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

GUANIDINE STRUCTURE AND HYPOGLYCEMIA:* SOME CARBOCYCLIC DIGUANIDINES

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Received May 31, 1938

INTRODUCTION

Certain reports in the literature on guanidine structure and hypoglycemic activity suggested the preparation of three carbocyclic diguanidines, *p*-phenylenediguanidine (I), 4,4′-diguanidobiphenyl (II) and 4,4′-diguanidodiphenylmethane (III), in order to compare their physiological action with 1,6-diguanidohexane which Kumagai, Kawai and Shikinami[1] reported as equal to synthalin (1,10-diguanidodecane) in hypoglycemic activity.

Para phenylenediguanidine (I) was of interest because in it the six skeletal carbon atoms of 1,6-diguanidohexane are combined in a benzene nucleus while the two guanido groups occupy comparable positions of maximum distance from each other. Previously it was shown by one of us[2] that when *p*-phenylenediamine was condensed with an *S*-alkylisothiourea salt only one amino group reacted, and a salt of *p*-aminophenylguanidine was obtained instead of *p*-phenylenediguanidine. The fact that *p*-aminophenylguanidine was physiologically inactive[3] made it even more desirable to obtain the corresponding guanyl derivative, *p*-phenylenediguanidine (I), for physiological study.

In 1928 Bischoff[4] obtained impure 4,4′-diguanidobiphenyl sulfate and made the observation that it lowered the blood sugar of normal rabbits. In view of the fact that his compound was admittedly impure, the question arises as to whether the hypoglycemic effects were due to 4,4′-diguanidobiphenyl (II) itself, or to some impurity. This issue could be settled only if pure 4,4′-diguanidobiphenyl (II) were available. Not only was the reported magnitude of the hypoglycemic effect[5] sufficiently great to justify

* For other papers in this series see BRAUN AND LUDWIG, J. ORG. CHEM., **2**, 442 (1937); **3**, 16 (1938).

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[1] KUMAGAI, KAWAI, AND SHIKINAMI, *Proc. Imp. Acad.* (Japan), **4**, No. 1, 23 (1928).

[2] BRAUN, *J. Biol. Chem.*, **89**, 97 (1930); *J. Am. Chem. Soc.*, **54**, 1511 (1932).

[3] PARKS, AND BRAUN, *J. Biol. Chem.*, **91**, 629 (1931).

[4] BISCHOFF, *ibid.*, **80**, 345 (1928).

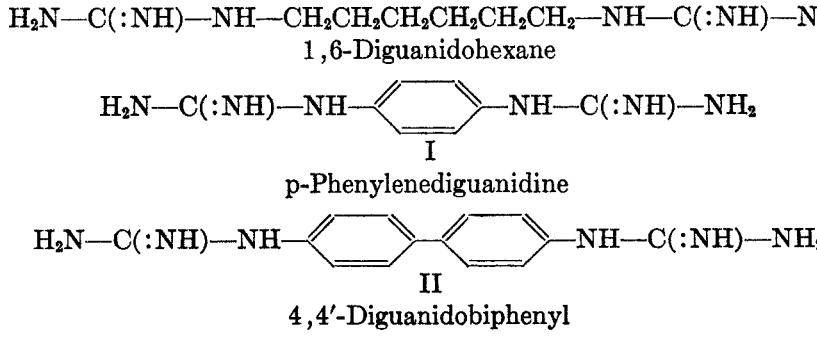
[5] BISCHOFF, SAHYUN, AND LONG, *ibid.*, **81**, 325 (1929).

attempts to prepare pure 4,4′-diguanidobiphenyl (II) and to study its hypoglycemic effects, but this compound would also offer the opportunity for direct comparison of its hypoglycemic activity with that of neosynthalin (1,12-diguanidododecane). In 4,4′-diguanidobiphenyl (II) the twelve skeletal carbon atoms are combined into two attached benzene nuclei, and in neosynthalin they are in a straight chain, while in both compounds the two guanido groups are at maximum distances from each other.

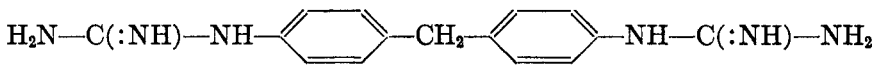
The attempted preparation of 4,4′-diguanidobiphenyl (II) by the Ullmann reaction[6] necessitated the synthesis of certain new salts of *p*-bromophenylguanidine and a new monoguanidine, *p*-iodophenylguanidine. All efforts to couple either *p*-bromophenylguanidine or *p*-iodophenylguanidine by means of copper or silver failed to produce any traces of II. In the case of *p*-iodophenylguanidine appreciable amounts of the starting compound were recovered from the reaction mass as well as some *p*-iodoaniline, the latter probably having been formed by decomposition of *p*-iodophenylguanidine, as evidenced by the continuous evolution of ammonia throughout the attempted coupling even under mild conditions.

