CHEMOTHERAPY OF MALARIA

Part I. A Study of the Methods of Synthesis of Diguanides

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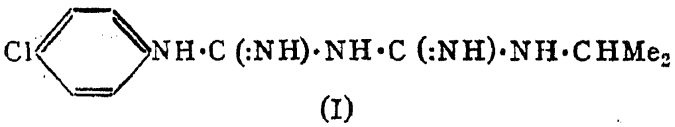
THE present state of knowledge of the problem of malaria, and the targets of the chemotherapy of malaria have been discussed elsewhere.[1] The present communication forms a part of the work undertaken along those lines. Of the thousands of compounds studied in recent years, only the 8-aminoquinolines and N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (paludrine, I) have been found to possess significant action on the exoerythrocytic form of *Plasmodium vivax* to be of any practical value. While the 8-aminoquinolines have little margin of safety, the action of paludrine is not intense enough. We have undertaken the synthesis of hybrids of these two structures to see if their properties could be reinforced in the same compound. In the synthesis of quinoline compounds with the substituted diguanide side chain, the problem initially turned out to be one of developing suitable methods for the construction of the required N^5-substituted side chain. So, we explored the various methods of synthesis of N^1: N^5-disubstituted diguanides, taking paludrine (I) as the model compound. This paper presents some of the results obtained.



(I)

Paludrine consists of two guanidine units linked in series and so the methods of synthesis of the guanidines could be used for its synthesis by choosing appropriate reactants. The three standard methods that can be employed for the synthesis of the guanidines are as follows:

(i) $R \cdot NH \cdot CN + NH_2R' \rightarrow R \cdot NH \cdot C(:NH) \cdot NH \cdot R'$ (Erlenmeyer's synthesis[2])

(ii) $R \cdot NH \cdot C(:NH) \cdot SMe + NH_2R' \xrightarrow{-HSMe}$

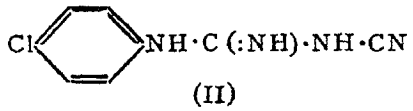
$R \cdot NH \cdot C(:HR') \cdot SMe + NH_3 \xrightarrow{-HSMe}$ } Rathke's synthesis[3, 5]

(iii) $R \cdot NH \cdot CS \cdot NH_2 + NH_2R' \xrightarrow{-H_2S}$

$R \cdot NH \cdot CS \cdot NH \cdot R' + NH_3 \xrightarrow{-H_2S}$ } [4]

Many permutations and combinations are possible with these three methods and we have tried a number of them in which the reactants are easily accessible.

At the time the work was started, two methods of syntheses of paludrine had been published. The first one based on the work of Slotta and Tschesche[6] was worked out by Curd and Rose[7] and consisted of the condensation of *p*-chlorophenyldicyandiamide (II) with isopropylamine. This method has

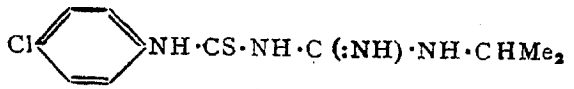


(II)

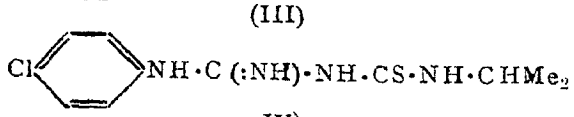
been studied in detail in this Institute and has been found to be the best and cheapest for the large-scale manufacture of this drug. We have found that N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (I) on crystallisation from dilute alcohol separates in colorless needles which melt at 96–97°, solidify and then melt again at 125–6°. Only the latter melting point has been recorded by Curd and Rose[7] who crystallised this compound from toluene. The other method is due to Dasgupta and Basu[8] which consists in the condensation of *p*-chlorophenylguanidine with *iso*propylcyanamide.

Choosing as one reactant *iso*propylguanidine sulphate, which was easily prepared by the condensation of *iso*propylamine with S-methylthiourea sulphate, we tried three methods by condensing it with (1) *p*-chlorophenylcyanamide, (2) S-methyl *p*-chlorophenylisothiourea and (3) *p*-chlorophenylthiourea. In all these three cases the required N^1-*p*-chlorophenyl-N^5-isopropyldiguanide (I) was obtained, the maximum yield being obtained in the first case and the minimum in the last. In the case of the condensation with *p*-chlorophenylcyanamide, when *iso*propylguanidine sulphate was used in the place of the free guanidine derivative, a compound, m.p. 125–6°, but not identical with the required diguanide (I) was obtained in poor yields. When this work was completed, we came to know that patent applications have been filed by the Imperial Chemical Industries, Limited, covering substantially the same processes.

