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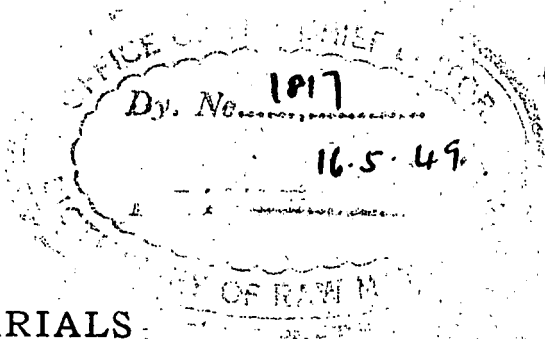
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PART III. PREPARATION OF PALUDRINE

BY

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AND PRAFULLA CHANDRA GUHA

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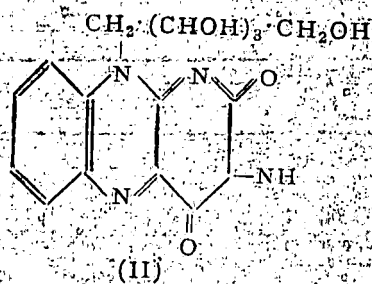
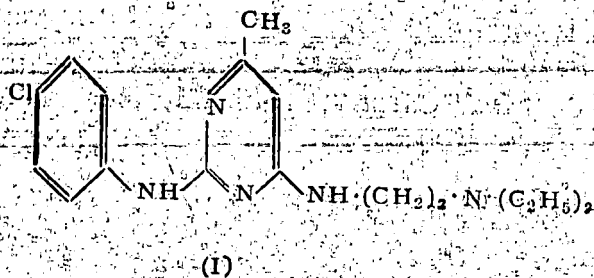
STUDIES IN ANTIMALARIALS

Part III. Preparation of Paludrine

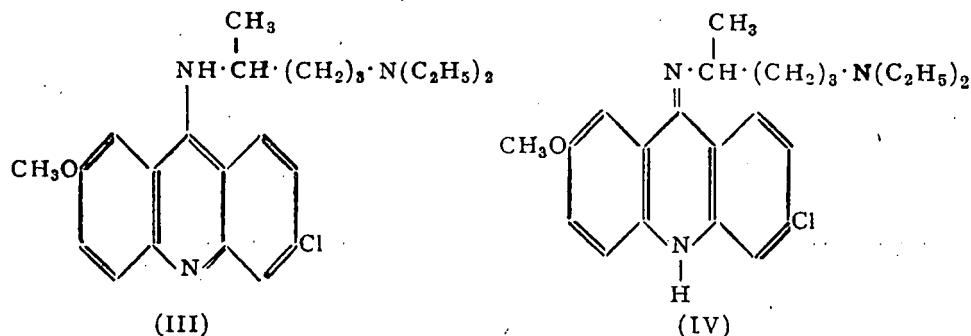
BY HARKRISHAN LAL BAMI, BALKRISHNA HARIHAR IYER
AND PRAFULLA CHANDRA GUHA

Quinine, plasmoquin and atebirin, the three well-known antimalarial drugs, are not to be considered perfect (Curd and Rose, *J. C. S.*, 1946, 343; Everette and Erich, *J. Org. Chem.*, 1946, 11, 1; Davey, *J. Bd. Sci. and Ind. Res.*, 1946, 4, 660; Temkin *et al.*, *Antimalarial Drugs*, Office of the Medical Information, Washington, 1946) due to their toxic and nonprophylactic nature. The efforts of chemists to discover a perfect antimalarial drug have therefore been ever in the increase.

