[CONTRIBUTION FROM THE CHRIST HOSPITAL INSTITUTE OF MEDICAL RESEARCH]

ISOLATION AND IDENTIFICATION OF A METABOLITE OF CHLORGUANIDE[1, 2]

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An investigation of the metabolic fate of the antimalarial drug, chlorguanide, N^1-*p*-chlorophenyl-N^5-isopropylbiguanide (1), was initiated in this laboratory in 1947 on the basis of previous studies (2) which indicated that this compound undergoes extensive degradation in the animal body. Interest in this problem was intensified by observations (3, 4) which suggested that the antimalarial activity of chlorguanide was due to a metabolite rather than to the parent drug. Subsequent studies (5) demonstrated the presence of two metabolites, as well as unchanged drug, in the livers of rhesus monkeys and in the sera and urine of humans and monkeys to whom chlorguanide had been administered. These metabolites were isolated from the urine of both humans and monkeys. One of the products was identified as N^1-*p*-chlorophenylbiguanide; the second was shown to be 2-amino-4, 4-dimethyl-6-(*p*-chlorophenylamino)-3,4-dihydro-1,3,5-triazine. The studies involved in the isolation and identification of the latter compound are the subject of the present report.

The methods employed in demonstrating the presence of unchanged chlorguanide and its metabolites in the body fluids of treated monkeys and humans (5) involved extraction of the alkalinized urine with ethylene dichloride, concentration of the extracted bases into a small volume of dilute hydrochloric acid, re-extraction into ethylene dichloride, then partition of the bases between this solvent and phosphate-borate buffer (*p*H 8.7) by counter-current distribution (6). This effectively separated unchanged chlorguanide from its metabolites. Although the presence of two different substances could be demonstrated by partitioning the residual bases between ethylene dichloride and 0.2 *N* sodium hydroxide, this procedure did not provide a usable method for separating the metabolites.

The metabolites were first isolated by application of the partition chromatography procedure of Martin and Synge (7), a technique not employed with biguanides heretofore. When this procedure was applied to the total bases recovered from the urine of treated monkeys, the parent drug passed down the column, while two more slowly moving and only partially resolved bands remained at the top. These partially resolved bases were removed from the adsorbent with dilute acetic acid, the free bases regenerated, and then separated by fractional crystallization from methanol. In further work chlorguanide was

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[2] Presented before the Division of Organic Chemistry at the 117th meeting of the American Chemical Society in Philadelphia on April 10, 1950.

separated from its metabolites by the modified counter-current distribution technique described above, after which the metabolites were separated from each other by fractional crystallization from methanol. The products of these two isolation procedures were the same. The methanol-soluble substance was identified as N^1-*p*-chlorophenylbiguanide (5).

The methanol-insoluble compound analyzed for $C_{12}H_{18-19}ClN_5O$. Further analysis revealed the absence of an N-methyl group and the presence of 0.73 of a C-methyl group compared to 0.83 C-methyl for chlorguanide. The presence of one O-methyl group was indicated. Supplemental observations suggested that this O-methyl originated from methanol present as solvate of crystallization.[3] The unknown yielded a positive Eegriwe test (9) for methanol; furthermore, when the unknown was acylated with benzoyl chloride in dilute alkali, the odor of methyl benzoate was detected. These findings suggested that the compound might be a methanolate with the formula $C_{11}H_{14}ClN_5 \cdot CH_3OH$.

Later work, involving concentration of ethylene dichloride extracts of urine after removal of unchanged chlorguanide, led to isolation of the metabolites as hydrates. The ethylene dichloride-soluble hydrate was identified as N^1-*p*-chlorophenylbiguanide (5). The insoluble hydrate analyzed for $C_{11}H_{14}ClN_5 \cdot H_2O$. It did not depress the melting point of the compound crystallized from methanol and in addition was identical with that substance insofar as ultraviolet absorption spectrum, distribution in a Craig system, and composition of picrate were concerned.

