treated with dilute aqueous hydrochloric acid (20 ml.). The precipitate which formed was separated by filtration; weight 8.5 g. dried (66%). Crystallization from a mixture of 200 ml. of methanol and 25 ml. of acetone gave 8 g., m.p. 200–201°.

Anal. Calcd. for $C_{24}H_{34}N_2O_6 \cdot 2HCl$: N, 5.4. Found: N, 5.4.

1,4-Bis-(1,4-benzodioxan-2-ylmethyl)-piperazine. (McN-181).—A mixture of 38.8 g. (0.2 mole) of piperazine hexahydrate, 81.4 g. (0.44 mole) of 2-chloromethylbenzodioxan and 16 g. (0.4 mole) of sodium hydroxide in 16 ml. of water was refluxed for 48 hours. The solid which separated on cooling was collected and crystallized from aqueous acetone; yield 45.2 g. (59%), m.p. 164–165°. This is the melting point of a mixture of the *dl* and *meso* forms.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.3. Found: C, 69.03; H, 6.94; N, 7.2.

The three isomers of this compound were separated by forming the bis-(*d*-camphorsulfonates) in methanolic solution (1 mole of the salts per 2800 ml. of methanol). Upon allowing the hot solution to cool to room temperature, about half of the salt crystallized. The solid was separated by filtration and crystallized repeatedly from methanol to give a product with m.p. 259.5–260° and $[\alpha]^{26}D$ +28.5 ± 0.5° (*c* 2, dimethylformamide), unchanged by further crystallization. This is the salt of the *meso* base, m.p. 173–174°, $[\alpha]^{26}D$ 0.0 ± 0.5° (*c* 2, dioxane). When the filtrate from the salt of the *meso* compound was chilled in a refrigerator, another crop of crystals was obtained, and upon further crystallization from methanol yielded a salt with m.p. 240–241°, $[\alpha]^{26}D$ −8.2 ± 0.5° (*c* 2, dimethylformamide); the regenerated base had m.p. 156–157°, $[\alpha]^{26}D$ −36.3 ± 0.5° (*c* 1, dioxane). The mother liquor from this salt, upon evaporation to dryness and crystallization of the residue from a mixture of methanol and ether and finally from dimethylformamide, yielded a salt with m.p. 255–256°, $[\alpha]^{26}D$ +65.0 ± 0.5° (*c* 2, dimethylformamide), unchanged after several further crystallizations. Regeneration gave the *d*-base, m.p. 156–157°, $[\alpha]^{26}D$ +36.0 ± 0.5° (*c* 2, dioxane).

Preparation of Unsymmetrical Piperazines. General Procedures.—The preparation of 1-phenylpiperazine[14] and its reaction with haloalkyl compounds presented no difficulties. The preparation of 1-(1,4-benzodioxan-2-ylmethyl)-4-(2-phenoxyethyl)-piperazine was accomplished by reaction of 2-phenoxyethyl bromide with 1-(1,4-benzodioxan-2-ylmethyl)-piperazine (III, R = R′ = H). The latter compound has been obtained by direct reaction of piperazine with 2-chloromethyl-1,4-benzodioxan.[15] We obtained it in 10% yield when this reaction was carried out in refluxing methanol. Better yields resulted by the method illustrated, reaction of 2-chloromethylbenzodioxan with 1-carbethoxypiperazine and hydrolysis of the resulting product.

1-(1,4-Benzodioxan-2-ylmethyl)-4-carbethoxypiperazine Hydrochloride. (McN-212).—A mixture of 13.4 g. (0.085 mole) of 1-carbethoxypiperazine[16] and 7.8 g. (0.043 mole) of 2-chloromethyl-1,4-benzodioxan was heated at 100° for 32 hours. The mixture was cooled, treated with ether, and the tan solid which remained undissolved was removed by filtration. Addition of hydrogen chloride to the dried ether filtrate precipitated 13.8 g. (94%) of a solid, m.p. 233° dec. Crystallization from a mixture of methanol and ether raised the m.p. to 235°.