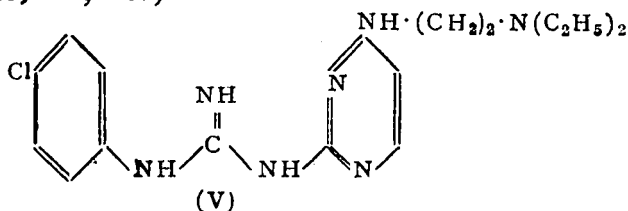
Heterocyclic rings like quinoline and acridine present in plasmoquin and atebirin molecules respectively, being foreign to human system, may be responsible for the toxic nature of these drugs to the host (Curd and Rose, *loc. cit.*). Rose (*Endeavour*, 1946, 5, 65) and Curd and Rose (*loc. cit.*) have postulated that substitution of the quinoline and acridine ring systems by pyrimidine rings may lead to the discovery of more useful and less toxic antimalarials, as the latter type of heterocyclic rings are present in biological products like nucleic acids, co-enzymes and vitamins. In support of their postulate, Curd and Rose (*J. C. S.*, 1946, 347) synthesised a number of substituted pyrimidine derivatives of which 2-*p*-chloroanilino-4- β -diethylaminoethylamino-6-methyl-pyrimidine (I) (serial No. 2666) was found to be active against experimental malarial infection. The activity of this drug was explained on the basis of Wood's theory (*Brit. J. Exptl. Path.*, 1940, 21, 74) according to which the drugs exert their action by interfering with the utilisation of essential metabolites or food factors necessary for the growth and life of disease-producing germs. In the case of this drug the growth-inhibitory action for *Lactobacillus casei* is antagonised by Riboflavin (II) (Madinaveitia, *Biochem. J.*, 1946, in press) which is having a formal resemblance to (I) and (III).



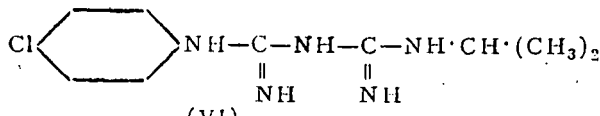
Schönhofer (*Z. Physiol. Chem.*, 1942, 274, 1) had postulated that the central nucleus of atebtrin (III) was capable of tautomeric changes through the migration of the hydrogen atom of 5-imino-group to ring nitrogen atom, giving rise to the *p*-quinoid arrangement of bonds as shown in (IV). The activity of atebtrin according to him was due to its tautomeric character. Compound (I) is also capable of such transformation.



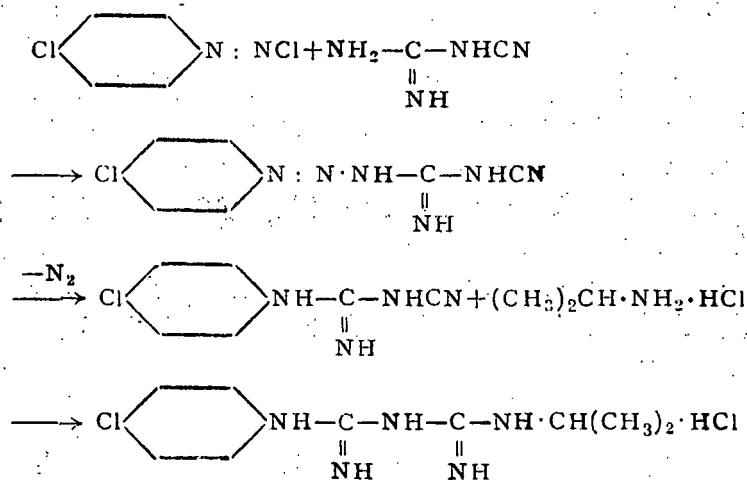
The next development on these lines was the synthesis of (V) (serial No. 3349) in which a guanidine residue acts as the connecting link between the benzene and the pyrimidine ring (Curd and Rose, *J. C. S.*, 1946, 362). This compound was a riboflavin antagonist and was found to possess considerable antimalarial activity in both clinical as well as experimental trials (Das Gupta, *et al.*, *Indian Med. Gaz.*, 1945, 80, 241; Curd *et al.*, *Ann. Trop. Med.*, 1945, 39, 165).