Several findings indicated that the unknown metabolite possessed a biguanide structure. It closely resembled N^1-*p*-chlorophenylbiguanide with respect to distribution in a Craig system. Its ultraviolet absorption spectrum (Figure 1) and titration curve (Figure 2) were similar although not identical with those of chlorguanide and N^1-*p*-chlorophenylbiguanide. Like the latter compounds and other guanidines the unknown yielded a positive Sakaguchi test. Its reactions with (a) ferric chloride, (b) bromine (either in water, glacial acetic acid, or chloroform), (c) acid, alkaline, or neutral potassium permanganate, (d) sodium hypoiodite, (e) ceric nitrate reagent, and (f) Tollens' reagent were the same as those of chlorguanide and N^1-*p*-chlorophenylbiguanide. The dibasic nature of the unknown was shown by preparation of a salt containing two molecules of *p*-toluenesulfonic acid and one molecule of base. The picrate of the unknown precipitated in a 1:1 mole ratio. Attempts to prepare hydrochloride, acetate, or carbonate salts were unsuccessful. The Slotta-Tscheche reaction (10) for N^1- and N^1, N^5-substituted biguanides was negative.

Acylation of the unknown, using the Schotten-Baumann method, yielded monoacyl derivatives. Under the same conditions, chlorguanide undergoes ring closure to form a triazine (11, 12). This indicated (a) substitution on either N^2 or N^4 of the metabolite, thus permitting acylation but preventing ring closure,

[3] Attempted isolation of the 3,5-dinitrobenzoate derivative was negative, but this result was not surprising, in view of the reported difficulties (8) in preparing micro quantities of the above derivative in dilute aqueous solution.

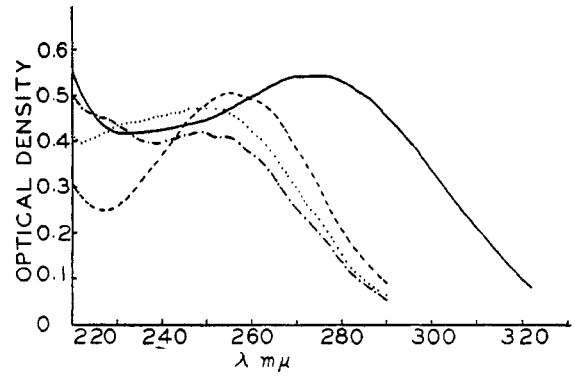


FIGURE 1. ULTRAVIOLET ABSORPTION SPECTRA OF CHLORGUANIDE, N^1-*p*-CHLOROPHENYLBIGUANIDE, 2-AMINO-4-METHYL-6-*p*-CHLOROPHENYLAMINO-1,3,5-TRIAZINE, AND 2-AMINO-4,4-DIMETHYL-6-*p*-CHLOROPHENYLAMINO-3,4-DIHYDRO-1,3,5-TRIAZINE METHANOLATE: -·-·-·-, chlorguanide, ϵ, 11,800, λ 250; . . . . . , N^1-*p*-chlorophenylbiguanide, 12,450, 250; ————, 2-amino-4-methyl-6-*p*-chlorophenylamino-1,3,5-triazine, 14,150, 272; - - - - - -, 2-amino-4,4-dimethyl-6-*p*-chlorophenylamino-3,4-dihydro-1,3,5-triazine methanolate, 17,000, 255.

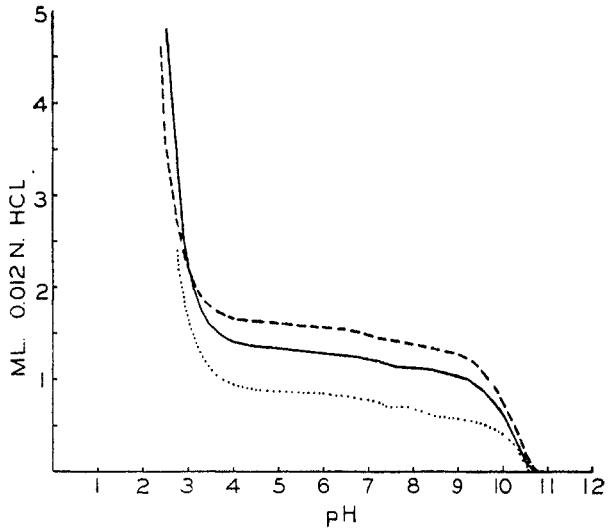


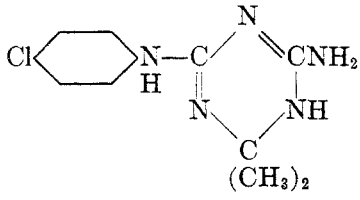
FIGURE 2. TITRATION CURVES OF CHLORGUANIDE, N^1-*p*-CHLOROPHENYLBIGUANIDE AND 2-AMINO-4,4-DIMETHYL-6-*p*-CHLOROPHENYLAMINO-3,4-DIHYDRO-1,3,5-TRIAZINE METHANOLATE: ————, chlorguanide; . . . . . , N^1-*p*-chlorophenylbiguanide; - - - - - -, 2-amino-4,4-dimethyl-6-*p*-chlorophenylamino-3,4-dihydro-1,3,5-triazine methanolate; sample, 1.85×10^{-5} mole.

