV)]
for $C_8H_{12}N_6O_2S_2$: N, 29.16*
[5,5′-(dithiodimethylene) diglycocyamidine (V)]
Found: (base from dihydrochloride *B-1*) N, 29.77
(base from dihydrochloride *B-2*) N, 29.59 (micro-Kjeldahl.)
(base from dihydrochloride *C-1*) N, 28.55

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[7] SAKAGUCHI, *J. Biochem. Tokyo*, **5**, 25, 133 (1925).

* The theoretical nitrogen content for 5,5′-(dithiodimethylene) diglycocyamidine (V) reported by Greenstein[3], page 37, should be 29.16%.

These data show that 5,5′-(dithiodimethylene) diglycocyamidine (V) was formed from β,β′-dithiobis(α-guanidopropionic acid) dihydrochloride (I) by reaction with ammonium hydroxide, and suggests that, in aqueous solution, an equilibrium exists between (I) and 5,5′-(dithiodimethylene) diglycocyamidine dihydrochloride (VI). Such an equilibriun would be similar to that which exists between glutamic acid and pyroglutamic acid in aqueous solution and which was recently discussed in detail by Wilson and Cannan[8].

If this equilibrium hypothesis is valid, then (I) in aqueous solution should give low values for primary amino nitrogen (assuming that the guanidine groups react) as determined by the method of Van Slyke[9] since the terminal amino groups are engaged in splitting out water to produce the anhydro form (VI). Conversely, if (VI), which contains no primary amino nitrogen, is partially converted into (I) in aqueous solution, then some amino nitrogen should be obtained when the method of Van Slyke is applied because the opening of the two meta diazine rings by the addition of two molecules of water must produce two primary amino groups at the terminal positions of each guanidine residue.

Amino nitrogen determinations carried out at 21° on freshly prepared sloutions of (I) and (VI) gave the following results:

For β,β′-dithiobis(α-guanidopropionic acid) dihydrochloride (I) $C_8H_{18}Cl_2N_6O_4S_2$:

Sample (mg.)	*Calcd. N, as NH_2 (mg.)*	*Found: N, as NH_2 (mg.)*
33.4 (15 min.)	2.35	0.592
33.4	2.35	0.542
33.4 (20 min.)	2.35	0.671
33.4	2.35	0.587
	Avge.	0.598 (25.45% of the theoretical)

For 5,5′-(dithiodimethylene) diglycocyamidine dihydrochloride (VI) $C_8H_{14}Cl_2N_6O_2S_2$

71.54	0.00	2.63
71.54 (20 min.)	0.00	2.68
71.54	0.00	2.85
	Avge.	2.72

The above data supported the suggestion that (I) and (VI) in aqueous solution exist in a state of equilibrium.

The results also showed, obviously, that the guanidine residues in the two compounds investigated reacted with nitrous acid to liberate nitrogen, a fact which is interesting in view of Van Slyke's observation[9] that the guanidine groups in guanidine, creatine, and arginine did not react with nitrous acid.

Preparation of 4,4′-diguanidodiphenyldisulfide sulfate.—4,4′-Dithioaniline prepared from *p*-chloronitrobenzene by the method of Lantz[10] melted at 78° (uncorr.) after recrystallization from 50% ethyl alcohol. The average yield from several runs was 50%. The picrate, recrystallized from 95% ethyl alcohol, melted at 183° (uncorr.).

The dihydrochloride was made by bubbling dry hydrogen chloride into an absolute ethyl alcohol solution of the base. The precipitated dihydrochloride was purified by dissolving it in hot ethyl alcohol and reprecipitating with acetone. The purified

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[8] WILSON AND CANNAN, *J. Biol. Chem.*, **119**, 309 (1937).

[9] VAN SLYKE, *ibid.*, **9**, 185 (1911); **12**, 275 (1912).

[10] LANTZ, U. S. Patent No. 1,933,217 (1933).

4,4'-dithioaniline dihydrochloride was obtained in the form of colorless needles, soluble in water, m.p. 231° (uncorr.).

Anal. Calc'd for $C_{12}H_{14}Cl_2S_2N_2$: Cl, 22.08. Found: Cl, 21.84.

Twenty grams (0.062 mole) of 4,4'-dithioaniline dihydrochloride and 7 grams (0.167 mole) of cyanamide (Eastman Kodak Co.) in 150 cc. of absolute methyl alcohol were heated under reflux on the steam bath for eighteen hours. The resulting solution was then concentrated to one-third of its original volume, diluted to about 425 cc. with water, treated with Norite, cooled in an ice bath, and made alkaline with a slight excess of an ice-cold 10% solution of sodium hydroxide. (This procedure was adopted after preliminary experiments had shown that the dihydrochloride of the diguanidine could not be isolated in crystalline condition from the reaction mass.) The base, 4,4'-diguanidodiphenyldisulfide (II), separated at once as a voluminous yellow precipitate, which was collected by filtration and air-dried at 100° (yield 17 g.).

