The compound is therefore the dibromide of 1-phenyl-4-bromobutadiene.

These crystals were dissolved in chloroform and a current of ozonized oxygen passed through the solution for about twenty-four hours. The chloroform was removed by means of suction and the residual ozonide decomposed with water. It was possible to isolate benzoic acid from the ozonization products. The benzoic acid was identified by its melting point and by the melting point of a mixture with known benzoic acid. The compound is therefore 1-phenyl-3,4,4′-tribromo-Δ^1-butene.

Summary

1. The two geometric isomers of 1-phenyl-4-bromobutadiene were isolated.

2. Hydrogen bromide is absorbed by each of the geometric isomers of 1-phenyl-4-bromobutadiene in the 3,4-positions to give the same 3,4-dibromide of phenylbutadiene.

3. 1-Phenyl-4-bromobutadiene absorbs a mole of bromine in the 3,4-positions to give 1-phenyl-3,4,4′-tribromo-Δ^1-butene.

4. The reactions of the 3,4-dibromide of phenylbutadiene with various alkaline reagents were studied and a number of new derivatives isolated.

CHICAGO, ILLINOIS RECEIVED JANUARY 30, 1933
PUBLISHED JULY 6, 1933

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

Aminoguanidine Derivatives

BY VERA A. CONARD AND R. L. SHRINER

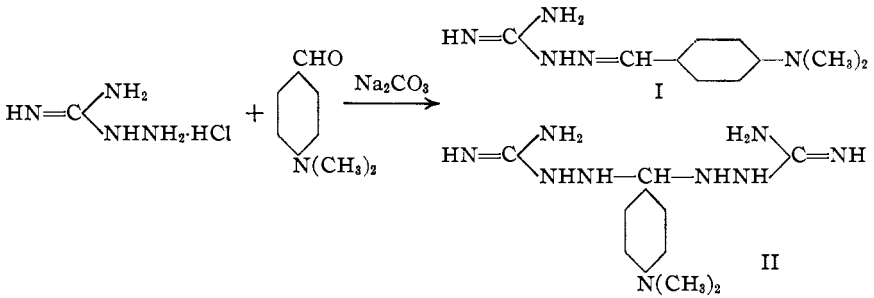
The most important types of compounds which have been studied in order to find pure substances possessing an action similar to that of insulin have been derivatives of guanidine. An excellent review of proposed insulin substitutes has been given by Braun.[1] Most of these compounds contain a guanidine and an amino group or two guanidine nuclei. The possibility that the guanidine grouping may be connected with the hypoglycemic action of insulin is suggested by the fact that arginine is one of the amino acids produced by the hydrolysis of insulin.[2] Since aminoguanidine appears to be less toxic than guanidine it was thought that certain of its derivatives might prove of value.

Accordingly aminoguanidine hydrochloride, prepared by the reduction of nitroguanidine, was condensed with *p*-dimethylaminobenzaldehyde in the presence of sodium carbonate. Both the mono and di condensation products were formed.

The two compounds were separated by fractional crystallization and their hydrochlorides prepared. The monohydrochlorides were used for the pharmacological tests since their aqueous solutions were nearly neutral and hence less irritating than the acidic solutions of the dihydrochlorides.

(1) C. E. Braun, *J. Chem. Ed.*, **8**, 2175 (1931).

(2) H. Jensen, O. Wintersteiner and V. du Vigneaud, *J. Pharmacol. and Exptl. Ther.*, **32**, 387 (1928).



It will be noted that Compound I above belongs to the agmatine type,[3] since it contains one guanidine nucleus whereas Compound II resembles synthalin[4] in that two guanidine groups are present. Although the aromatic substituted guanidines are generally quite toxic, the above compounds have the aromatic ring attached to a carbon side chain instead of to a guanidine nitrogen and hence should not be so toxic. This structure also does not contain the toxic *p*-phenylenediamine grouping[5] which was present in some aromatic guanidine derivatives studied.[6]

Through the courtesy of the Lilly Research Laboratories the influence of these compounds on the blood sugar of rabbits was determined by the same procedure as that used in testing insulin. The compounds were given by subcutaneous injection of solutions of the monohydrochlorides. If no hypoglycemia is produced by this method its gross reaction will not differ greatly by other modes of administration.