and (b) absence of a substitutent on N^3 which would have either prevented acylation or permitted diacylation.

The unknown metabolite was far more resistant to acid and alkaline hydrolysis than was chlorguanide. Thus the concentration of hydrochloric acid required

for hydrolysis of the unknown was ten times that required for chlorguanide. Alkaline hydrolysis of the latter compound was complete in 20 hours as contrasted with 60 hours for the unknown. Conversion of the products of hydrolysis to *p*-phenylazobenzamides,[4] followed by chromatographic separation and characterization of these derivatives, showed that the unknown yielded only ammonia and *p*-chloroaniline. This finding provided additional evidence against the presence of an N-alkyl substituent.

The composite findings set forth above, and particularly the results of hydrolysis studies, suggested that the unknown might be a heterocyclic compound. Of the various structures permitted, the dihydrotriazines, I and II, seemed to be the most probable: it has been shown that derivatives of this heterocycle are capable of forming alcoholates (13).



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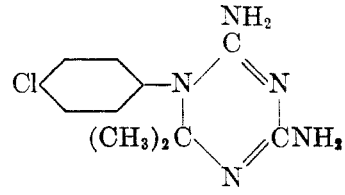

II

Inspection of the above structures suggested that both should yield acetone on hydrolysis. When the unknown was hydrolyzed in dilute hydrochloric acid, it yielded acetone which was detected qualitatively by the Lessaigne test (14) and characterized by isolation as the 2,4-dinitrophenylhydrazone.

Compound II did not appear a likely possibility since construction of this molecule with Hirschfelder models indicated restricted rotation resulting from conflict between the *p*-chlorophenyl group and the two methyl groups on the heterocyclic ring. On the other hand, existence of such hindrance would favor ring closure on N^2 as indicated in Compound I.

Compound I [2-amino-4,4-dimethyl-6-(*p*-chlorophenylamino)-3,4-dihydro-1,3,5-triazine] was synthesized by condensation of acetone with N^1-*p*-chlorophenylbiguanide in glacial acetic acid. The synthetic material proved to be identical with the unknown metabolite with respect to melting point, ultraviolet absorption spectrum, and behavior in a Craig distribution system. It likewise yielded a hydrate, methanolate, picrate, and monoacetyl derivative which were identical with the corresponding preparations of the unknown metabolite.[5]

[4] This procedure rested on development of a method which permitted separation and characterization of the components of a 20-mg. mixture of ammonia, isopropylamine, methylamine, and *p*-chloroaniline.

[5] Subsequent to completion of this identification, it was discovered that the same triazine had been synthesized by Birtwell and colleagues (15). The present work is described both because of intrinsic interest and because identification was effected without assistance from the above report.

EXPERIMENTAL

Isolation of the dihydrotriazine methanolate by partition chromatography. A 100-mg. mixture of the biguanide bases, isolated from the urine of chlorguanide-treated monkeys by ethylene dichloride extraction (5), was dissolved in 50 ml. of chloroform, purified from U.S.P. grade by fractional distillation. This solution was poured onto a $\frac{3}{8}'' \times 10''$ column of adsorbent,[6] previously wet with 5 ml. of chloroform, and was then forced through the column under air pressure (3″ Hg). Development of the chromatogram with 100 ml. of a solution containing 96 ml. of chloroform, 2 ml. of *n*-butanol (aldehyde free), and 2 ml. of pyridine yielded a light blue zone at the lower part of the column, a central pink area, and a graduated darker blue zone at the top. Elution of the bases from various segments of the column was accomplished by extraction with 0.1 *N* acetic acid. These eluates were made alkaline, 2 *N*, with 50% sodium hydroxide and extracted with ethylene dichloride, the respective extracts being evaporated to dryness under nitrogen. The residue from the lower zone of the column yielded chlorguanide. The residue from the graduated upper zone was dissolved in the minimal quantity of boiling methanol (under nitrogen). As this solution cooled, a crystalline compound appeared which proved to be the dihydrotriazine methanolate. This product melted[7] at 124–125°. After a second crystallization from methanol the m.p. was 128–129°. The compound was dried *in vacuo* at 25°.