Attempts were next made to prepare the two compounds, N′-(*p*-chloroanilinothioformyl)-N″-*iso*propylguanidine (III) and N′-(*p*-chlorophenyl)-N″-(*iso*propylaminothioformyl) guanidine (IV), and then to replace the sulphur atom in them by the imino groups by known methods.[4, 5] But so

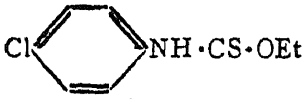


(III)



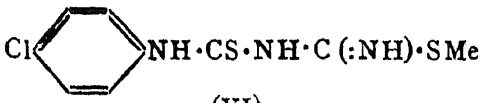
IX)

far, we have not succeeded in preparing either of these two compounds, though a number of methods were tried. Condensation of *p*-chlorophenylisothiocyanate with *iso*propylguanidine in alcoholic solution furnished a product, m.p. 104·5–5·5°, which was identified to be *p*-chlorophenylthiourethane (V). In benzene solution, no condensation product



(V)

could be isolated. Similarly, phenylguanidine also did not condense with *iso*propylisothiocyanate. This confirms the conclusion of Slotta, Tschesche and Dressler[9] that the mustard oils condense with guanidine and *syn*diphenylguanidines but not with mono-substituted guanidines. The condensation of *p*-chlorophenylcyanamide with either thiourea or *iso*propylthiourea furnished only the polymerised products of *p*-chlorophenylcyanamide. *p*-Chlorophenylisothiocyanate condensed easily with isomethylthiourea to yield S-methyl-N-(*p*-chloroanilino) thioformylisothiourea (VI), m.p. 134·5–5° Attempts to replace the methylmercapto group in this compound by the amino or substituted amino groups led to unexpected changes. On boiling the compound with alcoholic ammonia, *p*-chlorophenylthiourea was obtained. By using *iso*propylamine in the place of ammonia under a variety of conditions, three products were isolated: (1) a compound, m.p. 125–6°, identified to be *p*-chlorophenyl*iso*propylthiourea (VII), (2) a compound, m.p. 157–8° which appears to be *p*-chlorophenylcyanthiourea (VIII) and (3) a compound, m.p. 203–5°, which has not been identified.



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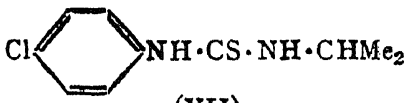
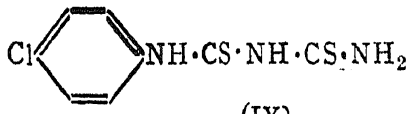


(VII)

(VIII)

Attempts were then made to prepare compounds of type (III) through the phenyldithiobiuret derivative. On condensing two molecular equivalents of *p*-chloraniline with isopersulphocyanic acid, *p*-chlorophenyldithiobiuret (IX), m.p. 163–4°, was obtained. On boiling this compound with two molecular equivalents of mercuric oxide and ammonia, *p*-chlorophenyldicyandiamide (II) was obtained in good yields. This method is of particular interest because in the case of many amines, particularly the heterocyclic compounds not easy to diazotise, the dicyandiamide derivative could be prepared by this route. We have actually applied this to prepare quinoline derivatives and the results obtained will be published in due course. But in the attempts to prepare a compound replacing only the end thioamide

group by the amidine group by using only one molecular equivalent of mercuric oxide and ammonia, the only crystalline compound isolated was the diacyandiamide derivative. On boiling *p*-chlorophenyldithiobiuret with two molecular equivalents of mercuric oxide and *iso*propylamine, N-*p*-chlorophenyl-N′-isopropyl-N″-cyanoguanidine (X), m.p. 151·5°, was



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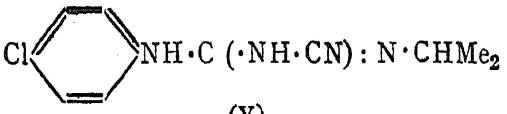
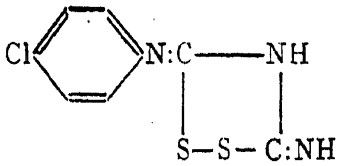