The availability of 4,4′-diguanidodiphenylmethane (III) for physiological study offers interesting considerations. In this compound (III) the two guanido groups are attached directly to aromatic nuclei as they are in 4,4′-diguanidobiphenyl (II). However, in III the two benzene residues are not directly attached as they are in II, but are separated by a single methylene group. It seems of interest to study the physiological action of 4,4′-diguanidodiphenylmethane (III) in order to determine to what extent, if any, the slight but very significant structural change of introducing one methylene group between the two benzene nuclei holding the guanido groups would have upon the hypoglycemic properties as compared directly with those of 4,4′-diguanidobiphenyl (II).

The details of the syntheses of these mono- and diguanidines and a brief comparison of the physiological activity of the latter with that of 1,6-diguanidohexane are presented in this paper. The structural relationships between the compounds under discussion are shown below.



[6] ULLMANN, *Ann.*, **332**, 38 (1904).



III

4,4′-Diguanidodiphenylmethane

EXPERIMENTAL

Synthetic Part

Preparation of 1,6-diguanidohexane sulfate.—Suberic acid (Kahlbaum) was converted in the usual manner through the dichloride into its diamide (m.p. 218–219° uncorr.).* The diamide through a double Hofmann degradation after the method of Van Breukeleveen[7] yielded hexamethylenediamine. 1,6-Diguanidohexane sulfate was obtained in 14.4% yield (purified compound) from the diamine and *S*-methylisothiourea sulfate according to Heyn[8]. The purified crystalline sulfate was discolored but not molten at 340°. (Heyn reported that this compound melts above 280°.)

Anal. Calc'd for $C_8H_{22}N_6O_4S$: S, 10.75; Found: S, 10.85.

Preparation of p-*phenylenediguanidine* (*I*).—Twenty-five grams (0.138 mole) of *p*-phenylenediamine dihydrochloride (Eastman Kodak Co.) and 18 g. (0.429 mole) of cyanamide (Eastman Kodak Co.) in 570 cc. of absolute ethyl alcohol were heated under reflux on a steam bath for ten hours. The resulting dihydrochloride (12.8 g.) was filtered off, washed with ether and dried. The crude salt was dissolved in 50 cc. of hot water, boneblacked, and to the cold filtrate was added 60 cc. of a 10% solution of sodium hydroxide. *p*-Phenylenediguanidine precipitated as lustrous white plates which were filtered off, washed with cold water and dried *in vacuo*. The base, after recrystallization from hot water, melted at 258–259° uncorr. with decomposition. The yield (purified crystalline compound) was 9.0 g. or 34.0%.

Anal. Calc'd for $C_8H_{12}N_6$: N, 43.79; Found: N, 43.55 (micro-Kjeldahl).

The base when first formed appeared as large lustrous white plates and probably was a hydrate. After standing *in vacuo* over sulfuric acid the lustre entirely disappeared and the material changed into a dull finely crystalline powder which was not a hydrate as shown by analysis. The base was soluble in hot absolute alcohol and hot water. Its aqueous solutions were strongly alkaline.

The picrate of *p*-phenylenediguanidine, after recrystallization from a large volume of boiling water, was bright golden yellow. It darkened at about 290° but was not molten at 317°.

Seven and one-half grams (0.039 mole) of the base was suspended in 470 cc. of warm 70% ethyl alcohol, and dry hydrogen chloride passed in until complete solution had been effected. The hot solution was boneblacked, filtered, and cooled, and dry ether was added until permanent turbidity was obtained. Upon standing in an ice-chest the dihydrochloride crystallized out. The white crystalline salt was filtered off, washed with dry ether and dried *in vacuo* at room temperature. The dihydrochloride was very soluble in cold water and melted at 315° uncorr. The yield (purified compound) calculated from the base was 7.3 g. or 70.9%.

Anal. Calc'd for $C_8H_{14}Cl_2N_6$: N, 31.70; Cl, 26.75.
Found: N, 31.39 (micro-Kjeldahl); Cl, 26.68.

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* The melting point reported for suberic diamide in Beilstein, 4th Ed., II, 694, is 216–217°.

[7] VAN BREUKELEVEEN, *Rec. trav. chim.*, **13**, 34 (1894).

[8] HEYN, *U. S. Patent* 1,737,192 (Nov. 26, 1929).

