RESEARCH PAPERS

ANTIMALARIALS : DERIVATIVES OF TETRAHYDROACRIDINE

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Received December 15, 1949

The activity of mepacrine in malaria infections has centred attention on the acridine nucleus, but the suggestion of Curd, Davey and Rose[1] that the activity of this type of compound depended upon the possibility of an electron displacement cannot be accepted, as compounds in which such a shift cannot take place—viz. 7-methoxy-1 : 2 : 3 : 4-tetrahydroacridone—were proved to be active as causal prophylactics by Stephen, Tonkin and Walker[2]. Several derivatives of tetrahydroacridine have been prepared and tested. Magidson and Travin[3] obtained compounds in which various basic side chains were attached to the meso carbon but even 2-chloro-5-(ω-diethylamino-α-methylbutyl)amino-1 : 2 : 3 : 4-tetrahydroacridine was found to be inactive. Sargent and Small[4] extended the series of this type of compound with no more success.

Consideration of the structure of the cinchona alkaloids led to the suggestion, by analogy with the quinuclidine fragment, that in derivatives of tetrahydroacridine the basic side chain should be incorporated in the reduced portion of the molecule. It was therefore decided to attempt the preparation of derivatives of 1 : 2 : 3 : 4-tetrahydroacridine in which the 1, 2, 3, or 4 position was substituted with a tertiary amino group, such compounds not having been previously reported.

The preparation by Glynn (unpublished report) of 1 : 5-dichloro-1 : 2 : 3 : 4-tetrahydroacridine provided an intermediate from which a start could be made. This intermediate was prepared by a modification of the original process by refluxing together 2-chlorocyclohexanone, anthranilic acid and phosphorus oxychloride. After decomposing with ice and basifying with ammonia the required compound was obtained in 95 per cent. yield. After recrystallisation from diethylamine the compound melted at 141°C. This compound proved to be very stable towards hydrolytic agents and was recovered unchanged after refluxing with (a) 10 per cent. hydrochloric acid (compare 5-chloroaminoacridines[5]); (b) concentrated hydrochloric acid for 3 hours (compare 5-chloroalkoxyacridines[6]); and (c) alcoholic potash for one hour. Heating with sodium carbonate decahydrate and with sodamide in boiling benzene were also without action.

Refluxing for 24 hours with 20 per cent. w/v sulphuric acid converted the compound into the monohydrate of 1-hydroxy-1 : 2 : 3 : 4-tetrahydroacridone which lost the molecule of water on heating at 120°C. *in vacuo*. This product proved to be a typical acridone, being yellow in colour, high melting and soluble in N/1 potassium hydroxide in alcohol (50 per

cent.) giving a yellow solution from which it could be recovered on acidification or by dilution with water. Attempts to reduce this compound to the corresponding acridine by the classical methods[7,8] using sodium in alcohol or sodium amalgam gave uncharacterisable products.

On adding sodium carbonate decahydrate to a gently boiling mixture of 1:5-dichloro-1:2:3:4-tetrahydroacridine in methylaniline, 3:4-dihydroacridone was obtained as a viscid orange oil which finally crystallised from diethylamine and formed a hydrochloride which was quickly hydrolysed by water, depositing the free base. On refluxing the dichlorotetrahydroacridine with glacial acetic acid for 3 hours the mono-acetate of 1-hydroxy-1:2:3:4-tetrahydroacridone was obtained in the form of buff crystals readily soluble in alcohol, the solution exhibiting a bright blue fluorescence. The alcoholic solution became yellow with a green fluorescence on addition of potassium hydroxide and the change was reversed on acidification.

Reactions of the dichloro compound with amines with or without a catalyst proved to be equally difficult, attention being concentrated upon the reaction with ω-diethylamino-α-methylbutylamine. Eventually success was attained by heating a mixture of the dichlorotetrahydroacridine, excess of amine and anhydrous potassium carbonate at 150°C. with stirring. The main product gave analytical figures required by a chloro-(ω-diethylamino-α-methylbutyl)aminotetrahydroacridine and occurred as a deep red viscous syrup, soluble in benzene and alcohol with a slight green fluorescence. It was readily soluble in acids forming yellowish-brown solutions. The *p*Ka for the base obtained by potentiometric titration against N/10 sodium hydroxide after solution in a mixture of 20 ml. of N/10 hydrochloric acid, 20 ml. of water and 80 ml. of methyl alcohol was found to be 8·5. It formed a dipicrate and a water-insoluble methylenebisoxynaphthoate, and a very hygroscopic dihydrochloride separated from solution in alcoholic hydrogen chloride.