Anal.[8] Calc'd for $C_{12}H_{18}ClN_5O$: C, 50.9; H, 6.35; N, 24.70; Cl, 12.52; O, 5.64; N-alkyl, none; C-methyl, 5.31; O-methyl, 10.9.
Found: C, 50.2, 50.8; H, 6.4, 6.58; N, 24.76; Cl, 12.33; O, 5.53;[9] N-alkyl, none; C-methyl, 3.86; O-methyl, 10.5.

Concentration of the original methanol solution from which the triazine had been obtained yielded a second crystalline compound which proved to be N^1-*p*-chlorophenylbiguanide.

Isolation of the dihydrotriazine as a hydrate. After application of the fractional extraction procedure (5) to the mixture of bases isolated from monkey urine, the buffer phase was made 2 *N* with respect to sodium hydroxide and was extracted repeatedly with an equal volume of ethylene dichloride. This extract was twice extracted with one-twentieth of its volume of 3% hydrochloric acid. These acid extracts were combined, made 2 *N* with respect to sodium hydroxide, and re-extracted into an equal volume of ethylene dichloride.[10] Crystals appeared in this solution upon standing overnight in the refrigerator. These crystals were filtered and washed successively with ethylene dichloride and water, m.p. 129–130°. A second crop of crystals was obtained by concentrating the ethylene dichloride to one-fifth of its volume by the extraction procedure described above. When recrystallized from hot water, needles were obtained which melted at 130–131°.

Anal. Calc'd for $C_{11}H_{16}ClN_5O$: C, 49.1; H, 5.95; N, 26.0; Cl, 13.2; O, 5.95; O-methyl, none.
Found: C, 48.64, 49.72; H, 5.56, 5.97; N, 26.35; Cl, 13.39; O, 5.31; O-methyl, none.

This crystalline hydrate did not depress the melting point of the previously isolated dihydrotriazine methanolate. The hydrate and the methanolate likewise yielded identical picrates and ultraviolet absorption spectra.

Titration curves. Samples of dihydrotriazine methanolate, chlorguanide, or N^1-*p*-chloro-

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[6] This adsorbent was prepared by thorough blending of 75 parts of 200 mesh silicic acid, U.S.P., and 25 parts of Celite (Johns Manville) with 50 parts of 0.1% aqueous azolitmin. This preparation retained activity for at least a month when kept in a tightly stoppered bottle.

[7] Melting points were determined on the Fisher-Johns apparatus.

[8] The analyses reported in this manuscript were performed by Clark Microanalytical Laboratories, Urbana, Illinois.

[9] All oxygen analyses reported in this manuscript have been estimated by difference.

[10] An emulsion will form if the concentration of the hydrate exceeds 200 mg. *per* 100 ml. of ethylene dichloride.

phenylbiguanide (1.85×10^{-5} mole) were dissolved in 0.5 ml. of ethanol and then diluted with 7 ml. of distilled water, both solvents having been boiled and cooled under nitrogen to exclude carbon dioxide. These biguanide solutions were titrated under nitrogen with 0.01 *N* hydrochloric acid, using a Beckman *p*H meter with glass electrode (Figure 2).

Ultraviolet absorption spectra. The ultraviolet absorption spectra of the dihydrotriazine methanolate, chlorguanide, and N^1-*p*-chlorophenylbiguanide were compared in a Model DU Beckman spectrophotometer (Figure 1). The solvent was 0.01 *N* hydrochloric acid in all cases. Data on the related triazine, 2-amino-4-methyl-6-*p*-chlorophenylamino-1,3,5-triazine were obtained for reference.

2-Amino-4,4-dimethyl-6-p-chlorophenylamino-3,4-dihydro-1,3,5-triazine-di-p-toluenesulfonate. A solution of 50 mg. of *p*-toluenesulfonic acid monohydrate in 1 ml. of ethanol was added to a solution of 47.5 mg. of the triazine methanolate in 0.5 ml. of alcohol. The salt (30 mg.), which crystallized upon combination of the above solutions, melted at 232° after one recrystallization from ethanol.