(X)

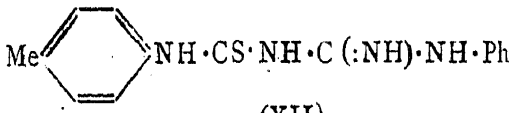
obtained. In this case also, using one molecular equivalent of mercuric oxide and *iso*propylamine, the anticipated compound (III) was not isolated. In *p*-chlorophenyldithiobiuret, the end thioamide group gets easily converted into the cyano group in the presence of desulphurising agents and it is not possible to replace the sulphur atom by the imino group. On the other hand, the sulphur atom in the thioamido group near the benzene ring undergoes this replacement quite easily. Thus, we have a good method of preparing compounds of formula (X) which we are utilising for the synthesis of many substituted diguanides by condensing them with the appropriate amines.

p-Chlorophenyldithiobiuret on oxidation with iodine in alcoholic solution yielded the hydroiodide of *p*-chlorophenylthiuret (XI). We could not

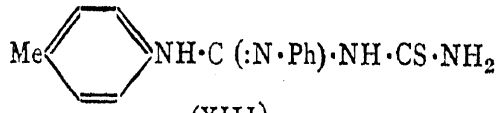


(XI)

crystalise the free base which is unstable. On boiling this with alcoholic ammonia, a deep seated change took place and no crystalline product could be isolated. Fromm and Veller[10] have condensed *p*-methylphenylthiuret with aniline and *p*-toluidine and have isolated the two isomeric products (XII) and (XIII). We expected to obtain the compound (III) by boiling

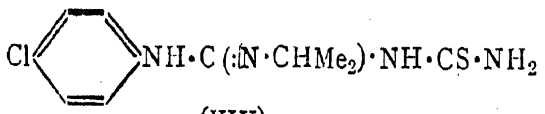


(XII)



(XIII)

p-chlorophenylthiuret with *iso*propylamine. Instead, the only compound isolated was found to be the isomer, N-*p*-chlorophenyl-N′-*iso*propyl-N″-cyanoguanidine (XIV). The structure of this is confirmed since this com-



(XIV)

pound on boiling with mercuric oxide in alcoholic solution furnished N-*p*-chlorophenyl-N′-*iso*propyl-N″-cyanoguanidine (X). On boiling the compound (XIV) with alcoholic ammonia and mercuric oxide, surprisingly

m.p. 96–7° (solidifies and then melts again at 125–6°). This sample gave no depression in m.p. on admixture with a commercial sample similarly crystallised.

(ii) Sodium (0·3 g.) was dissolved in butyl alcohol (20 c.c.) and to this *iso*propylguanidine sulphate (3·8 g.) was added and shaken well. To the mixture, *p*-chlorophenylcyanamide (1·9 g.) was added and refluxed for 3 hours. The resulting mixture was diluted with ether (200 c.c.) and extracted with dilute hydrochloric acid (75 c.c. of 1 : 3). The acid extract after clarification with charcoal was basified with a solution of sodium hydroxide when the diguanide derivative separated as an oil and gradually solidified to a crystalline mass (yield, 1·5 g.). On crystallisation from dilute alcohol, it separated as a mass of fine crystalline needles, m.p., 96–97°, and was found to be identical with the product obtained in experiment (i). Alternatively, the butyl alcohol was steam distilled off and the resulting acid solution basified. But the product obtained by this proceedure was not as pure as that obtained by acid extraction.

(iii) Sodium (0·4 g.) was dissolved in butyl alcohol (50 c.c.) and to this *p*-chlorophenyl*iso*methylthiourea hydroiodide (3·4 g., prepared by the action of methyl iodide on *p*-chlorophenylthiourea) and *iso*propylguanidine sulphate (3·0 g.) were added, the mixture refluxed for 4 hours and worked up as above described, whereby 1·0 g. of the diguanide derivative was obtained. In this experiment sodium bicarbonate was used in the place of sodium but the yield was considerably less than when sodium was used.