Anal. Calcd. for $C_{16}H_{22}N_2O_4 \cdot HCl$: N, 8.2; Cl, 10.4. Found: N, 8.2; Cl, 10.5.

1-(1,4-Benzodioxan-2-ylmethyl)-piperazine Hydrochloride. (McN-179).—A solution of 1-(1,4-benzodioxan-2-ylmethyl)-4-carbethoxypiperazine was prepared by dissolving 8.5 g. (0.025 mole) of the hydrochloride in 125 ml. of methanol and adding 1.4 g. (0.025 mole) of potassium hydroxide. The precipitate was removed by filtration, and the filtrate was treated with 11.2 g. (0.2 mole) of potassium hydroxide. The resulting solution was refluxed for 22 hours. Removal of the solvent, extraction of the residue with ether and addition of hydrogen chloride to the dried extract gave 5.4 g. (80%) of a hydrochloride. Crystallization from a mixture of acetone and ether gave 1.0 g., m.p. 197°.

Anal. Calcd. for $C_{13}H_{18}N_2O_2 \cdot HCl$: N, 10.3; Cl, 13.1. Found: N, 10.3; Cl, 13.4.

1-(1,4-Benzodioxan-2-ylmethyl)-4-(2-phenoxyethyl)-piperazine Dihydrochloride. (McN-324).—A mixture of 2.7 g. (0.01 mole) of 1-(1,4-benzodioxan-2-ylmethyl)-piperazine hydrochloride, 2 g. (0.01 mole) of 2-phenoxyethyl bromide, 2.1 g. (0.02 mole) of anhydrous sodium carbonate and 250 ml. of methanol was refluxed for 58 hours. The inorganic salts were removed by filtration. Addition of hydrogen chloride to the filtrate caused 2.4 g. (56%) of a white solid to separate. Crystallization from a mixture of methanol and ether gave 0.5 g., m.p. 235–239° dec.

Anal. Calcd. for $C_{21}H_{26}N_2O_3 \cdot 2HCl$: N, 6.6. Found: N, 6.5.

(14) C. B. Pollard and N. MacDowell, THIS JOURNAL, **56**, 2199 (1934).

(15) Mewburn, Ellis and Co., British Patent 420,078 (1934); (*Chem. Zentr.*, **106**, 2216 (1935)).

(16) T. S. Moore, M. Boyle and V. M. Thorn, *J. Chem. Soc.*, 39 (1929).

PHILADELPHIA, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND VITAL ECONOMICS, THE UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

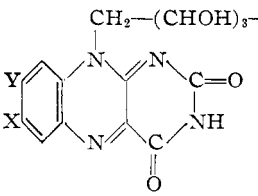
The Synthesis of 6-Chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine and 6-Methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine[1]

BY EDWARD E. HALEY AND JOHN P. LAMBOOY

RECEIVED APRIL 22, 1954

Two new isoalloxazines, 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine and 6-methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine have been synthesized. Both are inhibitors of riboflavin in *Lactobacillus casei*.

With Kuhn's demonstration that the substitution of both methyl groups on the benzene nucleus of the riboflavin molecule by chlorine atoms results in a compound which antagonizes riboflavin in certain microörganisms,[2] it was of interest to us to study the effects of replacing each methyl group individually by a chlorine atom. 6-Chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine (I) and 6-methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine (II) were synthesized and found to be effective as inhibitors of riboflavin in *Lactobacillus casei*.



I, X = Cl, Y = CH_3
II, X = CH_3, Y = Cl
III, X = Cl, Y = Cl

Weygand, *et al.*,[3] in a study of the specificity of

(1) This work was supported in part by Research Grant Number G 3326 C from the National Institute of Health, Public Health Service.

(2) R. Kuhn, F. Weygand and E. F. Möller, *Ber.*, **76**, 1044 (1943).

(3) F. Weygand, R. Löwenfeld and E. Möller, *Chem. Ber.*, **84**, 106 (1951).