The pharmacological data obtained showed that Compound I did not exert any marked hypoglycemic action until the amounts given approached the toxic dose. Administration of 4 mg./kilo of body weight of a rabbit lowered the blood sugar from 112 mg./100 cc. to 75 mg./100 cc. in three hours. The effect produced, however, was one of shock to the rabbits' system and not a true insulin-like action at all. The injection of glucose solutions in the rabbits at the time of convulsions failed to cause their recovery. Although the tests on the *bis*-aminoguanidine (II) were not as extensive as those on the mono derivative, the data obtained indicated no marked hypoglycemic effect in amounts approximately equal to the toxic doses of synthalin. The toxicities of both Compounds I and II were lower than that of synthalin.

Experimental

Nitroguanidine.—Dicyanodiamide was converted to guanidine nitrate in 96%

(3) E. Frank, M. Nothmann and A. Wagner, *Klin. Woch.*, **5**, 2100 (1926).

(4) E. Frank, *Naturwis.*, **15**, 213 (1927); *Deut. med. Woch.*, **53**, 1845 (1927); *Klin. Woch.*, **7**, 1996 (1928).

(5) P. J. Hanzlik, *J. Ind. Hyg.*, **4**, 386, 448 (1923); E. Erdmann and E. Vahlen, *Arch. exp. Path. Pharm.*, **53**, 402 (1905).

(6) F. Bischoff, *J. Biol. Chem.*, **80**, 345 (1928); F. Bischoff, M. Sahyun and M. L. Long, *ibid.*, **81**, 325 (1929); C. E. Braun, *ibid.*, **89**, 97 (1930); T. B. Parks and C. E. Braun, *ibid.*, **91**, 629 (1931).

yields according to the directions of Davis.[7] Guanidine nitrate was converted to nitroguanidine in 94% yield by treatment with concentrated sulfuric acid using recent modifications as to time and temperature.[8]

Aminoguanidine Hydrochloride.—A modification of the procedure described by Thiele[9] was used. Fifty grams of nitroguanidine and 170 g. of zinc dust were mixed together in a mortar with enough water (about 15 cc.) to make a thick paste. This paste was added slowly to 30 cc. of glacial acetic acid with stirring, the temperature was held below 40° all the time and between 0 and 10° most of the time. When all the material was added the temperature was allowed to rise slowly; finally, the mixture was warmed to 40° on a water-bath, and held at this temperature for about fifteen minutes, or until no red color was given with alkaline ferrous sulfate. The mixture must not be allowed to heat up too rapidly as the reaction becomes rapid and exothermic. The acetic acid solution was filtered and the filtrate evaporated in a vacuum at room temperature, as heat readily decomposes aminoguanidine. This concentrated solution contained aminoguanidine acetate.

The concentrated solution was treated with an equal volume of 6 *N* hydrochloric acid. Alcohol was added and the solution evaporated on a water-bath. When the volume had decreased about one half, more alcohol was added and the solution boiled, and filtered while hot. As the filtrate cooled, aminoguanidine hydrochloride separated as yellowish material. The precipitate was dissolved in hot alcohol and treated with norite. By cooling the solution, clear colorless needles of aminoguanidine hydrochloride were obtained which melted at 162–163°, which checked the value given by Thiele;[9] yield, 77.5 g.

Anal. Calcd. for $CH_5N_4 \cdot HCl$: Cl, 32.40. Found: Cl, 32.30.

Condensation of Aminoguanidine with *p*-Dimethylaminobenzaldehyde.—Twenty-five grams of aminoguanidine hydrochloride was dissolved in water and mixed with an alcohol solution of 33.3 g. of *p*-dimethylaminobenzaldehyde, not all of which was dissolved. An aqueous solution of 25 g. of sodium carbonate was added slowly with continuous stirring and at a temperature of 40–50°. The addition took thirty to forty-five minutes. The mixture was stirred until cool and allowed to stand about four hours. The *bis*-aminoguanidine derivative was less soluble and separated out first. It was recrystallized from hot water and yielded colorless needles; m. p. 178–179°; yield, 1.3 g.