A small amount of the crude base was recrystallized from boiling dilute (35%) ethyl alcohol. The purified compound was obtained in the form of pale yellow plates, m.p. 178° (uncorr.).

Anal. Calc'd for $C_{14}H_{16}N_6S_2$: N, 25.29. Found: N, 24.53 (micro-Kjeldahl).
S, 19.29. Found: S, 19.11 (Carius).

The remainder of the crude base was converted directly into the sulfate by heating it on the steam bath with a large volume (about 500 cc.) of very dilute sulfuric acid. The hot solution was treated with Norite, and filtered, and the filtrate was allowed to cool *very slowly*. Soon a heavy viscous oil settled out. The clear, pale yellow supernatant liquid was separated from the oil by decantation and allowed to stand for several days, after which crystals of the sulfate precipitated. These were collected by filtration and washed with ice water and absolute ethyl alcohol. The oily layer was taken up in a large volume of hot, very dilute sulfuric acid and allowed to crystallize *slowly*. The conversion of the oil into a crystalline solid was tedious and required many repetitions of the dilute sulfuric acid procedure.

The sulfate was finally purified by recrystallization from dilute ethyl alcohol. When first obtained, the sulfate appeared as fine white needles which became pale yellow upon standing. Storage of the colorless material either in a non-oxidizing atmosphere or in the dark failed to prevent the transition into the yellow variety. The melting points of both varieties were identical, 257–258° (uncorr.), and a mixture melting point showed no depression. Furthermore, there appeared to be no change in the crystalline form accompanying the transition, and analyses on each variety gave identical results. The final yield (purified yellow form) was 4.0 g.

Anal. Calc'd for $C_{14}H_{16}N_6S_2 \cdot H_2SO_4$: Sulfate S, 7.45. Found: S, 7.44
N, 19.53. Found: N, 19.41.
(Semi-micro Dumas).

The picrate of 4,4'-diguanidodiphenyldisulfide (II), after recrystallization from 95% ethyl alcohol, was bright orange and melted at 199° (uncorr.).

Preparation of 4,4'-diguanidodiphenylsulfide sulfate.—*p*-Thioaniline (*p*-aminophenylsulfide) was kindly supplied by E. I. duPont de Nemours & Company. It was purified by recrystallization from 35% ethyl alcohol until the melting point was 107–108° (uncorr.). The picrate, recrystallized from 95% ethyl alcohol, melted at 194° (uncorr.).

The corresponding dihydrochloride was obtained by bubbling dry hydrogen chloride into an absolute ethyl alcohol solution of the purified base. After recrystallization from 95% ethyl alcohol with the addition of ether, the dihydrochloride melted at 241° (uncorr.).

Anal. Calc'd for $C_{12}H_{14}Cl_2N_2S$: Cl, 24.53. Found: Cl, 24.69.

Twenty grams (0.069 mole) of *p*-thioaniline dihydrochloride and 6 g. (0.143 mole) of cyanamide in 230 cc. of absolute methyl alcohol were heated under reflux on the steam bath for fifteen hours. The resulting solution was then concentrated at room temperatiure *in vacuo* to one-fifth of its original volume. The concentrate was diluted with 600 cc. of water, treated with Norite, and cooled in an ice bath. It was then made alkaline with a slight excess of an ice-cold 10% solution of sodium hydroxide. The oil which first separated crystallized upon standing in the ice chest. The crystals were collected by filtration, washed with ice water, and dried at room temperature *in vacuo* over sulfuric acid.

A small sample of the crude 4,4′-diguanidodiphenylsulfide (III) was purified by recrystallization from boiling water. The purified base, glistening colorless prisms, melted at 203–204° (uncorr.) with decomposition.

Anal. Calc'd for $C_{14}H_{16}N_6S$: S, 10.67. Found: S, 10.77. (Carius).

The remainder of the crude base was converted directly into the sulfate by dissolving it in hot dilute sulfuric acid and allowing the solution to cool slowly. The sulfate crystallized out readily in glistening white plates. It was recrystallized without difficulty from hot dilute sulfuric acid, care being taken to wash the crystals thoroughly with ice water to remove any adhering sulfuric acid. The dried sulfate (6.0 g.) did not melt up to 290°.

Anal. Calc'd for $C_{14}H_{16}N_6S \cdot H_2SO_4$: N, 21.10. Found: N, 20.98 (micro Kjeldahl)
Sulfate S, 8.05. Found: 8.01.

The picrate of 4,4′-diguanidodiphenylsulfide, after recrystallization from 95% ethyl alcohol, was bright yellow and melted at 168° (uncorr.).