Anal. Calc'd for $C_{15}H_{30}ClN_5O_6S_2$: C, 50.49; H, 5.45; N, 11.76; S, 10.75; Cl, 5.98; O, 16.15.
Found: C, 50.49, 50.49; H, 4.97, 5.22; N, 11.5; S, 10.98; Cl, 5.08; O, 16.85.

2-Amino-4,4-dimethyl-6-p-chlorophenylamino-3,4-dihydro-1,3,5-triazine picrate. A solution of 25 mg. of the triazine methanolate, in 0.2 ml. of ethanol, was diluted with 0.6 ml. of benzene; to this was added 0.35 ml. of a saturated solution of picric acid in benzene. Crystal formation was induced by addition of 1 ml. of heptane. After standing for one-half hour, the mixture was centrifuged; the supernatant was removed, and the crystalline mass was washed twice with 0.2-ml. portions of benzene. The excess benzene was removed with a stream of nitrogen. The crystalline product melted at 232–233°. Recrystallization from methanol yielded 30 mg. of yellow needles, m.p. 238°.

Anal. Calc'd for $C_{17}H_{17}ClN_8O_7$: C, 42.5; H, 3.54; Cl, 7.4; N, 23.35.
Found: C, 42.53, 42.53; H, 3.62, 3.49; Cl, 7.3; N, 22.52.

Acylation of the dihydrotriazine. a. 2-Benzoylamino-4,4-dimethyl-6-p-chlorophenylamino-3,4-dihydro-1,3,5-triazine. Fifty mg. (0.000175 mole) of triazine methanolate was dissolved in a mixture of 0.2 ml. of dioxane and 0.3 ml. of ethylene dichloride, after which 0.1 ml. of 35% sodium hydroxide and 61 mg. (0.00043 mole) of benzoyl chloride were added.[11] The mixture was heated to 60° in a water-bath with stirring for five minutes, then was allowed to stand at room temperature for two hours. Quantities of sodium hydroxide and benzoyl chloride identical with those used originally were again added to the mixture and the heating process repeated. After the mixture had stood at room temperature for another two hours, 3 ml. of water was added. The mixture was centrifuged the next day, the supernatant being discarded. The residue was triturated with 0.4 ml. of water and centrifuged. The aqueous and thin oily layers were separated from the crystals which were next washed successively with two 0.3-ml. portions of heptane and two 0.4-ml. portions of water. The crystals, dried *in vacuo* at 50°, weighed 45 mg. and melted at 138–139°. Two crystallizations from ethylene dichloride yielded 23 mg. of colorless needles, m.p. 147–148°. This product was readily soluble in cold methanol and dilute acids.

Anal. Calc'd for $C_{18}H_{18}ClN_5O$(monobenzoyl): C, 61.6; H, 5.94; N, 18.85.
Found: C, 62.31; H, 6.48; N, 19.01.
Calc'd for $C_{25}H_{22}ClN_5O$ (dibenzoyl): C, 65.7; H, 5.48; N, 14.72.

Since the analyst reported that the high electrostatic charge of the material made accurate weighing impossible, the above analytical data are of interest chiefly because they point to formation of a monobenzoyl rather than a dibenzoyl derivative.

b. 2-Acetylamino-4,4-dimethyl-6-p-chlorophenylamino-3,4-dihydro-1,3,5-triazine. Twenty mg. of the triazine hydrate was dissolved in 0.2 ml. of warm dioxane, after which 0.13 ml. of 19 *N* sodium hydroxide and 0.04 ml. of acetic anhydride were added, with stirring. After disappearance of the odor of acetic anhydride, an additional 0.02 ml. of this reagent was added. The mixture was heated to 50° for five minutes then diluted with 0.7 ml. of water

[11] The odor of methyl benzoate was detected at this point.

and placed in the refrigerator. The product which separated on standing was filtered, washed with water, and dried in a desiccator. The crude material melted at 145–147°. Recrystallization from dioxane yielded 10 mg. of a product, m.p. 148.5°. The compound was a monohydrate since drying *in vacuo* at 76° led to loss of one mole of water.