The relative positions of the chloro group and the alkylamino group have not yet been definitely established but fortunately the structure of two by-products of the reaction support the contention that the compound should be described as 1-(ω-diethylamino-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine. These by-products were characterised as 3:4-dihydro-5-chloroacridine and 1-hydroxy-5-chloro-1:2:3:4-tetrahydroacridine.

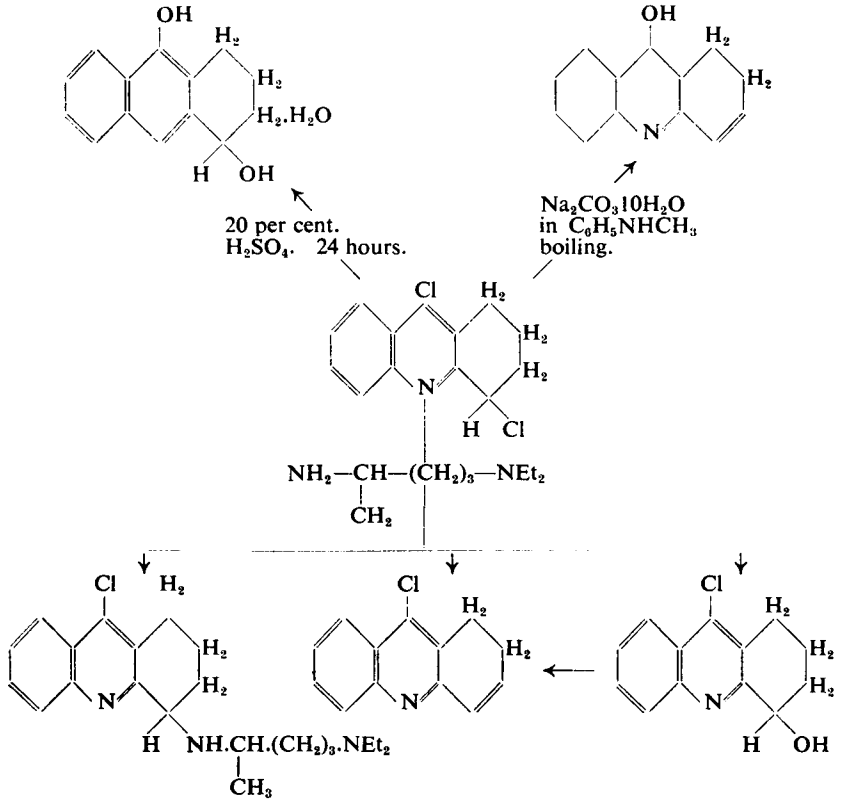
During these reactions exactly half the chlorine in the original dichlorotetrahydroacridine was obtained as ionisable chloride. Both the by-products possess a meso chloro group, thus indicating that under the conditions of this experiment the meso chloro group is the more stable. For these reasons the compound may be considered to be (1-(ω-diethylamino-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine. The reactions may be summarised as shown on page 147.

Thanks are due to Professor Buttle of this School and to the Wellcome Laboratories of Tropical Medicine for the pharmacological tests.

After toxicity tests had been carried out on mice the hydrochloride of (1-(ω-diethylamino-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine was administered at the rate of 100 mg./kg. to 10-day-old chicks

inoculated with 100 × 10^6 parasitised red cells. One dose was given on the day of injection, and then twice daily on 3 subsequent days. Blood smears were taken on the fifth day and the percentage of parasitised red cells found. Control birds were untreated. The compound was found to be inactive.

The inactivity may apply to all such derivatives of tetrahydroacridine but in this connection it should be remembered that acridine derivatives containing a 1-amino group are devoid of bacteristatic activity whereas amino groups in the other positions give compounds which exhibit activity[9].