On the basis of these observations Curd and Rose (*J. C. S.*, 1946, 729) put forward the hypothesis that the basic side chain and the benzene ring should be connected by a system of conjugated double bonds with alternate carbon and nitrogen atoms in order to have the required antimalarial activity. This fact is evident in the case of (I) and (V). In their opinion, if these conditions are satisfied it is not necessary that a heterocyclic ring should be present in the molecule. Further work on these lines led to the discovery of N^1 -*p*-chlorophenyl- N^5 -isopropylbiguanide (VI) commonly known as Paludrine (serial No. 4888) (Anon., *Ind. Chemist*, 1946, 22, 163; Curd and Rose, *loc. cit.*).

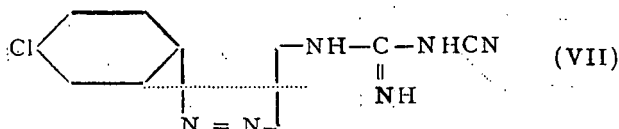


METHOD B



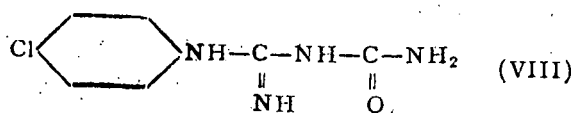
Method A.—As the details for the synthesis of *p*-chloro-phenyl-cyanamide and isopropyl cyanamide were not available in literature, they had to be prepared and characterised for the purpose. Paul Pierron's method (*Bull. Soc. Chim.*, 1906, III, 35, 1197; *J. C. S., Abst.*, 1907, 92, 121) was found most suitable for the preparation of *p*-chlorophenylcyanamide. Fritz Baum's (*Ber.*, 1908, 41, 523) modified method for the use of cyanogen bromide was employed for the preparation of isopropyl cyanamide. Isopropylamine itself was prepared by Hofmann's method (*Ber.*, 1888, 15, 768). Gabriel's method (*Ber.*, 1891, 24, 3104) for the preparation of isopropylamine was tried and found to be less suitable for the purpose.

Method B.—*p*-Chlorophenyl-azo-dicyanamide (referred to as triazine) was having the *cis*-structure (VII) and the two atoms of azo-nitrogen were removed to give *p*-chlorophenyl-cyanoguanidine according to Greishammer and Walther's method (*loc. cit.*). The yield in this case was not good but the unconverted material was capable of being treated over again for denitrogenation.



When the above work had been completed, Curd and Rose's (*loc. cit.*) method for denitrogenation was available to us. These authors have used β -ethoxy-ethanol as a solvent while we have successfully replaced it by acetone and alcohol. Curd and Rose (*loc. cit.*) have drawn attention to the fact that although Walther and Grieshammer (*loc. cit.*) have converted (VII) into (VIII) by evaporating a solution of triazine in ethanol and hydrochloric acid, they

have not made it clear whether hydrolysis of cyano-group precedes or follows the elimination of azo-nitrogen.



We have observed that under suitable conditions it is possible that only removal of azo-nitrogen should take place because according to our observation the denitrogenation precedes the hydrolysis of cyano-group. The final condensation has been brought about without solvent by fusion of *p*-chloro-cyano-guanidine and isopropylamine hydrochloride. The yield was less due to certain side reactions discussed by Werner and Bell (*Trans. Chem. Soc.*, 1922, **121**, 1792). The product has been isolated as its picrate as well as its acetate salt.

According to our observations the latter scheme is better as it involves less number of steps which are comparatively easy and gives fairly good yields.

Further work on substituted biguanides is in progress.

Experimental

METHOD A

p-Chloro-phenyl-cyanamide (I).—Chloro-benzene was nitrated and the *p*-chloro-nitro-benzene isolated in 60% yield was reduced by iron and hydrochloric acid according to the method of Bashiaum and Power (*Ind. Eng. Chem.*, 1923, **52**, 407). *p*-Chloro-aniline (18 g.) was dissolved in alcohol (50 c.c.) and potassium bicarbonate (15 g.) was added to it. Cyanogen bromide (15 g.) was freshly prepared (Gilman, *Organic Synthesis*, Vol. II, p. 30) and added to the above cooled mixture. The contents were kept for 2 hours and later refluxed for 1 hour on the water-bath. The solution was filtered and the filtrate diluted with water (200 c.c.), made alkaline with sodium hydroxide solution (10%), and the alkaline solution acidified after filtration and treatment with charcoal. On cooling the product was collected by filtration, washed and dried. Crystallised from alcohol, m.p. 101°; yield 25 g. (Found: N, 18.1. $\text{C}_7\text{H}_5\text{N}_2\text{Cl}$ requires N, 18.3 per cent.)