(In carrying out the chloride determinations, the silver chloride precipitates were removed by filtration at 60–70° to prevent the precipitation of *p*-phenylenediguanidine nitrate which is insoluble in cold water.)

Preparation of p-*bromophenylguanidine.*†—Thirty-six grams (0.173 mole) of *p*-bromoaniline hydrochloride and 8 g. (0.190 mole) of cyanamide in 100 cc. of absolute ethyl alcohol were heated for thirteen hours under reflux on a steam bath. The resulting solution, after being cooled, was treated with dry ether until permanent turbidity resulted. After standing in an ice-chest a crystalline mass of *p*-bromophenylguanidine hydrochloride formed. The product was filtered off, washed with dry ether, and dried *in vacuo* over sulfuric acid. After purification by re-solution in absolute alcohol and slow precipitation with petroleum ether the hydrochloride melted at 175° uncorr. The yield (purified crystalline salt) was 33 g. or 76.3%.

Anal. Calc'd for $C_7H_8BrClN_3$: Cl, 14.20; Found: Cl, 14.18.

The free base (m.p. 121–123° uncorr.), produced by the addition of a dilute solution of sodium hydroxide to an aqueous solution of the hydrochloride, gradually absorbed carbon dioxide from the air and formed the normal carbonate. After recrystallization from hot water the carbonate was obtained as small clusters of fine colorless needles, melting at 145–149° uncorr. with decomposition.

Anal. Calc'd for $(C_7H_8BrN_3)_2 \cdot H_2CO_3$: Br, 32.61; Found: Br, 32.48 (micro-Carius).

The picrate *of p-bromophenylguanidine, after recrystallization from boiling* dilute ethyl alcohol, was deep yellow and melted at 220° uncorr.

Preparation of p-*iodophenylguanidine.*—Twelve and one-half grams (0.049 mole) of *p*-iodoaniline hydrochloride and 3.5 g. (0.083 mole) of cyanamide in 50 cc. of absolute ethyl alcohol were heated under reflux on a steam bath for seventeen hours. The resulting solution was boneblacked, filtered hot, and then diluted with water. After the alcohol had been distilled off, the remaining aqueous solution was treated with a dilute solution of sodium hydroxide, whereupon an oily layer formed at once. The latter solidified after standing in the cold. The solid material was collected by filtration and treated with 100 cc. of boiling water. The oily layer of unreacted *p*-iodoaniline was allowed to settle, and the clear supernatant liquid was removed by decantation. After concentration to about 70 cc. the aqueous layer upon standing in the cold deposited white crystalline *p*-iodophenylguanidine, which was collected on a filter, washed with ice water and dried. The free base, as in the case of *p*-bromophenylguanidine, absorbed carbon dioxide and formed a stable normal carbonate. The latter, after purification by repeated recrystallizations from hot water, melted at 147–149° uncorr. The yield (purified compound) was 3.5 g. or 12.2%.

Anal. Calc'd for $(C_7H_8IN_3)_2 \cdot H_2CO_3$: I, 43.46; Found: I, 43.69 (Carius).

The picrate of *p*-iodophenylguanidine, after recrystallization from boiling dilute ethyl alcohol, was bright yellow and melted at 235° uncorr.

Some of the carbonate was suspended in absolute ethyl alcohol, and dry hydrogen chloride was passed in until all of the solid had dissolved. The resulting solution was boneblacked, filtered hot, and concentrated to a small volume (5 cc.). After cooling, anhydrous ether was added until permanent turbidity resulted. Upon

† Harwood[9] first prepared the free base (m.p. 122–124°) and the nitrate (m.p. 185–186°) of *p*-bromophenylguanidine by heating under pressure an alcoholic solution of *p*-bromoaniline hydrochloride and cyanamide, but did not isolate the hydrochloride, carbonate or picrate.

[9] HARWOOD, Thesis, Iowa State College, **1931.**

standing in the ice-chest for several hours the white *p*-iodophenylguanidine hydrochloride crystallized. It was purified by re-solution in absolute alcohol and reprecipitation with dry ether. The pure hydrochloride melted at 151–153° uncorr.

Anal. Calc'd for $C_7H_9ClIN_3$: Cl, 11.92; Found: Cl, 12.01.

Preparation of 4,4'-diguanidobiphenyl (II).—Twelve grams (0.0467 mole) of benzidine dihydrochloride and 5 g. (0.119 mole) of cyanamide in 75 cc. of freshly distilled isoamyl alcohol were heated under reflux on an oil bath for twenty-three hours. (There was never complete solution of the reactants during the condensation.) The white, insoluble reaction product was filtered off, washed with ether, and dried *in vacuo* at room temperature. The free base was obtained from the crude dihydrochloride by treating the latter in hot aqueous solution with an excess of dilute sodium hydroxide. The base, after three recrystallizations from a large volume of boiling water and drying at 100° *in vacuo*, melted at 234–236° uncorr. with decomposition. The yield (crystalline base) was 4.5 g. or 36%.

