A NOTE ON THE IDENTIFICATION OF SOME ANTIMALARIAL DRUGS

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Received November 22, 1957

Crystal and colour tests are described for 14 antimalarial drugs.

Since the identification of quinine as the active constituent of cinchona bark, many attempts have been made to produce a substitute that would combine an enhanced plasmodicidal effect with diminished toxicity. Of the many hundred substances which have been tested for antimalarial activity only about a dozen have come into clinical use. These are all

TABLE I

Substance	Reagent	Crystals	Sensitivity (μg.)
Primaquine (Avlon, 6-methoxy-8-(4-amino-1-methylbutylamino) quinoline diphosphate)	Picric acid Styphnic acid	Rosettes of curved needles Rosettes of rods	0·1 0·1
Pentaquine (6-methoxy-8-(5-*iso*-propylaminoamylamino) quinoline phosphate)	Potassium cadmium iodide Picrolonic acid	Dendrites ON Dense rosettes	0·25 0·1
Isopentaquine (6-methoxy-8-(4-*iso*propylamino-1-methyl butylamino) quinoline)	Potassium cadmium iodide Gold cyanide	Curved needles Dendrites ON	0·1 1·0
Rhodoquine (6-methoxy-8-(diethylaminopropylamino) quinoline)	Picric acid Potassium mercury iodide	Rosettes of plates Rods ON	0·25 0·1
Amodiaquine, (Camoquin 7-chloro-4-(3′-diethylamino-methyl-4-hydroxyanilino) quinoline HCl)	Gold cyanide Potassium cadmium iodide Picric acid	Snowflake rosettes Burrs of fine needles Rosettes of plates	0·1 0·1 0·5
Chloroquine (Nivaquine, 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline diphosphate)	Styphnic acid	Rosettes of plates	0·5
Hydroxychloroquine, (Plaquenli, 7-chloro-4-(4-(*N*-ethyl-*N*-2-hydroxyethylamino) - 1 - methylbutylamino) quinoline sulphate	Picric acid Styphnic acid	Small irregular plates Snowflake rosettes	0·1 0·25
Nivaquine C (Sontoquine, 7-chloro - 3 - methyl - 4 - (diethyl aminopentylamino) quinoline HCl)	Platinum bromide Gold cyanide	Rosettes of irregular needles Dense feathery rosettes ON	0·1 1·0
Mepacrine (Atabrine, Quinacrine, 2-chloro-5(4-diethylamino-1-methylbutylamino)-7-methoxy acridine HCl)	Potassium tri-iodide (2) Gold cyanide	Rosettes of needles, often dense ON Clumps of hair-like needles	0·5 0·1
Proguanil (Paludrine, N^1-*p*-chlorophenyl-N^5-*iso*propyl diguanidine HCl)	Gold bromide/HCl Picrolonic acid	Long plates Short rods some in rosettes	0·25 0·25
Pyrimethamine (Daraprim, 2:4-diamino-5-(4′chlorophenyl)-6-ethylpyrimidine)	Gold bromide/HCl Potassium chromate	Serrated needles Irregular blades	0·1 0·1
377C54 (2:5 bis(*cyclo*hexylamino-methyl) naphthalene-1:6 diol di HCl)	Potassium tri-iodide (3) Ammonium thiocyanate	Rosettes of plates ON Rosettes of plates	0·1 1·0
Compound 5943 (N^1-3:4-dichlorophenyl-N^5 *iso*propyl diguanide HCl	Gold bromide/HCl Platinum chloride	Long plates, often serrated Rosettes of irregular rods	0·25 0·25

ON (overnight) indicates that the crystals do not usually form until the following day.

basic nitrogenous substances of alkaloidal type, the majority being aminoquinoline derivatives. As they are of low toxicity, few tests for their identification have been described.

This paper describes crystal and colour tests for fourteen of these compounds. Compound 5943 is a derivative of proguanil and is stated to be more active than the parent substance[1]. The substance 377C54

(2:5-bis(*cyclo*hexylaminomethyl) napthalene-1:6-diol diHCl) has recently undergone clinical trials[2]. Although metachloridine has been used in the treatment of malaria, it is actually a sulphonamide, and has therefore not been included in this study.

Experimental Procedure

Microcrystalline Tests

The hanging microdrop technique developed by Clarke and Williams[3,4] was used. Mepacrine and pyrimethamine were dissolved in 1 per cent acetic acid, the other compounds in 1 per cent hydrochloric acid.