Anal. Calc'd for $C_{13}H_{16}ClN_5O$ (monohydrate): C, 53.25; H, 5.45; N, 23.82.
Calc'd for $C_{13}H_{14}ClN_5$ (triazine): C, 56.8; H, 5.09; N, 25.4.
Found: C, 53.28; H, 6.06; N, 23.78.

Characterization of the products of acid and alkaline hydrolysis of the dihydrotriazine and chlorguanide. a. Preparation of p-phenylazobenzamides for reference purposes.

p-Phenylazobenzamide was synthesized by a method described previously (16), m.p. 228°.

N-Methyl-p-phenylazobenzamide was prepared using the Schotten-Baumann procedure. Crystallization of the crude material from benzene yielded red needles, m.p. 157°.

Anal. Calc'd for $C_{14}H_{13}N_3O$: N, 17.6. Found: N, 17.65.

N-Isopropyl-p-phenylazobenzamide was prepared by the above procedure. Crystallization, first from benzene, then from methanol yielded red needles, m.p. 192°.

Anal. Calc'd for $C_{16}H_{17}N_3O$: N, 15.7. Found: N, 15.60.

N-(p-Chlorophenyl)-p-phenylazobenzamide was also synthesized by the above method. The benzamide was separated by Soxhlet extraction of the crude product with toluene. The material which crystallized from this extract melted at 247°.

Anal. Calc'd for $C_{19}H_{14}ClN_3O$: N, 12.58. Found: N, 12.45.

b. Method for separation and recovery of a known mixture of ammonia, methylamine, isopropylamine, and p-chloroaniline. One ml. of an aqueous solution containing *p*-chloroaniline (8.9 mg.), ammonia (4.8 mg.), methylamine (2.2 mg.), and isopropylamine (4.2 mg.) as their hydrochlorides was placed in a 10-ml. ground-glass centrifuge tube, and evaporated to dryness on a steam-bath with nitrogen. The residue was redissolved in 0.3 ml. of dioxane after which 150 mg. of *p*-phenylazobenzoyl chloride and 0.2 ml. of 5 *N* sodium hydroxide were added. The reaction mixture was shaken for ten minutes and allowed to stand overnight. The dioxane was removed with a stream of nitrogen at 50°. The residue suspended in 3–4 ml. of water was filtered through a Soxhlet thimble and washed with water until the filtrate was no longer alkaline to phenolphthalein. After drying at 60° for ten hours, the thimble and contents were extracted continuously with 60 ml. of benzene. This extract was poured on a 1.5 × 25 mm. column of adsorbent composed of equal parts of Celite and alumina (17). The chromatogram was developed with a 60:40 mixture of benzene and *n*-heptane until the *p*-chloroaniline derivative had been washed from the column. Development was completed with a 75:25 mixture of benzene and *n*-heptane. The column, containing three bands, was divided into appropriate segments, each of which was extracted continuously with ethanol. The upper band contained the ammonia derivative, 53 mg., m.p. 228°, the second band the methylamine derivative, 20 mg., m.p. 128°, and the lower band the isopropylamine derivative, 24 mg., m.p. 175–184°. The filtrate yielded the *p*-chloroaniline derivative, 24 mg., m.p. 245–246°. The second and third bands were rechromatographed separately to give pure compounds.

c. Products of the acid hydrolysis of the dihydrotriazine methanolate. A solution of 30 mg. of the triazine methanolate in 4 ml. of 3 *N* hydrochloric acid was placed in a Pyrex bomb and heated for 16 hours, at 160°. After cooling, this acid solution was evaporated to dryness. The residue, dissolved in 0.3 ml. of dioxane, was acylated with 161 mg. of *p*-phenylazobenzoyl chloride and chromatographed according to the method described in the preceding section. The solution which passed through the column yielded 20 mg. of a crystalline product, m.p. 247–248°, which did not depress the melting point of an authentic specimen of N-(*p*-chlorophenyl)-*p*-phenylazobenzamide. Extraction of the column yielded 21 mg. of a second crystalline product, m.p. 227–228°, which did not depress the melting point of an authentic sample of *p*-phenylazobenzamide. There was no indication of the presence of another amide.