Anal. Calc'd for $C_{14}H_{16}N_6$: N, 31.33; Found: N, 30.64‡ (Semimicro-Dumas).

The picrate of 4,4'-diguanidobiphenyl (II) was obtained from a large volume of boiling water as an orange, finely crystalline material which, upon being heated, gradually discolored (badly discolored at 290°) and finally decomposed without melting at 308° uncorr. This orange picrate presents an interesting contrast with the picrates of the other diguanidines described in this paper, all of which were bright yellow.

The dihydrochloride was prepared by suspending the free base in boiling absolute ethyl alcohol and adding dilute hydrochloric acid until complete solution had been effected. The hot solution was boneblacked, filtered, and cooled. Acetone and ether were then added until permanent turbidity resulted. Upon standing in the cold, the white crystalline dihydrochloride precipitated out. It was filtered off, washed with dry acetone and ether and dried at 100° *in vacuo*. It did not melt up to 300°.

Anal. Calc'd for $C_{14}H_{18}Cl_2N_6$: N, 24.64; Cl, 20.79.
Found: N, 24.13 (Kjeldahl); Cl 20.60.

The sulfate was obtained by adding dilute sulfuric acid to a suspension of the free base in warm water. The crude sulfate, after being washed free of sulfuric acid with cold absolute ethyl alcohol, was recrystallized from boiling water. The purified sulfate (glistening prisms) melted at 318–320° uncorr., with decomposition.

Anal. Calc'd for $C_{14}H_{16}N_6 \cdot H_2SO_4$: S, 8.75; Found: S, 8.73.

Preparation of 4,4'-diguanidodiphenylmethane (III).—4,4'-Diaminodiphenylmethane (Eastman Kodak Co.) was converted into its dihydrochloride by heating it on a steam bath with concentrated hydrochloric acid. The dihydrochloride, recrystallized from hot concentrated hydrochloric acid, melted at 282° uncorr.§

Anal. Calc'd for $C_{13}H_{16}Cl_2N_2$: Cl, 26.16; Found: Cl, 26.02.

Twenty-three grams (0.0848 mole) of 4,4'-diaminodiphenylmethane dihydrochloride and 10 g. (0.238 mole) of cyanamide in 40 cc. of absolute ethyl alcohol were heated under reflux on the steam bath for five hours. The resulting solution was

‡ This compound was very difficult to burn. Although the nitrogen analysis was only in fair agreement with the calculated value, subsequent analyses on the dihydrochloride and sulfate salts prepared from this base left no doubt as to its identity.

§ The melting point reported for 4,4'-diaminodiphenylmethane dihydrochloride in Beilstein, 4th Ed., XIII, 239, is 285°.

filtered, diluted with water and made alkaline by the addition of an excess of dilute aqueous sodium hydroxide solution, whereupon the free base, 4,4′-diguanidodiphenylmethane (III), precipitated out at once. It was collected by filtration, washed with ice water until free of alkali and dried at 105–110°. After four recrystallizations from boiling water (200 cc.) the base was obtained as small colorless plates which melted at 199–200° uncorr., with decomposition. The yield (purified base) was 8 g. or 34%.

The picrate, slender, light-yellow needles from boiling dilute ethyl alcohol, softened and turned orange at 200–202° and melted at 229–230° uncorr.

The sulfate was prepared by the addition of dilute sulfuric acid to a hot aqueous solution of the free base (III). Upon concentrating the solution and cooling, the sulfate crystallized in glistening, colorless prisms. These were filtered off, washed with absolute ethyl alcohol, and recrystallized from boiling dilute alcohol. The purified sulfate melted at 254–256° uncorr., with decomposition.

Anal. Calc'd for $C_{15}H_{18}N_6 \cdot H_2SO_4$: S, 8.43; Found: S, 8.44.

Both 4,4′-diguanidobiphenyl (II) and 4,4′-diguanidodiphenylmethane (III) absorbed carbon dioxide from the air and formed stable carbonates.

The free base and the salts of 4,4′-diguanidodiphenylmethane (III) were much more readily soluble in water than the corresponding derivatives of 4,4′-diguanidobiphenyl (II).