6,7-dichloro-9-(1′-D-ribityl)-isoalloxazine (III) as an antagonist of riboflavin prepared the monohalogen analogs, 6-chloro-9-(1′-D-ribityl)-isoalloxazine and 6-fluoro-9-(1′-D-ribityl)-isoalloxazine. These and other flavins closely related to III which differed in the side chain at the 9-position, in the halogen, or in both were found to be less effective than III in the inhibition of growth of *Streptobacterium plantarum P 32*. Others have been interested in halogenated analogs of riboflavin due to the activity of some to enhance regression of lymphosarcoma implants in mice.[4-6] Riboflavin activity and inhibition studies of these analogs have not been reported, with the exception of 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine which is inactive for *L. casei* and rats. Snell, *et al.*,[7] have reported that III neither promotes growth nor is inhibitory for *L. casei*.

Several methods of synthesis were employed in attempts to prepare 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine and 6-methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine, and the first analog was actually synthesized by two methods. Attempts to prepare both analogs by the classical method through the catalytic reduction of the corresponding methyl chloro-2-nitro-aniline-N-D-ribosides and condensation of the resulting N-D-ribityl-2-amino-chloromethylanilines with alloxan were not successful due to the failure of D-ribose to condense with 4-chloro-5-methyl-2-nitroaniline or 4-methyl-5-chloro-2-nitroaniline under a variety of modifications of conditions.[8,9]

The next attempted preparation of the 6-chloro-7-methyl analog was through the reaction of 1-(D-ribitylamino)-2-*p*-nitrophenylazo-4-chloro-5-methylbenzene, and also 1-(D-ribitylamino)-2-*p*-tolylazo-4-chloro-5-methylbenzene, with barbituric acid. No reaction took place with the first azo compound. Reaction of the second azo compound resulted in a condensation product which did not have the physical properties of the isoalloxazines. Too little of the material was available to attempt to identify it. 1-(D-Ribitylamino)-2-phenylazo-4-chloro-5-methylbenzene was reduced catalytically and reacted with alloxan to form 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine. Inasmuch as Tishler, *et al.*,[10] had reported that the products formed from the diazotization of an aryl amine and coupling with N-D-ribityl-3,4-dimethylaniline were not pure 2-azo compounds, but contained significant amounts of 6-azo compounds, it appeared that the compounds formed from N-D-ribityl-3-methyl-4-chloroaniline were probably mixtures of 2- and 6-azo compounds, and that following reduction their condensation with alloxan resulted in two isomeric flavins being formed. The 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine preparation was chromatographed in a butanol, acetic acid, water system by the descending method. Two spots developed, indicating that the material contained some of the 5-methyl-6-chloro isomer.

Since a feasible method for the separation of the two azo compounds or of the flavins on a preparative scale was not available, another method of synthesis was pursued which yielded the desired intermediates and the isoalloxazine free of contaminating isomers. In this method 2,5-dichloro-4-nitrotoluene was treated with D-ribamine to give 2-nitro-4-chloro-5-methyl-N-D-ribitylaniline. This material was catalytically reduced, and the resulting 2-amino-4-chloro-5-methyl-N-D-ribitylaniline condensed with alloxan monohydrate yielding 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine. The isoalloxazine was obtained from the reaction mixture in a remarkable state of purity. No other flavin preparation was found to be cited in which the compound abundantly precipitates from the acetic acid reaction mixture in crystalline form.

An attempt was made to synthesize the 6-methyl-7-chloro analog by the method of Karrer, *et al.*[11] 3-Methyl-4-chloro-carbethoxyanilide was nitrated and the resulting nitrocarbethoxyanilide reduced to yield 4-chloro-5-methyl-2-aminocarbethoxyanilide. This compound, however, when reacting with D-ribose under the usual conditions[12] failed to produce 2-ribityl-amino-4-chloro-5-methyl-carbethoxyanilide.

The method which had finally led to the synthesis of the 6-chloro-7-methyl analog was successful in producing 6-methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine. First 4,6-dichloro-3-nitrotoluene reacted with D-ribamine to yield 2-nitro-4-methyl-5-chloro-N-D-ribitylaniline. This was reduced and the resulting 2-amino-4-methyl-5-chloro-N-D-ribitylaniline condensed with alloxan monohydrate yielding 6-methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine.