Anal. Calcd. for $C_{11}H_{21}N_9 \cdot H_2O$: N, 42.42. Found: N, 41.98. Calcd. for $C_{11}H_{21}N_9 \cdot H_2O$: H_2O, 6.06. Found: H_2O, 5.45 (dried 24 hrs.); H_2O, 7.12 (dried 48 hrs.).

There was some decomposition when the material was dried for forty-eight hours.

An equal volume of alcohol was added to the filtrate and the solution allowed to stand overnight. A second precipitate formed which was filtered and recrystallized from hot water. A light yellow powder was obtained which lost its water of crystallization at 105° and finally melted at 149°. This was the monoaminoguanidine derivative; yield, 17.88 g.

Anal. Calcd. for $C_{10}H_{16}N_6 \cdot 2H_2O$: N, 29.05. Found: N, 29.45. Calcd. for $C_{10}H_{15}N_5 \cdot 2H_2O$: H_2O, 14.9. Found: H_2O, 13.08.

Hydrochlorides.—When hydrogen chloride was passed into an acetone solution of the *p*-dimethylaminobenzalaminoguanidine the precipitate which formed was the dihydrochloride. The latter is a yellow solid, m. p. 221–227° (decomp.). In order to obtain the monohydrochloride an acetone solution of 2.47 g. of *p*-dimethylaminobenzalaminoguanidine was treated with 11.5 cc. of 0.888 *N* hydrogen chloride in acetone. This

(7) T. L. Davis, "Organic Syntheses," John Wiley and Sons, New York, Collective Vol. I, 1931, p. 295.

(8) G. B. L. Smith, V. J. Sabetta and O. F. Steinbach, *Ind. Eng. Chem.*, **23**, 1124 (1931).

(9) J. Thiele, *Ann.*, **270**, 23 (1892).

is the calculated 1:1 ratio. A light orange, crystalline precipitate formed; m. p. 205–210° (decomp.); yield, 1.4 g. The product was readily soluble in water.

Anal. Calcd. for $C_{10}H_{15}N_5 \cdot 2H_2O \cdot HCl$: Cl, 12.8. Found: Cl, 12.07.

In the case of the bis-guanidine derivative a solution of the monohydrochloride was prepared by dissolving a weighed amount in the calculated volume of standard hydrochloric acid and diluting to a definite concentration. This solution was used for the pharmacological tests which were carried out according to the international methods adopted for the standardization of insulin.[10]

Summary

Two derivatives of aminoguanidine have been prepared and their hypoglycemic action studied. Neither compound exerted an insulin-like action. Hypoglycemia was produced only when the amount of the compound administered approached the toxic dose, but this drop in blood sugar was due to shock to the rabbit's system as shown by the fact that injection of glucose did not cause recovery.

(10) "The Biological Standardization of Insulin," League of Nations, III, Health Report, 7 (1926).

URBANA, ILLINOIS RECEIVED JANUARY 30, 1933
PUBLISHED JULY 6, 1933

[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY]

Beta-Ergostenol. II

BY FREDERICK W. HEYL, MERRILL C. HART AND HAROLD EMERSON

In a recent publication[1] from this Laboratory we described the separation of β-ergostenol from the mixture of isomeric sterols obtained by the saponification of α-ergostenol acetate which had been treated in chloroform solution with dry hydrogen chloride. Later Heilbron and Wilkinson[2] reported a better yield of β-ergostenol by the isomerization of the α-benzoate.

In our study[1] we obtained evidence of the presence of a third isomer; this was more soluble and had a higher rotation than β-ergostenol. We have now carried out a rather comprehensive survey of the esters of both α- and β-ergostenol.

Experimental

The β-ergostenol used had a melting point of 141° and $[\alpha]_D$ 21.2°. It was our purpose in this study to accumulate data indicating the best ester to use in the separation of α- and β-ergostenol from each other and the third isomer present in the isomerized mixture. From the data summarized in Table I it is seen that the *p*-nitrobenzoates show in alcohol the greatest differential in solubilities between the α- and β-esters, and consequently crystallization of the *p*-nitrobenzoates from this solvent should afford a satisfactory separation of these two isomers. This experiment was

(1) Hart and Emerson, THIS JOURNAL, **54**, 1070 (1932).
(2) Heilbron and Wilkinson, *J. Chem. Soc.*, 1708 (1932).