EXPERIMENTAL

1:5-Dichloro-1:2:3:4-tetrahydroacridine was prepared by Glynn's method with important modifications. Freshly distilled (69° to 70°C. at 9 mm.). 2-chlorocyclohexanone (23 g.), anthranilic acid (23 g.), and phosphorus oxychloride (115 ml.) were refluxed together for 1½ hours. The dark liquid was poured, with vigorous stirring, on to ice and the whole left to stand for 2 hours with occasional stirring. The resinous precipitate described by the original author redissolved on standing and on carefully basifying the filtered liquid with strong ammonia in the

presence of ice dichlorotetrahydroacridine was precipitated in almost theoretical yield. It was washed with cold water, and dried.

Yield 40 g. (=95 per cent. of theory). M.pt. 135°C. On recrystallising from acetone the melting-point rose to 138°C. Yield 27 g. (=70 per cent. of theory). A small quantity recrystallised from diethylamine gave a constant melting-point of 141°C. Found C, 61·74; H, 4·24; N, 5·37; Cl, 28·00 per cent. $C_{13}H_{11}NCl_2$ requires C, 62·15; H, 4·38; N, 5·57; Cl, 27·89 per cent. Pale cream needles from diethylamine. Insoluble in water and in acetic acid (30 per cent.) but soluble to yellow solutions in mineral acids.

1 : 5-Dihydroxytetrahydroacridine (1-hydroxytetrahydroacridone). 1 : 5-dichlorotetrahydroacridine (2 g.) was refluxed for 24 hours with sulphuric acid (20 per cent. w/w) and the solution then poured on to ice, and basified with ammonia solution. The pale yellow precipitate, when washed and dried, melted over a range of 160° to 200°C. (Yield 1·7 g.). After removing unchanged starting material by refluxing with acetone the residual product melted at 226°C. and after twice recrystallising from alcohol a pale yellow substance of melting-point 280° to 284°C. (sealed tube) was obtained which proved to be the monohydrate of dihydroxytetrahydroacridine. Found C, 66·83; H, 6·33; N, 6·23; Cl, 0·65 per cent. $C_{13}H_{13}NO_2,H_2O$ requires C, 66·95; H, 6·44; N, 6·01 per cent. Loss in weight on heating at 120°C. *in vacuo* for 2 hours was 7·75 per cent. One molecule of water requires a loss of 7·72 per cent.

The yellow colour, high melting-point, and solubility of the product in 50 per cent. alcoholic potassium hydroxide (N/1) to form a yellow solution from which it is reprecipitated by acids or by water are together sufficient proof of the acridine structure. The loss of only one molecule of water on heating *in vacuo* favours the formulation of the product as the monohydrate of dihydroxytetrahydroacridine, as against the dihydrate of 3 : 4-dihydroacridone.

Reaction of dichlorotetrahydroacridine with methylaniline. Formation of 3:4-dihydroacridone. Dichlorotetrahydroacridine (1 g.) and methylaniline (2 ml.) were heated together until just boiling, and finely powdered sodium carbonate decahydrate added in small quantities over a period of 15 minutes. The contents of the flask were then subjected to steam distillation until no more methylaniline distilled and the flask left to cool. An orange brown residue was obtained which, after unsuccessful attempts to recrystallise from various solvents, was dissolved in benzene and poured on to an alumina column, followed by development with benzene. A broad orange-yellow band capped by a small pink band resulted, and development was continued until all the former had been removed from the column. Upon removal of the benzene the dihydroacridone was obtained as a viscous orange oil which refused to crystallise. Yield 0·6 g. (76 per cent. of theory). The hydrochloride was obtained as a blue hygroscopic solid by passing dry hydrogen chloride into an ethereal solution of the base. Found C, 66·0; H, 5·9; N, 6·0; Cl, 15·48 per cent.: $C_{13}H_{11}NO.HCl$ requires C, 66·8; H, 5·14; N, 6·00; Cl, 15·2 per cent.