Both isoalloxazines were found to be potent antagonists of riboflavin in *L. casei*, with inhibition indices for the 6-chloro-7-methyl and 6-methyl-7-chloro compounds of 85 and 22, respectively. These are the only ribityl isoalloxazines reported to have this property. All others either have riboflavin activity or are inactive in this organism. There is evidence that these analogs inhibit some microorganisms which do not require riboflavin.

Experimental

3-Methyl-4-chloroaniline-N-D-riboside.—The riboside was prepared following the method of Berger and Lee.[8] D-Ribose, 1.0 g., was dissolved with heating in 10 ml. of 95% ethyl alcohol. In this solution were dissolved 10 drops of glacial acetic acid and 1.0 g. of 3-methyl-4-chloroaniline.[13] The mixture was allowed to stand at room temperature for 7 hours, then placed in the refrigerator overnight. A white solid, 0.68 g. (37%), was obtained, m.p. 99–104° dec.[14]

(4) F. W. Holly, E. W. Peel, R. Mozingo and K. Folkers, THIS JOURNAL, **72**, 5416 (1950).

(5) F. W. Holly, E. W. Peel, J. J. Cahill, F. R. Koniuszy and K. Folkers, *ibid.*, **74**, 4047 (1952).

(6) C. H. Shunk, F. R. Koniuszy and K. Folkers, *ibid.*, **74**, 4251 (1952).

(7) E. E. Snell, O. A. Klatt, H. W. Bruins and W. W. Cravens, *Proc. Soc. Exptl. Biol. Med.*, **82**, 583 (1953).

(8) L. Berger and J. Lee, *J. Org. Chem.*, **11**, 84 (1946).

(9) R. Kuhn and R. Ströbele, *Ber.*, **70B**, 773 (1937).

(10) M. Tishler, K. Pfister, 3rd, R. D. Babson, K. Ladenburg and A. J. Fleming, THIS JOURNAL, **69**, 1487 (1947).

(11) P. Karrer, K. Schöpp, F. Benz and K. Pfaehler, *Helv. Chim Acta*, **18**, 69 (1935).

(12) P. Karrer and F. M. Strong, *Helv. Chim. Acta*, **18**, 1343 (1935).

(13) J. P. Lambooy and E. E. Haley, THIS JOURNAL, **74**, 1087 (1952).

(14) All melting points were observed on thermometers calibrated against U.S.P. Melting Point Reference Standards.

Anal. Calcd. for $C_{12}H_{16}O_4NCl$: C, 52.6; H, 5.9; N, 5.1; Cl, 12.9. Found: C, 51.4; H, 6.0; N, 5.1; Cl, 12.8.[15]

N-D-Ribityl-3-methyl-4-chloroaniline.—3-Methyl-4-chloroaniline-N-D-riboside, 1.5 g., was dissolved in 50 ml. of absolute ethyl alcohol, 0.6 g. of Raney nickel catalyst was added and the mixture hydrogenated at 60 p.s.i. and 70° for 5 hours. After filtration and refrigeration a white crystalline solid precipitated. The liquor was reduced in volume to obtain another crop, thus increasing the yield to a total of 1.0 g. (67%). A sample for analysis was recrystallized from 50% ethyl alcohol, m.p. 145.5–146°.

Anal. Calcd. for $C_{12}H_{18}O_4NCl$: C, 52.3; H, 6.6; Cl, 12.9. Found: C, 53.0; H, 6.5; Cl, 11.9.[15]

