The action of the cinchona and certain other alkaloids in bird malaria. Pt. 2 / by G.A.H. Buttle, T.A. Henry and J.W. Trevan.

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THE ACTION OF THE CINCHONA AND CERTAIN OTHER ALKALOIDS IN BIRD MALARIA. PART II.

BY

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(From the Biochemical Journal, 1934)

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[From THE BIOCHEMICAL JOURNAL, Vol. XXVIII, No. 2, pp. 426–441, 1934]

PRINTED IN GREAT BRITAIN

LXI. THE ACTION OF THE CINCHONA AND CERTAIN OTHER ALKALOIDS IN BIRD MALARIA. II.

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(*Received February 16th, 1934.*)

In the previous paper [Goodson *et al.*, 1930], it was shown that Roehl's method of investigating the relative therapeutic efficiencies of antimalarial drugs, by trial in bird malaria, might prove sufficiently delicate to show quantitative differences in action between the eight principal alkaloids of cinchona bark, *viz.* *l*-quinine, *l*-cinchonidine, *d*-quinidine, *d*-cinchonine (which may conveniently be called the primary cinchona alkaloids) and the reduction products yielded by each of these four bases. In these reduction products the vinyl side-chain of each parent alkaloid is reduced to an ethyl group: they are therefore dihydro-derivatives and are so named throughout this paper. Commercially they are known as hydroquinine, hydrocinchonine, *etc.*

The determination of the relative antimalarial values of the several cinchona alkaloids has now assumed greater practical importance owing to the introduction, by the Health Organisation of the League of Nations, of "totaquina," a product designed as an economical substitute for quinine in the mass treatment of indigent, malarial populations. "Totaquina" is defined [Malaria Commission, League of Nations, 1931], as a mixture of cinchona alkaloids containing a minimum of 15 % of quinine and 70 % of crystallisable cinchona alkaloids, with a maximum of 20 % of amorphous cinchona alkaloids, 5 % of moisture and 5 % of ash.

This innovation, based on the view that there is not a great deal to choose between the crystalline cinchona alkaloids as antimalarial drugs, does not meet with universal acceptance among malariologists, but the opinion of the majority is probably not unfairly represented by the following quotation:

"There is no conclusive evidence to show that any one of the crystallisable alkaloids is so markedly superior to any other as to justify its exclusive use in ordinary cases of malaria in preference to any other, taking into account the shortage of these alkaloids and their cost" [Sinton, 1930].

The misgivings aroused by proposals to substitute for quinine any one of the secondary cinchona alkaloids, or of mixtures such as "totaquina," may be indicated by the following quotation:

"En *premier* lieu il était nécessaire de démontrer que la malaria *peut* être guérie au moyen des alcaloïdes mineurs. Nous n'avons pas besoin d'insister sur ce point qui est, pensons nous, universellement admis.

"En *second* lieu il était nécessaire de donner des préceptes nets au sujet de la façon dont ces alcaloïdes doivent être appliqués pour donner le degré de sécurité auquel on était habitué par un emploi pendant presqu'un siècle de la quinine. Il fallait établir (*a*) à quelles exigences de pureté les nouvelles préparations à

introduire doivent satisfaire et quelle doit être leur composition et (*b*) quelle est la dose à laquelle il faut les administrer pour le succès soit assuré" [Van der Sleen and Drossart Lulofs, 1931].

The definition of "totaquina" allows considerable latitude in the relative proportions of the various cinchona alkaloids present, and in consequence there is much variation in this respect in commercial "totaquina," as the following analyses show:

Table I. *Composition of "totaquina."*

	Type I % *	Type II % †	Type III % *
Quinine	32·5	15·3	17·0
Cinchonidine	34·2	5·7	24·0
Quinidine	—	5·2	10·0
Cinchonine	20·1	55·4	23·0
Total crystallisable alkaloids	86·8	81·6	74·0
Amorphous alkaloids	9·9	13·4	17·0
Moisture	2·6	1·9	5·0
Ash	0·2	2·1	4·0

* Manufacturer's analysis. † Analysis by Mr J. A. Goodson.

It is obvious that if there is a marked difference in the action of, say quinine and cinchonine, the therapeutic values of "totaquina" of types I and II will be different and some evidence for this view has already been brought forward as a result of trials of these two types in bird malaria [Giemsa, 1932] and in human malaria [Malaria Commission, League of Nations, 1933].