The substance occurred as a bright orange oil becoming more viscous and finally recrystallisable from diethylamine to yield an orange powder melting at 192°C. and soluble in N/1 alcoholic potash. Solutions in organic solvents were bright orange with a green fluorescence. It formed a deep blue hydrochloride which was soluble in dilute hydrochloric acid, but hydrolysed in water to the orange oily base. Picrate, orange, m.pt. 205°C.

Both chlorine atoms of the original compound have been removed, and from the analytical evidence only one has been replaced by a hydroxyl group. Hence the other must have been lost as hydrochloric acid. This could only occur to the halogen in the 1-position. Furthermore the orange colour and the solubility in alcoholic potash are evidence for the acridine structure.

Preparation of 1-(ω-diethylamine-α-methylbutyl)amino-5-chloro-1:2:3:4-tetrahydroacridine. Dichlorotetrahydroacridine (12 g.), ω-diethylamino-α-methylbutylamine (24 ml. freshly distilled) and potassium carbonate (6 g.) (oven-dried at 110°C.) were heated together at 140° to 150°C. with stirring for 3 hours and, after cooling, the whole was extracted with an ether-water mixture and allowed to separate. The ionisable chloride content of the aqueous layer was determined gravimetrically, 7 g. of AgCl, equivalent to one chlorine atom, being obtained.

The dark ethereal layer was washed several times with water to remove the excess of water-soluble diamine, and then filtered to remove a little tar. The required base was then extracted by shaking with acetic acid (33 per cent.). The residual yellow ethereal solution was washed well with water and evaporated when a dark crystalline residue of 3:4-dihydro-5-chloroacridine (5·55 g.) was obtained (See A below). The deep-red acetic acid solution after being filtered from a little white solid (see B below) was basified with caustic soda and the bases extracted with ether. After washing the ether layer with water, drying with anhydrous sodium sulphate, and removing the ether, a deep red viscous oil was obtained (yield 4·05 g.).

This oil was dissolved in benzene and chromatographed through a column of alumina, followed by development of the column with benzene. Liquid chromatograms were collected in the following order and the solvent removed from each *in vacuo* to give the product and the yield indicated. (1) Deep red solution yielding 2·17 g. of red oil. (2) Orange solution yielding 0·4 g. of orange oil.

The development was now continued with 2 per cent. ethyl alcohol in benzene, when the following fractions were collected: (3) Pale straw solution yielding 0·13 g. orange oil. (4) Deep red solution yielding 0·7 g. red oil. (5) Brownish-straw solution yielding 0·13 g. dark solid, finally, on clearing the column with alcohol (97 per cent.) there was obtained (6) Orange yellow solution yielding 0·01 g. of solid. These six fractions accounted for 3·71 g. of the original 4·05 g. of red oil.

The 2·17 g. of red oil (1) was redissolved in benzene and passed through a second column of alumina. The column exhibited a 9-inch red band over a 2-inch bright orange band. After the latter had been washed

through with benzene and collected separately, the top of the column, showing a dirty brown zone, was removed mechanically and the bright red zone washed out with alcohol. On removal of the solvent *in vacuo* the bright red oily residue weighed 1·03 g., and after a final treatment on a fresh alumina column using benzene to develop, a homogeneous deep red treacle was obtained (yield 0·91 g.; 5 per cent. of theory, calculated on the acridine component).

The bright orange benzene solution, collected separately from the second column, on evaporation yielded a very viscous orange syrup (0·91 g.), which after treatment on a third column and evaporation of the benzene at a 100°C. *in vacuo* weighed 0·73 g. This substance has not yet been identified, but from the analytical evidence is very similar in composition to the red product. Found C, 71·41; 71·18; H, 7·23; 7·611; N, 9·60; 9·78; Cl, 7·0 per cent.

Identification and Properties of the red syrup. For analysis, the product obtained from the third alumina column was freed from benzene vapours by heating at 100°C. at 2 cm. pressure for half an hour. Found C, 72·53; 72·15; H, 8·30; 8·37; N, 11·2; 11·5; Cl, 7·55 per cent. $C_{22}H_{32}N_3Cl$ requires C, 70·68; H, 8·56; N, 11·24; Cl, 9·50 per cent. A picrate was obtained as a greenish yellow powder melting at 164°C. Found C, 48·91; H, 4·48; N, 14·6; Cl, 4·37 per cent. $C_{22}H_{32}N_3Cl.2C_6H_3N_3O_7$ requires C, 49·1; H, 4·57; N, 15·1; Cl, 4·27 per cent. None of the other products was characterised except A and B.