Confirmatory evidence for the liberation of *p*-chloroaniline through acid hydrolysis of the triazine was obtained as follows. The hydrolysis described in the preceding section was repeated. The resulting solution was made alkaline to phenolphthalein with sodium hydrox-

ide and was then extracted with benzene. This extract was concentrated to a small volume under nitrogen; 0.1 ml. of acetic anhydride and 1 ml. of 2 *N* sodium hydroxide were then added. The remaining benzene was evaporated and the crude material crystallized from heptane. This crystalline product melted at 175–176° and did not depress the melting point of an authentic sample of *p*-chloroacetanilide, m.p. 176–177°.

d. Products of alkaline hydrolysis of chlorguanide. A solution of 23 mg. of chlorguanide and 800 mg. of barium hydroxide octahydrate in 10 ml. of water was placed in a two-necked 50-ml. flask. A stream of nitrogen, regulated with a bubble counter, was passed over the surface of the solution. The reaction mixture was refluxed under a 20-cm. Liebig condenser, the top of which was fitted with an inverted U-tube having one end drawn to a 1-mm. capillary. The end of this capillary was led to the bottom of a tube (10 × 100 mm.) which contained 3.46 ml. of 0.0228 *N* hydrochloric acid and a drop of Methyl Red indicator. After the acid in this tube was neutralized by trapped amine, a second tube containing another equivalent of acid was substituted. This substitution was repeated with third and fourth tubes, each containing 3.46 ml. of hydrochloric acid, and a fifth tube containing 3.12 ml. Each neutralization required approximately four hours. At the end of the total reaction period, the contents of each tube were evaporated to dryness; the residue was dissolved in dioxane and treated with *p*-phenylazobenzoyl chloride as in the acid hydrolysis studies. Purification of these acylated preparations by chromatography showed that tube 1 contained chiefly isopropylamine and some ammonia, tube 2, ammonia, tube 3, chiefly *p*-chloroaniline and some ammonia, and tubes 4 and 5, chiefly ammonia.

e. Products of alkaline hydrolysis of the dihydrotriazine methanolate. The procedure described for use with chlorguanide was applied to the hydrolysis of a 23-mg. sample of the triazine. Fifteen hours' refluxing were required to liberate sufficient amine to neutralize 3.60 ml. of 0.228 *N* hydrochloric acid. Acylation and chromatographic analysis of the material in this solution showed that the liberated amine was chiefly ammonia contaminated with a small amount of *p*-chloroaniline. Attempts to isolate crystalline materials from the refluxing mixture were unsuccessful.

A second hydrolysis with 10 ml. of 0.5 *N* sodium hydroxide was attempted. Fifteen hours were required for liberation of each of the first three equivalents of amine. Liberation of a fourth equivalent could not be accomplished in an additional fifteen hours. Acylation of the various fractions and separation of the benzamides by chromatography indicated that the only amines evolved were ammonia and *p*-chloroaniline.

Liberation of acetone through acid hydrolysis of the dihydrotriazine hydrate. Forty mg. of the hydrate, dissolved in 3 ml. of 4 *N* hydrochloric acid and sealed in a Pyrex bomb under nitrogen, was heated for 12 hours at 155°. A drop of the cooled hydrolysate gave a positive Lessaigne test (12) for acetone. The remainder of this hydrolysate was diluted to 10 ml. with water and an 8-ml. portion was distilled until 3 ml. of condensate had been collected in a solution of 28 mg. of 2,4-dinitrophenylhydrazine in 1 ml. of dioxane. This distillation was accomplished with the tip of the condenser immersed in the dioxane solution. The distillate mixture was heated to 60° for one minute, then diluted with 9 ml. of water. One drop of concentrated hydrochloric acid was added, the tube reheated to 60° for three minutes and allowed to stand overnight. The precipitate was filtered, washed with water, and dried *in vacuo* at room temperature. The product (m.p. 120–121°) was then extracted with two 0.5-ml portions of cold benzene. These extracts were combined, the benzene removed, and the residue re-extracted with three 0.3-ml. portions of a cold mixture of equal volumes of benzene and *n*-heptane. The crystalline product obtained from these extracts melted at 124–125° and did not depress the melting point of acetone-2,4-dinitrophenylhydrazone, m.p. 125–126°.

Anal. Calc'd for $C_9H_{10}N_4O_4$: C, 45.75; H, 4.2; N, 23.5.
Found: C, 45.82; H, 4.02; N, 23.56.