It appeared to the authors that definite quantitative evidence on this matter could best be obtained by comparative trials of pure specimens of the individual alkaloids in bird malaria.

The four primary cinchona alkaloids have been purified by processes described later, and a method of controlling these processes, so far as the elimination of the dihydro-bases is concerned has been devised. From the primary bases the pure dihydro-bases have been prepared by catalytic hydrogenation. As there was no certainty that any of these eight alkaloids had been obtained pure previously the principal constants of each have been determined and are recorded and compared with the best of the known previous determinations in Tables II, III and IV.

The biological trials gave results which are summarised in Table A. The figures represent the dose of quinine necessary to produce the same amount of protection as unit dose of the other alkaloids. The figures are only approximate, but the authors think that (*a*) the distinction between quinine and dihydroquinine is certain, (*b*) that dihydroquinidine, cinchonidine and quinidine are definitely less active than quinine and (*c*) that dihydrocinchonine and dihydrocinchonidine form a group of much lower activity than any of the others. The samples of quinidine and cinchonine used were not the purest available; the quinidine contained 1·8 % of dihydroquinidine and cinchonine 1·4 % of dihydrocinchonine. Trials with pure alkaloids were spoilt by the outbreak of a *B. gaertner* epidemic in the stock of canaries, but it will be seen that the removal of these amounts of the dihydro-alkaloids would not affect the order of activity given in the table.

It is not claimed that the order in which the drugs are arranged in this table necessarily represents the order of antimalarial activity in man. But it is logical to assume that, if the alkaloids differ so much in their activity on the parasite experimented with, they will not be of equal therapeutic value in man.

The final decision as to which is the most active must rest on clinical evidence, which is notoriously difficult to assess. It is however, with the present evidence, unjustifiable to assume that all the crystallisable alkaloids are of equal value.

The same order can be deduced from the results of previous workers [Goodson *et al.*, 1930; Giemsa *et al.*, 1926; Giemsa and Oesterlin, 1933] except as regards quinidine. Goodson *et al.* [1930] obtained results, which suggest that quinidine has a higher activity than that now assigned to it. The number of birds on which quinidine is given its place, is small, owing to the fact that some died during the experiment. It is probable that 5 mg. daily is too toxic a dose for quinidine, though the large majority of canaries survive these doses of the other alkaloids. In this respect our canaries are less sensitive than those used by Giemsa. The apparent difference cannot be explained by any difference in purity of the alkaloids used. The point is however being further investigated together with the proper place of cinchonine.

The effect of hydrogenation is astonishingly different for different alkaloids. For quinine and possibly quinidine, hydrogenation increases the activity; for cinchonine the effect is probably to diminish the activity. No possible explanation can be offered and yet another instance of lack of correspondence between biological activity and chemical structure must be left on record.

Table A.

Dihydroquinine	1–2	Quinidine	About 0·5*
Quinine	1	Cinchonine	Less than 0·2
Dihydroquinidine	0·5–1	Dihydrocinchonine	,,
Cinchonidine	About 0·5	Dihydrocinchonidine	,,

* Further experiments with the pure alkaloids suggest that this is an overestimate rather than an underestimate of the relative activity of quinidine.

Modified cinchona alkaloids. In the previous paper it was shown that although quitenine is itself inactive it recovers antimalarial activity on esterification; this activity is slight for the methyl and ethyl esters and only becomes well-marked in the butyl and amyl esters. Observations with two of these esters have been repeated with the results recorded in Table B, which are given in a form readily comparable with those of Goodson *et al.*

These results are interesting in two ways; (1) they are both positive, whereas in the former more extensive series negative results were occasionally obtained with each substance; (2) the improvement in activity from ethyl to amyl is not so well-marked as in the previous experiments. These alkylquitenines are somewhat readily hydrolysed, and it is suggested that the apparent variation in antimalarial activity is caused by varying capacity for hydrolysing the drugs shown by different canaries. Less variable results would probably be obtained by intravenous injection, a method not well suited for use with canaries.