2,5-Dichloro-4-nitrotoluene.—The synthesis of 2,5-dichloro-4-nitrotoluene was based on the method of Gunstone and Tucker.[16] Sodium nitrite, 4.1 g., was added with stirring to 45 ml. of concentrated sulfuric acid over a period of about 20 minutes. When the addition was completed the temperature was raised to 70° to dissolve the sodium nitrite. The solution was cooled in an ice-bath to 25–30° and a solution of 10.0 g. of 4-chloro-5-methyl-2-nitroaniline[13] in 110 ml. of hot glacial acetic acid was added at a rate such that the temperature did not exceed 40°. The solution was stirred at 40° for an additional 30 minutes, then added slowly with vigorous stirring to a cold solution of 11.8 g. of cuprous chloride in 110 ml. of concentrated hydrochloric acid over a period of about 5 minutes. The mixture was heated on a steam-bath with occasional stirring until the evolution of nitrogen ceased. Water, 300 ml., was added and the mixture cooled in an ice-bath and allowed to stand in the refrigerator overnight. The yellow product was separated by centrifugation, washed three times with water by this means, and extracted with ether. The ether solution was washed with water, 5% sodium bicarbonate, and again with water and the product isolated in the usual manner. The residue was recrystallized from 60 ml. of *n*-hexane to yield 5.9 g. of yellow crystalline solid, m.p. 47–48°. The liquor was evaporated to dryness and the residue recrystallized from 38 ml. of 80% ethyl alcohol to give a second crop, 2.2 g., m.p. 47–48°, for a total yield of 8.1 g. (74%).

2-Nitro-4-chloro-5-methyl-N-D-ribitylaniline.—2,5-Dichloro-4-nitrotoluene, 5.0 g., and 10 g. of 77% D-ribamine[17] were refluxed in 180 ml. of pyridine in a nitrogen atmosphere for 6 hours. The reaction mixture was placed in the refrigerator overnight, then the solution decanted from unreacted D-ribamine and evaporated to dryness *in vacuo* at room temperature. The residue was extracted three times with 50-ml. portions of hot *n*-hexane to remove unreacted dichloronitrotoluene and then dissolved in 110 ml. of hot methyl alcohol. The orange crystalline solid which precipitated from the methanol solution on cooling in an ice-bath for three hours was filtered and washed with cold methyl alcohol. The yield was 2.85 g. (37%), m.p. 176–177°. Recrystallization from methyl alcohol improved the appearance of the material but did not alter the melting point.

Anal. Calcd. for $C_{12}H_{17}O_6N_2Cl$: C, 44.9; H, 5.3; N, 8.7. Found: C, 45.0; H, 5.6; N, 8.6.

6-Chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine.—The synthesis was a modification and combination of procedures previously described.[4,20] 2-Nitro-4-chloro-5-methyl-N-D-ribitylaniline, 1.50 g., was suspended in a mixture of 26 ml. of glacial acetic acid and 5.4 ml. of water. After addition of 100 mg. of platinum oxide catalyst the mixture was hydrogenated at 60 p.s.i. and room temperature for 45 minutes. The catalyst was removed by filtration and the filtrate added to a suspension of 0.86 g. of alloxan monohydrate and 1.74 g. of boric acid in 75 ml. of glacial acetic acid. Flavin synthesis began immediately. The mixture was warmed at 45–50° with constant agitation for 40 minutes. An orange crystalline solid began precipitating after the first 20 minutes of heating. The mixture was allowed to stand at room temperature in the dark for two days, then filtered, and the orange solid washed with 95% ethyl alcohol and ether to yield 1.16 g. On recrystallization from 1100 ml. of hot water 0.87 g. of long orange micro needles was obtained, m.p. 274–275° dec. The liquor from the reaction mixture was evaporated to dryness *in vacuo* at room temperature and the residue recrystallized twice from hot water, then once from 5% acetic acid to yield another crop of orange micro needles, 0.22 g., m.p. 273–274° dec. The total yield was 1.09 g. (59%).

Anal. Calcd. for $C_{16}H_{17}O_6N_4Cl$: C, 48.4; H, 4.3; N, 14.1. Found: C, 48.3; H, 4.4; N, 13.7.