(iv) A mixture of *iso*propylguanidine sulphate (2·9 g.), *p*-chlorophenylthiourea (3·0 g.) and lead oxide (4·5 g.) in butyl alcohol (50 c.c.) was refluxed for six hours with good shaking. The mixture was filtered, the lead sulphide washed with butyl alcohol and the combined butanol solution worked up as indicated above, when about 0·25 g. of the diguanide derivative was obtained.

p-Chlorophenylthiourethane (*V*).—This compound was obtained by refluxing *p*-chlorophenyl*iso*thiocyanate for thirty minutes in alcoholic solution. It crystallised from dilute alcohol in beautiful needles and had m.p. 104–5·5°. (Found: N, 6·09; 6·20. $C_9H_{10}NSCl$ requires N, 6·49 per cent.). This compound was identical with that obtained when *p*-chlorophenyl*iso*thiocyanate was refluxed with *iso*propylguanidine in alcoholic solution.

S-Methyl-N-(p-*chloroanilino*)-*thioformylisothiourea* (*VI*).—To isomethylthiourea sulphate (4·9 g.) dissolved in water (20 c.c.) was added sodium bicarbonate (2·9 g.) followed by acetone (40 c.c.); to the solution *p*-chlorophenylisothiocyanate (4·8 g.) was added and the mixture refluxed for about

two hours. The mixture which had separated into two layers was allowed to evaporate and the crystalline product that separated was collected (7·2 g.) and recrystallised from dilute alcohol. The condensation product was obtained in prismatic needles and had m.p. 134·5–135° (dec.). (Found: N, 16·05. $C_9H_{10}N_3S_2Cl$ requires N, 16·18 per cent.).

Action of alcoholic ammonia on S-methyl-N-(p-*chloroaniline*)*-thioformyl-*iso*thiourea.*—S-Methyl-N-(*p*-chloroanilino)-thioformylisothiourea (1·0 g.) in alcoholic ammonia (50 c.c.) was gently refluxed for four hours and the solution evaporated to dryness. The residue on crystallisation from alcohol, yielded a product crystallising in thin needles, m.p. 175–76·5°. (Found: N, 14·9; 15·1. *p*-Chlorophenylthiourea, $C_7H_7N_2SCl$, requires N, 15·02 per cent.), which showed no depression in m.p. on admixture with a genuine sample of *p*-chlorophenylthiourea.

Action of iso*propylamine on S-methyl-N-*(p-*chloroanilino*)*-thioformyl-*iso*thiourea.*—To S-methyl-N-(*p*-chloroanilino)-thioformylisothiourea (1 g.) in alcohol (20 c.c.) were added water (5 c.c.) and *iso*propylamine (2 c.c.); the mixture was refluxed for three hours, the solution evaporated to dryness and the residue crystallised from a mixture of benzene and little alcohol. A product crystallising in plates, m.p. 157–8°, (Found: N, 19·6; 20·1; 19·8 per cent.) was obtained, which appears to be *p*-chlorophenylcyanothiourea ($C_8H_6N_3SCl$ requires N, 19·85 per cent.).

In the above experiment, when a large excess of *iso*propylamine was used and the time of refluxing prolonged, two other products were obtained: (1) a compound crystallising in needles, m.p. 202·5–3° (Found: N, 16·75; 16·55; 16·8; 17·03 per cent.) and (2) a compound crystallising in fine needles, m.p. 124–5°, which was identified to be *p*-chlorophenyl*iso*propylthiourea by comparing it with a specimen prepared as indicated below.

*p-Chlorophenyl*iso*thiocyanate.*—This compound was prepared by Losanitch[11] by the action of iodine on bis-(*p*-chlorophenyl)-thiourea. The following method is a rapid one but the yield is only about 30 per cent. of the theoretical.

To *p*-chloroaniline (40 g.) suspended in alcohol (75 c.c.) and ammonium hydroxide (75 c.c.), was added carbon disulphide (45 c.c.) with good shaking. When the mixture warmed up, it was cooled in ice-bath. A clear solution was obtained and then the crystalline ammonium dithiocarbamate derivative separated. It was allowed to stand overnight, the crystalline product filtered, dissolved in water (about 1 litre) and a solution of lead nitrate (100 g.) added and the mixture subjected to steam distillation. *p*-Chlorophenyl*iso*thiocyanate passed over as an oil and solidified as a snow white crystalline mass; m.p. 46–7° (yield, 19 g.).