TABLE II

Substance	Colour	Sensitivity (μg.)
Sulphuric acid-formaldehyde test (Marquis)		
Primaquine	Orange	0·5
Pentaquine	Orange	0·5
Isopentaquine	Orange	0·5
Rhodoquine	Orange	0·5
Mepacrine	Bright yellow	0·1
377C54	Yellow-brown	0·5
Ammonium vanadate test		
Primaquine	Purple—orange	0·5
Pentaquine	Purple—orange	0·5
Isopentaquine	Purple—orange	0·5
Rhodoquine	Purple—orange	0·5
Mepacrine	Purple—bright yellow	0·25
377C54	Grey—purple—orange	0·5
Ammonium molybdate test		
Primaquine	Pale blue	1·0
Pentaquine	Pale blue	1·0
Isopentaquine	Pale blue	1·0
Rhodoquine	Pale blue	1·0
Amodiaquine	Green—*blue—green	0·5
Mepacrine	Yellow-green	0·25
377C54	Blue—green—yellow	0·5
Selenium dioxide test		
Primaquine	Greenish yellow	1·0
Pentaquine	Greenish yellow	1·0
Isopentaquine	Greenish yellow	1·0
Rhodoquine	Greenish yellow	1·0
Amodiaquine	Light brown	0·5
Mepacrine	Bright yellow	0·25
377C54	Green—orange brown	0·5

* This blue colour is not seen if drug is in excess.

Pamaquin will be discussed later. The results obtained are shown in Table I. It should be noted that compounds which differ only in their non-functional groups may give crystals that are similar in appearance.

Colour Tests

Colour tests are made with microdrops on opal glass as described previously[3]. Chloroquine, hydroxychloroquine, nivaquine C, proguanil, Compound 5943 and pyrimethamine give no colours with any of the reagents. The results obtained with the other compounds are given in Table II. With Vitali's test most of these substances give indefinite shades of brown and yellow which are valueless for purposes of identification. A further colour test that may be employed is a modification of the diazo test originally described by Sanchez[5,6]. To a microdrop of a saturated solution of *p*-nitroaniline in 2N hydrochloric acid is added a

microdrop of a 10 per cent solution of sodium nitrite, followed by a similar drop of a 1 per cent solution of the drug. Pamaquin, primaquine, pentaquine, isopentaquine, rhodoquine and 377C54 all give an orange colour. A microdrop of 4N sodium hydroxide solution is then added. The 6-methoxy-8-aminoquinoline derivatives all turn purple, 377C54 turns green, while amodiaquine gives a greenish-brown precipitate. The other substances remain colourless.

Discussion

The identification of pamaquin, which is the 2:2′-dihydroxy-1:1′-dinaphthylmethane-3:3′-dicarboxylic acid salt of 6-methoxy-8-(4-diethylamino-1-methylbutylamino)quinoline, calls for special consideration. The test most frequently described for its identification is that in the B.P. 1953 monograph which is the formation of a green colour with the formaldehyde-sulphuric acid reagent. This green colour is, however, due to the acidic, not the basic, part of the molecule, as the free acid gives the same green colour, while pamaquin base with this reagent gives an orange colour similar to that given by the other 6-methoxy-8-aminoquinoline derivatives. The green colour given by pamaquin with the other sulphuric acid reagents is also due to the acid, as the free base gives the same colours as are given by primaquine (Table II).

Pamaquin is insufficiently soluble in dilute acids for the solution to give crystal tests. If a microdrop of a solution of this compound in acetone containing 5 per cent of water is added to a microdrop of a solution of potassium cadmium iodide, small rosettes of needles are formed; similarly the potassium tri-iodide reagent No. 3 gives transparent tablets. But in each case, similar crystals are formed from these reagents with an ethanolic solution of the free acid. Pamaquin base, dissolved in 1 per cent hydrochloric acid, gives amorphous precipitates or oils only with the usual reagents, although in the case of styphnic acid the oil will sometimes crystallise slowly into aggregates of rods.

All the tests described above were carried out on the microgram scale with pure substances supplied by the manufacturers.

Acknowledgements. I wish to express my thanks to Professor E. C. Amoroso, F.R.S. for his continued encouragement, and to Mr. T. D. Whittet for help in obtaining material. I acknowledge most gratefully gifts of drugs from Messrs. Abbott Laboratories Ltd., Boots Pure Drug Co. Ltd., Burroughs Wellcome & Co., Cyanamid Ltd., Imperial Chemical (Pharmaceuticals) Ltd., May & Baker Ltd., Parke-Davis & Co. Ltd., Societé des Usines Chimiques Rhône-Poulenc, and the Sterling Winthrop Research Institute. I am also much indebted to Mrs. A. Williams for technical assistance.

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