4-Chloro-5-methyl-2-nitrocarbethyoxyanilide.—The procedure was one recently reported by Karrer and Becker.[18,19] Ethyl chlorocarbonate, 3.0 g., was treated with 2.8 g. of 3-methyl-4-chloroaniline in a mixture of 7.5 ml. of acetone, 4.5 ml. of water and 3.5 ml. of 24% sodium hydroxide. The urethan was nitrated following a method described by Lambooy,[20] making use of a mixture of 12.1 ml. of concentrated nitric acid and 4.4 ml. of concentrated sulfuric acid cooled to −9° in a salt–ice-bath. The product was recrystallized from 15 ml. of 95% ethyl alcohol to yield 3.7 g. of yellow needles, m.p. 59–67°. This material, together with that from other preparations (total 8.8 g.) was purified by fractional crystallization from 95% ethyl alcohol to yield 7.9 g. of yellow needles, m.p. 75–76°. A small amount of higher melting material (m.p. 85–91°) was separated but was not identified.

Anal. Calcd. for $C_{10}H_{11}O_4N_2Cl$: C, 46.4; H, 4.3; Cl, 13.7. Found: C, 46.5; H, 4.7; Cl, 13.3.

4-Chloro-5-methyl-2-aminocarbethoxyanilide.—4-Chloro-5-methyl-2-nitrocarbethoxyanilide, 1.0 g., was dissolved in 50 ml. of absolute alcohol; 50 mg. of platinum oxide catalyst was added and the mixture hydrogenated at 60 p.s.i. and room temperature for one hour. After removal of the catalyst by filtration, the solution was reduced in volume to about 10 ml. *in vacuo* at room temperature in an atmosphere of hydrogen and placed in the refrigerator. The precipitated material was recrystallized from 200 ml. of *n*-hexane to yield 0.35 g. of shiny white needles, m.p. 145–146°. Further recrystallization from *n*-hexane and from 50% ethyl alcohol did not raise the melting point. Another crop was filtered from the reaction mixture and recrystallized and decolorized from 50% ethyl alcohol to yield 0.14 g. of crystals, m.p. 144.5–145°; total yield 0.49 g. (56%).

Anal. Calcd. for $C_{10}H_{13}O_2N_2Cl$: C, 52.5; H, 5.7; Cl, 15.5. Found: C, 53.2; H, 5.7; Cl, 15.6.

4,6-Dichloro-3-nitrotoluene.—The method of synthesis was the same as that described for 2,5-dichloro-4-nitrotoluene. 4-Methyl-5-chloro-2-nitroaniline,[13] 10.0 g., was diazotized and reacted with cuprous chloride. The residue from the ether extracts was recrystallized from 72 ml. of *n*-hexane to yield 6.5 g. of yellow needles, m.p. 51–52°. The liquor was evaporated to dryness and the residue recrystallized from 32 ml. of 80% ethyl alcohol, yielding an additional 2.5 g. of product, m.p. 51–52°. The reported m.p. is 54–55°.[21] The total yield was 9.0 g. (82%).

2-Nitro-4-methyl-5-chloro-N-D-ribitylaniline.—The method of synthesis was essentially the same as that described for 2-nitro-4-chloro-5-methyl-N-D-ribitylaniline, with the exception that the refluxing time in this case was 10 hours. 4,6-Dichloro-3-nitrotoluene, 5.0 g., reacted with 10 g. of 77% D-ribamine. The residue after the hot *n*-hexane extractions was dissolved in 90 ml. of hot methyl alcohol, then cooled in an ice-bath. Orange needles, 2.6 g. (33%) were produced, m.p. 169–170°. Repeated recrystallization from methyl alcohol did not alter the melting point.

Anal. Calcd. for $C_{12}H_{17}O_6N_2Cl$: C, 44.9; H, 5.3; N, 8.7. Found: C, 45.2; H, 5.4; N, 8.7.

6-Methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine.—The method of synthesis was similar to that of 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine. 2-Nitro-4-methyl-5-chloro-N-D-ribitylaniline, 2.56 g., was reduced and reacted with 1.46 g. of alloxan monohydrate as described. Flavin synthesis began immediately. The mixture was refluxed for 5 minutes, then allowed to stand in the dark at room temperature for two days. The solution was evaporated to dryness and the residue recrystallized from 780 ml.

(15) The procedure involving these materials had been abandoned before the analytical results were available. While the identity of these compounds seems beyond question, we were unable to obtain further analytical data because of lack of materials.