As in the case of 4,4′-diguanidodiphenyldisulfide (II), it was found by preliminary experimentation that the dihydrochloride of 4,4′-diguanidodiphenylsulfide (III) could not be isolated from the reaction mass, and therefore the procedure, as described above, was adopted. It should also be noted, that, recrystallization of the sulfate of 4,4′-diguanidodiphenylsulfide (III) presented none of the difficulties encountered in recrystallization of the sulfate of the corresponding disulfide (II). There was no transition of 4,4′-diguanidodiphenylsulfide sulfate from the white to the yellow variety as was noted with 4,4′-diguanidodiphenyldisulfide sulfate. Similar transitions between white and yellow forms of the same compound have been reported in the literature on amino derivatives of aromatic sulfides.

The Sakaguchi reaction for guanidine residues could not be applied to either of these two aromatic sulfur-containing diguanidines, as the formation of a black precipitate completely masked the color of the solution.

Physiological Part

The physiological investigation of β,β′-dithiobis(α-guanidopropionic acid) dihydrochloride (I), 4,4′-diguanidodiphenyldisulfide sulfate and 4,4′-diguanidodiphenylsulfide sulfate was carried out at The Lilly Research Laboratories.* The data obtained are recorded in the accompanying table.

It is evident from these results that no hypoglycemia followed administration of these three sulfur-containing diguanidines even in doses as high as 100 mg. (calculated as free base) per kilo of body weight. Also, there was no evidence of acute toxicity with any of the three diguanidines. The failure to obtain hypoglycemia is

* For an outline of the method used for the physiological assay, the reader is referred to a previous paper: BRAUN AND LUDWIG, J. ORG. CHEM., **2**, 442 (1937).

consistent with the observations of Greenstein and Friedgood[11] who reported that α,α-dithiobis(ϵ-guanidocaproic acid) produced no hypoglycemia, but in high doses gave marked hyperglycemia accompanied by death of some of the animals. On the other hand, these negative results are not in accord with the observations of Ackermann and Heinsen[1] who reported that diguanylcystamine produced marked hypoglycemia. However, these investigators noted also that tetramethyldiguanylcystamine caused a sharp increase in the blood sugar.

Since it has been shown here that β,β'-dithiobis(α-guanidopropionic acid) dihydrochloride (I) and 5,5'-(dithiodimethylene) diglycocyamidine dihydrochloride (VI) both gave the same base, 5,5'-(dithiodimethylene) diglycocyamidine (V), when their aqueous solutions were treated with ammonium hydroxide, and since administration of (I) produced no hypoglycemia, it might reasonably be presumed that the anhydro form, (VI), is also devoid of hypoglycemic properties.

TABLE

COMPOUND	DOSE IN MG. FREE BASE PER KILO	BLOOD SUGAR IN MG. PER 100 CC. BLOOD TIME AFTER ADMINISTRATION			
		Initial	1.5 hr.	3 hr.	5 hr.
β,β'-Dithiobis(α-guanidopropionic acid) dihydrochloride (I)	17.5	88.0	107.0	93.0	102.0
	17.5	102.0	109.0	100.0	110.0
	100.0	117.2	108.8	105.9	114.4
4,4'-Diguanidodiphenyldisulfide (II) (Sulfate)	17.5	105.0	117.0	105.0	114.0
	17.5	107.0	114.0	107.0	110.0
	100.0	111.6	103.1	101.7	105.9
4,4'-Diguanidodiphenylsulfide (III) (Sulfate)	17.5	89.0	103.0	88.0	99.0
	17.5	107.0	127.0	105.0	105.0
	100.0	91.8	107.4	104.5	103.1

While entirely too few diguanidines containing the dithio linkage have been prepared and studied for hypoglycemic properties to permit the formulation of any conclusion regarding a possible relationship between structure and physiological activity in this type of compound, nevertheless, it appears evident from the data now available that the mere presence of guanidine residues and dithio linkages in a molecule will not render that compound capable of producing hypoglycemia.

The authors wish to thank Dr. H. A. Lubs of E. I. du Pont de Nemours & Company, Wilmington, Delaware, for the *p*-thioaniline used in this work, and Mr. H. A. Shonle and Dr. E. D. Campbell of The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, for the physiological assays.

SUMMARY

1. Methods for the preparation of β,β'-dithiobis (α-guanidopropionic acid) dihydrochloride (I) have been described, and some evidence for a

[11] GREENSTEIN AND FRIEDGOOD, *J. Biol. Chem.*, **114**, Proc. xliv (1936).

possible equilibrium relationship between (I) and its anhydro form, 5,5′-(dithiodimethylene) diglycocyamidine dihydrochloride (VI) in aqueous solution has been presented.

2. The methods of synthesis for 4,4′-diguanidodiphenyldisulfide (II), its picrate and sulfate, and for 4,4′-diguanidodiphenylsulfide (III), its picrate and sulfate, have been described.

3. The physiological behavior of these three diguanidines has been investigated, and the observations are recorded and briefly discussed in relation to the general problem of guanidine structure and hypoglycemic activity.