p-Chlorophenyl-guanidine nitrate (II).—*p*-Chlorophenyl cyanamide (15 g.) and alcoholic ammonia (30 c.c.) were charged in a soda-water bottle and heated under pressure for 8 hours at 120–30° in an oil-bath. Afterwards the contents of the flask were removed and excess of ammonia allowed to

escape. The mixture was acidified with dilute nitric acid, treated with norite, filtered and allowed to cool. The product obtained was crystallised from dilute alcohol, m.p. 142–43°; yield 12 g.

Isopropylamine hydrochloride (III).—Gabriel's method.—Potassium salt of phthalimide (20 g.) and isopropyl iodide (30 g.) (Morkownikoff, *Annalen*, 1866, 138, 364) were heated together under pressure in a sodawater bottle for 8 hours at 180–90° in an oil-bath. The excess halide was removed by steam distillation and the residue ground with dilute sodium carbonate solution, filtered, washed and dried. The product was crystallised from alcohol, m.p. 85°. This product was heated with C.P. hydrochloric acid, (30 c.c.) for 6 hours. The mixture was filtered and the filtrate on evaporation gave isopropylamine hydrochloride (8 g.). The product was finally dried over sulphuric acid.

Isopropylamine hydrochloride (III).—Hoffman's method.—Isobutyric acid (88 g.) was converted into its acid chloride with thionyl chloride (140 g.) and the amide was obtained by reacting this with excess of ammonia (800 c.c.) at 0°. The product was crystallised from water and further purified by leaching it with absolute alcohol and removing the alcohol to get the pure isobutyramide, m.p. 128°; yield 60 g. Isobutyramide (44 g.) and bromine (80 g.) were treated with sodium hydroxide solution (15%) at 0° till the colour of the solution was light yellow. Sodium hydroxide (70 g.) in water (100 c.c.) was taken in a flask and the above solution was added to it through a dropping funnel at 70°. The mixture became clear by 1 hour and the free amine was removed by steam distillation and collected in hydrochloric acid (15%; 100 c.c.). A litre of the distillate was collected and evaporated to dryness. The mass was extracted with alcohol and the pure isopropylamine hydrochloride obtained after removal of alcohol. Product was dried over sulphuric acid. Hygroscopic solid; yield 30 g.

Isopropyl cyanamide (IV).—Isopropylamine hydrochloride (10 g.) was dissolved in water (15 c.c.) and added through a dropping funnel on to sodium hydroxide (30 g.) taken in a distillation flask. The amine generated was expelled by heating and collected in ether (20 c.c.) kept at 0°. The ether solution of the amine was reacted in cold with cyanogen bromide (10 g.) in ether (20 c.c.). After 1 hour the reaction was complete when the ether was removed and the product distilled under reduced pressure; b.p., 130°/30 mm.; yield 6 g. (Found: N, 33.06. $C_4H_8N_2$ requires N, 33.33 per cent.)

Paludrine nitrate (V).—Isopropyl cyanamide (1.2 g.) and *p*-chlorophenylguanidine nitrate (3.5 g.) were heated together for 6 hours at 140–45°.

The fused mass was kept over calcium chloride in a desiccator for 2-3 days when a solid mass was obtained. The product was extracted with hot water and the residue obtained after evaporation, was further crystallised from water; m.p. 158-60°; yield 2 g. (Found: N, 23.82. $C_{11}H_{16}N_5Cl.HNO_3$ requires N, 23.51 per cent.).