(16) F. D. Gunstone and S. H. Tucker, *Org. Syntheses*, **32**, 23 (1952).

(17) We are especially indebted to Dr's. K. Folkers and F. W. Holly of Merck and Company, Inc., not only for the gift of this material but for having synthesized it for our use.

(18) P. Karrer and B. Becker, *Helv. Chim. Acta*, **18**, 1435 (1935).

(19) J. P. Lambooy, THIS JOURNAL, **71**, 3756 (1949).

(20) J. P. Lambooy, *ibid.*, **72**, 5225 (1950).

(21) J. B. Cohen and H. D. Dakin, *J. Chem. Soc.*, **79**, 1129 (1901).

of 5% acetic acid to yield 2.31 g. of orange micro crystals. This material was recrystallized again from 700 ml. of 5% acetic acid, producing 2.03 g. (64%) of micro needles, m.p. 261–262° dec.

Anal. Calcd. for $C_{16}H_{17}O_6N_4Cl$: C, 48.4; H, 4.3; N, 14.1. Found: C, 48.4; H, 4.5; N, 14.0.

Biological Data.—The microbiological assays were carried out in the usual manner.[22] The 6-chloro-7-methyl analog did not support the growth of *L. casei* at levels up to 75 μg./10 ml., nor the 6-methyl-7-chloro analog at levels up to 100 μg./10 ml. To study the inhibition of riboflavin by the analogs culture tubes containing 0.3 μg./10 ml. of riboflavin plus graded amounts of the analogs from 0 to 100 μg./10 ml. were prepared. Acid production was measured by the glass electrode in the 6-chloro-7-methyl analog study and by both glass electrode and titration in the 6-methyl-7-chloro analog study. The inhibition index[23] for 6-chloro-7-methyl-9-(1′-D-ribityl)-isoalloxazine was found to be 85, while the values for 6-methyl-7-chloro-9-(1′-D-ribityl)-isoalloxazine were 22 and 28, respectively, by the two methods of acid measurement.

In studies with the 6-methyl-7-chloro analog the basal medium and flavin solutions were autoclaved separately, then mixed and inoculated, since it was found that the analog undergoes a reversible reduction when autoclaved in the presence of the medium.

(22) Association of Vitamin Chemists, "Methods of Vitamin Assay," Interscience Publishers, Inc., New York, N. Y., 1951.

(23) Inhibition indices were calculated in both cases from the *p*H represented by the hydrogen ion concentration at half maximal growth, and in the latter case also from titration values.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE CHRIST HOSPITAL INSTITUTE OF MEDICAL RESEARCH]

1-*p*-Chlorophenyl-2,4-diamino-6,6-dimethyl-1,6-dihydro-1,3,5-triazine

BY TI LI LOO[1]

RECEIVED FEBRUARY 25, 1954

1-*p*-Chlorophenyl-2,4-diamino-6,6-dimethyl-1,6-dihydro-1,3,5-triazine (I) was prepared by condensation of *p*-chlorophenylbiguanide with acetone in the presence of concentrated hydrochloric acid. In an effort to obtain unequivocal proof of structure, an attempt was made to synthesize the above compound *via* 1-*p*-chlorophenyl-2-methylthio-4-amino-6,6-dimethyl-1,6-dihydro-1,3,5-triazine (V); this failed at the final stage because of unsuccessful aminolysis.

In the course of studies on the metabolic fate of the antimalarial drug chlorguanide (N^1-*p*-chlorophenyl-N^5-isopropylbiguanide), Crounse[2] isolated a crystalline material from the urine of rhesus monkeys receiving this drug. He identified the substance as 2-*p*-chloroanilino-4-amino-6,6-dimethyl-1,6-dihydro-1,3,5-triazine (II) by comparison with the product resulting from condensation of *p*-chlorophenylbiguanide with acetone in glacial acetic acid. The same triazine had been prepared independently by Birtwell, *et al.*,[3] using essentially the above procedure.