Iso*propyl*iso*thiocyanate*.—The preparation of this compound has been reported by Jahn[12]; the following method gives a better yield. *Iso*propylamine (84 c.c.) in water (150 c.c.) was cooled in an ice-bath and carbon disulphide (60 c.c.) was run in and shaken well. To this, under cooling, a solution of sodium hydroxide (40 g.) in water (230 c.c.) was added gradually in the course of about half an hour. A white precipitate separated. After allowing the mixture to stand for about two hours more, water was added to dissolve the crystalline solid, followed by a concentrated solution of mercuric chloride (136 g.). After shaking the mixture well, it was subjected to steam distillation. *Iso*propyl*iso*thiocyanate passed over as an oil; it was collected and fractionated; b.p. 138° (yield, 38 g.).

N[1]*-Chlorophenyl-N*[5]-iso*propylthiourea* (*VIII*).—This compound was obtained by refluxing (1) *p*-chlorophenyl*iso*thiocyanate with *iso*propylamine and (2) *p*-chloraniline with *iso*propyl*iso*thiocyanate, in alcoholic solution. On crystallisation from dilute alcohol it separated in beautiful needles, m.p. 124–5°. (Found: N, 12·17. $C_{10}H_{13}N_2SCl$ requires N, 12·26 per cent.)

p-Chlorophenyldithiobiuret (*IX*).—A mixture of *iso*persulphocyanic acid (3·0 g.) and *p*-chloraniline (5·1 g.) in alcohol (75 c.c.) was refluxed for two hours. The yellow *iso*persulphocyanic acid gradually disappeared and a crystalline product separated. The mixture was cooled, the solid obtained dissolved in cold 5 per cent. sodium hydroxide solution to free it from the accompanying sulphur, filtered and the clear filtrate acidified with concentrated hydrochloric acid whereby the condensation product separated as a bulky mass. It was separated by filtration (yield, 2·3 g.) and crystallised from dilute alcohol, whereby *p*-chlorophenyldithiobiuret separated in clusters of fine needles, m.p. 163–4°. (Found: N, 17·23; 17·41. $C_8H_8N_3S_2Cl$ requires N, 17·1 per cent.)

Action of alcoholic ammonia and mercuric oxide on p-*chlorophenyldithiobiuret*.—*p*-Chlorophenyldithiobiuret (2 g.) in alcoholic ammonia (50 c.c.) and mercuric oxide (3·8 g.) was refluxed on the steam-bath. The yellow oxide rapidly turned black. After refluxing for about four hours, it was filtered, the alcohol evaporated from the filtrate and the residue crystallised from dilute alcohol. The compound crystallising in thin leaflets, m.p. 202–3°, was identified to be *p*-chlorophenyldicyandiamide by comparing it with an authentic specimen.

In the above experiment, even when one molecular equivalent of mercuric oxide was used, the only product that could be isolated in crystalline form was the same *p*-chlorophenyldicyandiamide.

Action of iso*propylamine and mercuric oxide on p-chlorophenyldithiobiuret.*—*p*-Chlorophenyldithiobiuret (2 g.) in alcohol (50 c.c.) and *iso*-propylamine (5 c.c.), was treated with mercuric oxide (3·8 g.) and the mixture refluxed on the steam-bath for four hours. The black precipitate was filtered off and the filtrate evaporated to dryness. On crystallisation from alcohol, a product separating in rectangular plates, m.p. 151·5°, (Found: N, 23·68 per cent.), was obtained which was identified to be N-*p*-chlorophenyl-N′-isopropyl-N″-cyanoguanidine (X) (see below).

p-*Chlorophenylthiuret* (*XI*)-*hydroiodide.*—*p*-Chlorophenyldithiobiuret (10 g.) in alcohol (100 c.c.) warmed to obtain a solution and to this a concentrated solution of iodine (10 g.) in alcohol was added till the decolorisation was complete and a faint colour of iodine was permanent. A bulky crystalline precipirate separated which rapidly became granular. This was cooled, filtered and washed with alcohol. The hydroiodide of *p*-chlorophenylthiuret thus obtained (10 g.) was used as such without any further purification.

Action of alcoholic ammonia and mercuric oxide on p-*chlorophenylthiuret.*—*p*-Chlorophenylthiuret hydroiodide (2 g.) in alcoholic ammonia (50 c.c.) was treated with mercuric oxide (1·8 g.) and the mixture gently refluxed for 3 hours. The black precipitate was filtered off, and the mother liquor, on working up as usual, furnished a product crystallising in thin plates, m.p. 202–3°, which showed no depression in m.p. on admixture with *p*-chlorophenyldicyandiamide.