Physiological Part

The physiological assays of 1,6-diguanidohexane, *p*-phenylenediguanidine (I), 4,4′-diguanidobiphenyl (II) and 4,4′-diguanidodiphenylmethane (III) were carried out in part at The Lilly Research Laboratories and in part at the Department of Physiology, College of Physicians and Surgeons, Columbia University, normal rabbits serving as the experimental animals in all of the work. The compounds were administered subcutaneously, and at various time intervals following injection the blood sugar was determined by the micro method of Shaffer and Somogyi[10] or by the macro procedure of Shaffer and Hartmann[11] using the conversion tables of Duggan and Scott.[12] In the micro procedure 0.25 cc. of blood was diluted 1 to 15 with the zinc reagent, and 2 cc. of the filtrate was used for each determination.

1,6-Diguanidohexane was hypoglycemic in doses greater than 30 mg.* per kilo of body weight but in smaller doses produced no hypoglycemia up to five hours following administration. This agreed with the observations of Kumagai, Kawai, and Shikinami[1].

Para phenylenediguanidine (I), in doses as high as 50 mg. per kilo, exhibited no hypoglycemic activity but was hyperglycemic at half this dosage, the hyperglycemia being especially marked shortly (up to about two hours) after injection of the compound. It appeared therefore, that cyclization of the six carbon atoms of 1,6-diguanidohexane into a benzene nucleus, as in *p*-phenylenediguanidine (I), destroyed the hypoglycemic properties and imparted hyperglycemic activity but without apparent increase in toxicity.

Pure 4,4′-diguanidobiphenyl (II) produced hypoglycemia in doses of 35 mg. per kilo. This definitely established the hypoglycemic properties of this compound, and confirmed the work of Bischoff[4] and Bischoff, Sahyun, and Long[5], who, after

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[10] Shaffer, and Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

[11] Shaffer, and Hartmann, *ibid.*, **45**, 365 (1920).

[12] Duggan, and Scott, *ibid.*, **67**, 287 (1926).

* All of the doses reported are calculated as free base.

working with impure 4,4′-diguanidobiphenyl sulfate (they named this compound diguanylbenzidine and also guanylbenzidine) reported that "certain of these fractions had a physiological action similar to synthalin." Unfortunately, however, 4,4′-diguanidobiphenyl proved to be very toxic and caused death with hypoglycemia in most of the animals.

4,4′-Diguanidodiphenylmethane (III) showed practically no hypoglycemic activity but was about as toxic as 4,4′-diguanidobiphenyl (II). It was interesting to compare the physiological properties of 4,4′-diguanidodiphenylmethane (III) with those of two similar aromatic diguanidines, 4,4′-diguanidodiphenyldisulfide and 4,4′-diguanidodiphenylsulfide[13] which were shown to produce no hypoglycemia and were not toxic in doses of 100 mg. per kilo. These observations suggested that the presence of the biphenyl nucleus is essential to the hypoglycemic properties of 4,4′-diguanidobiphenyl (II) since rupture of this nucleus through the introduction of a methylene group, a dithio linkage or a sulfide linkage between the two benzene residues destroyed the activity despite the presence of the two guanido groups in comparable positions in the molecules.

The physiological results indicated that the three carbocyclic diguanidines, *p*-phenylenediguanidine (I), 4,4′-diguanidobiphenyl (II) and 4,4′-diguanidodiphenylmethane (III), possessed less hypoglycemic activity, and, with the exception of *p*-phenylenediguanidine (I), were much more toxic than the straight-chain diguanidine, 1,6-diguanidohexane.

The major part of the synthetic work presented in this paper was supported by funds from a Sigma Xi Research Grant.

The authors wish to thank Mr. H. A. Shonle and Dr. E. D. Campbell of the Lilly Research Laboratories, and Professor E. L. Scott and Dr. L. B. Dotti of the Department of Physiology, College of Physicians and Surgeons, Columbia University, for carrying out the physiological assays.

SUMMARY

1. The methods of synthesis for *p*-phenylenediguanidine, *p*-bromophenylguanidine, *p*-iodophenylguanidine, 4,4′-diguanidobiphenyl, 4,4′-diguanidodiphenylmethane, and certain of their salts have been described.

2. The effects of *p*-phenylenediguanidine, 4,4′-diguanidobiphenyl and 4,4′-diguanidodiphenylmethane upon the blood sugar of normal rabbits have been studied and compared directly with the hypoglycemic properties and toxicity of a compound of the synthalin type, 1,6-diguanidohexane, and the results are briefly discussed from the point of view of hypoglycemia and chemical constitution in the guanidine field.

[13] BRAUN, AND LUDWIG, J. ORG. CHEM., **3**, 16 (1938).