Subsequently, Carrington and co-workers[4] isolated from the urine of men and the feces of rabbits given chlorguanide a base which, largely on the basis of X-ray crystallographic studies, was assigned the structure 1-*p*-chlorophenyl-2,4-diamino-6,6-dimethyl-1,6-dihydro-1,3,5-triazine (I). This base was believed to be isomeric with II and was shown to be converted to II during exposure to alkali. Later, work by the present author,[5,6] and independent studies of Modest,[7] showed that replacement of glacial acetic acid by concentrated hydrochloric acid in the Crounse condensation formed a product which appeared on chemical grounds to be identical with I and isomeric with II and which could be converted to II by heating in dilute alkali.[7c] Studies of antimalarial properties[6] also indicated that the synthetic product was identical with the "active metabolite" of chlorguanide isolated by Carrington.

With a view to proving unequivocally the structure of the new isomeric compound, designated I, an alternate scheme of synthesis was devised, which, together with the molecular rearrangement of this compound to II, is illustrated in the following diagram. The present investigation, conceived in its entirety independently of Birtwell[8] whose paper on essentially the same subject appeared at the last stage of this work, is reported hereby as a confirmation of some of his results.

The starting material, N-*p*-chlorophenyl-N′-amidinothiourea (III), was prepared by a modified Slotta procedure.[9,10] This product was caused to

(1) Research Division, National Distillers Chemical Co., 1275 Section Rd., Cincinnati 37, Ohio.

(2) N. N. Crounse, *J. Org. Chem.*, **16**, 492 (1951).

(3) S. Birtwell, F. H. S. Curd, J. A. Hendry and F. L. Rose, *J. Chem. Soc.*, 1645 (1948).

(4) (a) H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi and F. L. Rose, *Nature*, **168**, 1080 (1951); the synthesis of I was also reported in very general terms in this communication; (b) A. F. Crowther and A. A. Levi, *Brit. J. Pharmacol.*, **8**, 93 (1953); (c) since the submission of this paper for publication, the excellent and thorough work of the British workers has appeared: H. C. Carrington, A. F. Crowther and G. J. Stacey, *J. Chem. Soc.*, 1017 (1954).

(5) This work grew out of attempts to improve the synthesis of II according to the Crounse method.[2] During recrystallization of *p*-chlorophenylbiguanide hydrochloride from acetone, a crystalline product altogether different from the starting material was obtained. Its elementary analysis corresponded very well with the monohydrochloride of II, yet it had an utterly different ultraviolet absorption spectrum. However, on warming with dilute alkali, the spectrum changed to that of II. This sequence of reactions, similar to those made available to us in a private communication from Dr. H. C. Carrington, suggested that this new compound might be identical with the active metabolite of chlorguanide reported later by Carrington and co-workers.[4]

(6) L. H. Schmidt, T. L. Loo, R. Fradkin and H. B. Hughes, *Proc. Soc. Exptl. Biol. Med.*, **80**, 367 (1952).

(7) (a) E. J. Modest, G. E. Foley, M. M. Pechet and S. Farber, THIS JOURNAL, **74**, 855 (1952); (b) E. J. Modest, Abstract of Papers, Am. Chem. Soc., 122, 9-L (1952); (c) E. J. Modest and H. Kangur, *ibid.*, **124**, 26 (1953).

(8) S. Birtwell, *J. Chem. Soc.*, 1279 (1952).

(9) K. H. Slotta, R. Tschesche and H. Dressler, *Ber.*, **63**, 208 (1930).

(10) This method also yielded a small quantity of a higher m.p. crystalline material which had the composition $C_{15}H_{11}Cl_2N_5S$. Although its structure has not been established conclusively, the expression 1-*p*-chlorophenyl-2-*p*-chloroanilino-4-amino-6-thio-1,6-dihydro-1,3,5-triazine (VIII) appears to be a reasonable assumption. Its existence may be due to the equimolecular condensation of N-*p*-chlorophenyl-N′-amidinothiourea with *p*-chlorophenylisothiocyanate and the elimination of a molecule of hydrogen sulfide. A similar conclusion had been reached earlier by A. F. Crowther, *et al.*, *J. Chem. Soc.*, 1636 (1948), and Birtwell (reference 8).