Methylene bisoxynaphthoate of the base. A 5 per cent. solution of ammonium methylenebisoxynaphthoate was prepared by boiling 5 g. of methylenebisoxynaphthoic acid with water and ammonia until the free acid began to precipitate. The yellow solution was then placed in a stoppered bottle and the clear liquid decanted when required. 0·2 g. of the red base was dissolved in 20 ml. of hydrochloric acid (N/10) and the calculated volume of caustic soda (N/10) added to give a solution containing the dihydrochloride of the base. 5 ml. of the clear solution of ammonium methylenebisoxynaphthoate was added with stirring and, after leaving a few moments, the pink precipitate was removed from the colourless solution, washed with water, and dried at 100°. Melting point, indefinite, about 260°C. Found C, 69·40; H, 6·08; N, 5·50; Cl, 4·61. $C_{45}H_{48}O_6N_3Cl$ requires C, 70·91; H, 6·30; N, 5·51; Cl, 4·672 per cent.

Examination of A. Yield 5·55 g. After recrystallising once from acetone and twice from diethylamine the melting-point was constant at 142°C., but a mixed melting-point with the original dichloro compound indicated a new substance. (Mixed m.pt.—122° to 123°C.) Analysis indicated that a chlorine atom had been lost from the original dichlorotetrahydroacridine, but had not been replaced by a hydroxyl group. This could only happen to the chlorine in the l-position, hence the chlorine atom at position 5 is intact and the substance A is probably 3:4-dihydro-5-chloroacridine. Found C, 69·35; H, 4·83; N, 6·87; Cl, 15·55 per cent. CHNCl requires C, 72·4; H, 4·64; N, 6·5; Cl, 16·47 per cent.

Examination of B. This weighed only 0·12 g., and as it melted fairly sharply at 166°C. no attempt was made to recrystallise it. Analysis

showed it to be a chlorohydroxytetrahydroacridine, one of the chlorine atoms of the original dichlorotetrahydroacridine having been replaced by a hydroxyl group. As the substance has a low melting-point and is not appreciably more soluble in alcoholic potassium hydroxide (50 per cent., N/1) than in alcohol (50 per cent.) and was therefore not 1-chlorotetrahydroacridone, it must be 1-hydroxy-5-chlorotetrahydroacridine. Its very pale cream colour, not deepened to yellow by alcoholic potash, was further evidence for this formulation as was the occurrence of Compound A. Found C, 66·63; H, 5·18; N, 6·43; Cl, 15·52 per cent. $C_{13}H_{12}NOCl$ requires C, 66·83; H, 5·14; N, 6·0; Cl, 15·21 per cent.

Reaction between the same two components in glacial acetic acid and acetic anhydride. Dichlorotetrahydroacridine (0·5 g.) and ω-diethylamine-α-methylbutylamine (0·5 g.) were refluxed together in solution in glacial acetic acid (10 ml.) and acetic anhydride (5 ml.) for 3 hours, and the mixture poured into water. After standing for 3 days the buff precipitate was collected, dried and weighed. Yield 0·4 g.

After recrystallisation from alcohol (50 per cent.) the cream crystals melted at 276°C. (sealed tube). The compound was soluble in alcohol, even dilute, with a bright blue fluorescence. Readily soluble in alcoholic potash (50 per cent. N/1) with a bright green fluorescence and a yellow colour, the fluorescence being turned blue and the yellow colour discharged by acids. The high melting-point and behaviour with alcoholic potash indicated the presence of the acridone structure, and the analytical figures showed the substance to be 1:5-dihydroxytetrahydroacridine-1-acetate. Found C, 71·63; H, 5·86; N, 5·65 per cent. $C_{15}H_{13}NC_3$ requires C, 70·05; H, 5·84; N, 5·45 per cent. On repeating the experiment without the diamine, the same product was obtained. (Identified by melting-point and mixed melting-point with above.)

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