Action of iso*propylamine and mercuric oxide on* p-*chlorophenylthiuret.*—In the above experiment when *iso*propylamine was used in the place of alcoholic ammonia, a compound, m.p. 150–1°, was obtained which was identified to be N-*p*-chlorophenyl-N′-*iso*propyl-N″-cyanoguanidine.

N-(p-*Chlorophenyl*)-*N*′-iso*propyl-N*″-*aminothioformylguanidine* (*XIV*).—*p*-Chlorophenylthiuret hydroiodide (15 g.) suspended in alcohol (50 c.c.) was treated with *iso*propylamine (15 c.c.) and the mixture boiled for three hours. The alcohol was distilled off and the residue boiled with a small quantity of water and filtered hot to free it from the sulphur accompanying the product. From the filtrate, the condensation product crystallised out in fine plates (yield, 7 g.). On repeated crystallisation from water, it had m.p. 153–4·5°. (Found: N, 21·02; 21·12; 20·99; 21·19. $C_{11}H_{15}N_4SCl$ requires N, 20·70 per cent.)

p-*Chlorophenyl-N*′-iso*propyl-N*″-*cyanoguanidine* (*X*).—The abovementioned compound (0·5 g.) dissolved in alcohol (20 c.c.) was treated with mercuric oxide (0·6 g.) and the mixture refluxed for about three hours. The black precipitate was filtered off and the residue on working up yielded the

desulphurised product which crystallised from dilute alcohol in shining prisms and had m.p. 150–151·5°. (Found: N, 23·72; 23·94; 23·75. $C_{11}H_{13}N_4Cl$ requires N, 23·67 per cent.)

Action of alcoholic ammonia and mercuric oxide on the compound (XIV).—In this experiment, the compound that was isolated crystallised from alcohol and had m.p. 201–2° (Found: N 29·2. *p*-Chlorophenyldicyandiamide. $C_8H_7N_4Cl$. requires N, 28·6 per cent.) showing no depression in m.p. on admixture with a genuine sample of *p*-chlorophenyldicyandiamide.

Action of alcoholic iso*propylamine and mercuric oxide on the compound (XII).*—In this case, the compound isolated was identified to be N-*p*-chlorophenyl-N′-*iso*propyl-N″-cyanoguanidine.

We are indebted to Mr. M. H. Shah of the Department who carried out the analyses recorded in this paper. We also thank Major-General Sir Sahib Singh Sokhey for his kind interest in these investigations.

Summary

In the course of attempts to prepare quinoline derivatives with substituted diguanide side chains, the methods of syntheses of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (I) as a model compound have been investigated. The diguanide (I) was obtained by the condensation of *iso*propylguanidine with (1) *p*-chlorophenylcynamide, (2) S-methyl-*p*-chlorophenyl*iso*thiourea and (3) *p*-chlorophenylthiourea in the presence of lead acetate. Attempts to prepare the compound (III) by the condensation of *p*-chlorophenyl*iso*-thiocyanate with *iso*propyl guanidine were unsuccessful. *p*-Chlorophenyl-*iso*thiocyanate condensed with S-methyl *iso*thiourea to yield S-methyl-N^1-(*p*-chloroanilino)-thioformyl*iso*thiourea (VI but the methylmercapto group of this compound could not be replaced by either imino or *iso*propylimino groups. *p*-Chloraniline condensed with *iso*persulphocyanic acid to yield *p*-chlorophenyldithiobiuret which on boiling with ammonia and *iso*propylamine in the presence of mercuric oxide furnished respectively *p*-chlorophenyldicyandiamide and N-*p*-chlorphenyl-N′-*iso*propyl-N″-cyanoguanidine (X). *p*-Chlorophenyldithiobiuret on oxidation with iodine yielded *p*-chlorophenylthiuret which on treatment with ammonia and *iso*propylamine in the presence of mercuric oxide furnished respectively *p*-chlorophenyldicyandiamide and N-*p*-chlorophenyl-N′-*iso*propyl-N″-cyanguanidine. On treatment with *iso*propylamine, *p*-chlorophenylthiuret yielded only N-(*p*-chlorophenyl)-N′-*iso*propyl-N″-aminothioformylguanidine (XII), which easily undergoes desulphurisation to yield the compound (X); however, in the presence of alcoholic ammonia and mercuric oxide, *p*-chlorophenyldicyanamide was obtained.

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