METHOD B

p-Chlorophenyl-azo-cyanoguanidine (I).—*p*-Chloroaniline (32 g.) was dissolved in hydrochloric acid (55 c.c.) in water (50 c.c.) and diazotised with sodium nitrite (18 g.) in water (30 c.c.) at 0°. The clear solution of the diazonium salt was added to cyanoguanidine (22 g.) (obtained from calcium cyanamide according to the method of Soll and Stutzer, *Ber.*, 1909, 42, 4533) dissolved in water (1.5 litres) at 10°. The solution was made alkaline when a red coloured solution of the triazine resulted. The mixture was kept for 2 hours and acidified with acetic acid. The yellow precipitate was collected by filtration, washed with water and dried in an electric air-oven. Light yellow amorphous powder, decomposing at 136°; yield 50 g.

p-Chlorophenyl-cyanoguanidine (II).—(a) *Using ether as solvent.*—*p*-Chlorophenyl-azo-cyanoguanidine (15 g.) was suspended in ether (300 c.c.) and dried hydrochloric acid gas was passed for 2 hours under cooling. The ether in the mixture was evaporated in a shallow dish at room temperature in about 3 hours. The solid thus obtained was stirred with water (20 c.c.) and filtered. The product was heated with water (30 c.c.) at 70-80° for 1 hour when the nitrogen evolution took place. Afterwards, the mixture was cooled, filtered and the residue crystallised from water (1 lit.). The product was obtained as crystalline powder, m.p. 197-98°; yield 4 g. The unconverted triazine weighed 7 g.

(b) *Using acetone as solvent.*—*p*-Chloro-aniline (32 g.) was diazotised and reacted with cyanoguanidine (22 g.) as described under (I). The triazine obtained was dried well on the filter paper and directly used for denitrogenation as follows: To a mixture of acetone (150 c.c.) and hydrochloric acid (30 c.c.) the triazine was added at room temperature in about half-an-hour with good stirring. The evolution of nitrogen was spontaneous and considerable heat developed, which was controlled by outer cooling. The reaction was over after 1 hour and the solution was diluted with water (1200 c.c.) and cooled. The crystalline precipitate obtained was filtered and dissolved in dilute alkali. The alkali solution was treated with norite, filtered and acidified. The product was finally filtered and crystallised from alcohol. White amorphous powder, m.p. 200°. yield, 20 g.

(c) *Using alcohol as solvent*.—*p*-Chlorophenyl-azo-cyanoguanidine obtained from *p*-chloroaniline (32 g.) and cyano-guanidine (22 g.) as detailed in (II b) was added to a mixture of alcohol (150 c.c.) and hydrochloric acid (35 c.c.) with good stirring in half-an-hour. The mixture was warmed on a water-bath when the evolution of nitrogen commenced. The nitrogen evolution was over in 1 hour and the solution thus obtained was worked up as described in (II b) and the product was finally obtained as detailed before; m.p., 200°; yield, 18 g.

Paludrine hydrochloride (III).—*p*-Chlorophenyl-cyanoguanidine (5 g.) and isopropylamine hydrochloride (4 g.) were heated together for 4 hours at 130–40°. The solid mass was extracted with hot water (20 c.c.) twice, and after treatment with norite, was filtered and concentrated to a smaller volume (8 c.c.). On cooling, the product was obtained as white crystalline solid which was collected and recrystallised from water; m.p. 241°; yield 2 g. (Found: N, 24.12. $C_{11}H_{16}N_5Cl \cdot HCl$ requires N, 24.13 per cent.)

Paludrine acetate (IV).—Paludrine hydrochloride (2 g.) was dissolved in water (10 c.c.) and treated with excess of sodium hydroxide solution (20%). The base separated as a sticky solid which was washed repeatedly with cold water and dissolved in acetic acid (1 c.c.) in water (5 c.c.). The solution was evaporated and the product obtained crystallised from water as white crystalline powder; m.p., 186°; yield, 1.2 g.

The *Picrate* of the Paludrine base was obtained from its hydrochloride and was crystallised from alcohol twice. Crystalline powder, m.p. 174–76°.

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