[12] The methanol filtrate was evaporated to dryness with a stream of nitrogen. The residue was shown to be N^1-*p*-chlorophenylbiguanide by formation of its acetate salt, m.p. 183–185°.

Synthesis of 2-amino-4,4-dimethyl-6-p-chlorophenylamino-3,4-dihydro-1,3,5-triazine hydrate and methanolate from acetone and N^1-p-chlorophenylbiguanide. A solution containing 4.26 g. (0.02 mole) of N^1-*p*-chlorophenylbiguanide, 1.16 g. (0.2 mole) of dry acetone, and 13 ml. of glacial acetic acid was refluxed for 3.5 hours. The solution was cooled to room temperature, then diluted with 45 ml. of water. The slight precipitate which formed was removed. Addition of 15 ml. of 50% sodium hydroxide to the filtrate yielded an oil. After decanting the aqueous layer the oil was redissolved in 45 ml. of water and 10 ml. of 50% sodium hydroxide was added. An oil separated out again; on standing in the refrigerator, this material yielded 2 g. of a crystalline compound, m.p. 110–120°. Purification of this product by continuous extraction with 15 ml. of methanol yielded 1 g. (18.5%) of needles, m.p. 130–131°.[12] Recrystallized from water, it melted at 131.5–132°.

Anal. Calc'd for $C_{11}H_{16}ClN_5O$: C, 49.2; H, 5.95.
Found: C, 49.27; H, 5.90.

A methanolate of the above triazine was prepared in the following manner. A 0.5-g. sample of the purified hydrate was dissolved in 10 ml. of dry boiling methanol, after which 50 mg. of sodium dissolved in 3 ml. of methanol was added. Three crops of crystals were obtained from this material, each crop being washed free of mother liquor with methanol. The second crop melted at 129–130°.

Anal. Calc'd for $C_{12}H_{18}ClN_5O$: C, 50.9; H, 6.37.
Found: C, 51.02; H, 6.22.

The synthetic dihydrotriazine hydrates and methanolates did not depress the melting points of the corresponding natural products. The synthetic and natural products gave identical ultraviolet absorption spectra and identical behaviors in a Craig counter-current distribution system. The picrates and acetyl derivatives of the synthetic and natural products were also identical. Neither product exhibited measurable activity against infections induced by inoculation of rhesus monkeys with *Plasmodium cynomolgi*.

SUMMARY

A compound isolated from the urine of monkeys receiving chlorguanide has been identified through degradative and synthetic studies as 2-amino-4,4-dimethyl-6-*p*-chlorophenylamino-3,4-dihydro-1,3,5-triazine.

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REFERENCES

(1) CURD AND ROSE, *J. Chem. Soc.*, 729 (1946).
(2) SCHMIDT, HUGHES, AND SMITH, *J. Pharmacol. and Exptl. Therap.*, **90**, 233 (1947).
(3) HAWKING, *Nature*, **159**, 409 (1947).
(4) HAWKING AND PERRY, *Brit. J. Pharmacol.*, **3**, 320 (1948).
(5) HUGHES, SCHMIDT, AND CROUNSE, to be published.
(6) CRAIG, *J. Biol. Chem.*, **155**, 519 (1944).
(7) MARTIN AND SYNGE, *Biochem. J.*, **35**, 1358 (1941).
(8) LIPSCOMB AND BAKER, *J. Am. Chem. Soc.*, **64**, 179 (1942).
(9) EEGRIWE, *Mikrochim. Acta*, **2**, 329 (1937).
(10) SLOTTA AND TSCHECHE, *Ber.*, **62**, 1390 (1929).
(11) CURD AND ROSE, *J. Chem. Soc.*, 362 (1946).
(12) CURD, LANDQUIST, AND ROSE, *J. Chem. Soc.*, 154 (1947).
(13) ANKER AND COOK, *J. Chem. Soc.*, 323 (1941).
(14) KAMM, *Qualitative Organic Analysis*, 2nd Ed., John Wiley and Sons, New York, 1932, p. 155.
(15) BIRTWELL, CURD, HENDRY, AND ROSE, *J. Chem. Soc.*, 1645 (1948).
(16) FREUNDLER, *Compt. rend.*, **142**, 1155 (1906).
(17) ZECHMEISTER AND CHOLNOKY, *Principles and Practices of Chromatography*, John Wiley and Sons, New York, 1941, p. 47.