Certain of the cinchona alkaloid "chlorides" being available for other purposes, the opportunity was taken to try them in bird malaria. Quinine chloride, dihydroquinine chloride and cinchonidine chloride all proved inactive in six daily doses of 5 mg. as was to be expected from the recorded inactivity of quinine chloride [Giemsa *et al.*, 1926]. It is however of interest to note that whilst in the authors' experiments no difficulty was experienced in giving doses of 5 mg. of these chlorides, Giemsa was only able to give 1·5 mg., a further example of variability in canaries. The disappearance of activity as a result of replacing the —CHOH— group by —CHCl— is a striking example of the important part played by this group in this set of alkaloids, activity disappearing

it to be a trihydrate. It is possible that in this case also Emde's lower figures are in part due to incomplete drying. Assuming that Emde really used a monohydrate instead of the anhydrous salt for his determination, his figure would be raised to $+217{\cdot}9°$, which on the basis of the present authors' results would correspond to quinidine containing 6 % of dihydroquinidine. This calculation assumes that the change in rotation due to dilution is the same and remains constant for quinidine and dihydroquinidine dihydrobromides. This is approximately true so far as the dilutions from $M/10$ to $M/40$ given in Table III are concerned, the two figures being 3·7 and 3·9°. The same assumption is made in the calculation for cinchonine given above and in this case the difference due to dilution of the dihydrobromides from $M/10$ to $M/40$ is 2·6° for both cinchonine and dihydrocinchonine.

Two further comparisons are possible with the results recorded in Table III. The Dutch Pharmacopoeia permits a range of $-17{\cdot}7°$ to $-18°$ for the observed rotation of $M/10$ solutions of quinine and its salts taken under specified conditions as to solvent and temperature. Under these conditions $M/10$ solutions of the quinine salts described in Table III gave the following results: monohydrochloride $-18{\cdot}06°$; neutral sulphate $-18{\cdot}095°$ and acid sulphate $-18{\cdot}085°$.

Realising the lack of any method of eliminating the large quantity of dihydroquinidine present in ordinary quinidine, a successful attempt was made recently [Butler and Cretcher, 1933] to prepare pure quinidine by re-arrangement of quinine [Rabe *et al.*, 1932]. The yield was apparently small. The product had $[\alpha]_D + 323{\cdot}8°$ ($c = 2$ in 1·8 % HCl). The pure quinidine used in the present investigation had $[\alpha]_D + 323{\cdot}1°$ observed under the same conditions.

The specific rotations, calculated as base in Tables III and IV, bring out the interesting point that all eight alkaloids in the form of dihydrobromides show a lower specific rotation in $M/10$ than in $M/40$ solution in water. It has not been possible to extend this comparison to other acid salts of all eight bases owing to the difficulty of getting satisfactory preparations of the complete series; the acid sulphates, for example, are too soluble to yield good preparations in some instances unless large quantities of the pure bases are available, and in other cases they cannot be dried completely without decomposition. It is however of interest to note that whilst quinine acid sulphate, like the dihydrobromide, shows a higher rotation at $M/40$ than at $M/10$, cinchonidine acid sulphate shows the same specific rotation at both concentrations. Determinations of p_H of these solutions, for which the authors are indebted to Mr C. G. Pope, throw no light on these differences. Anomalies of this kind in connection with the optical rotation of cinchona alkaloids have long been recorded [Oudemans, 1876] and have frequently been investigated, but so far no satisfactory and final explanation of their occurrence has been given.

Preparation of the drugs.

Separation of the primary alkaloids. It is easy to detect mere traces of quinine in cinchonidine, or of quinidine in cinchonine by the fluorescence of solutions in dilute sulphuric acid; and in purifying cinchonidine and cinchonine, each base was crystallised until a 1 % solution in $N/10$ sulphuric acid examined in bright, diffused daylight in a 2 dm. polarimeter-tube, showed no greater intensity of fluorescence than a 1 in 1,000,000 solution of quinine or quinidine respectively, examined in like manner. Under these conditions the fluorescence of quinine is just visible at 1 in 2,000,000.

The inverse operation, *viz.* the detection of cinchonidine in quinine has been much investigated, and in practice there are two methods in use, *viz.* the

ammonia test, which probably does not detect less than 2 % of cinchonidine, and the polarimetric test, which is of no value for the present purpose, since quinine is contaminated not only with cinchonidine, but also with the optically active bases, dihydroquinine and dihydrocinchonidine. Cinchonidine is less soluble in benzene than quinine, whilst its acid salts are more soluble in water than those of quinine: there is no reason to doubt that by rejection of appropriate fractions in fractional crystallisation from such solvents the small amount of cinchonidine in commercial quinine is eliminated.

The detection of cinchonine in quinidine is also difficult; there is a considerable difference between the optical rotations of the two alkaloids, but there has been until now no method of distinguishing between cinchonine and dihydroquinidine as the cause of low rotation in commercial quinidine. Cinchonine is less soluble in alcohol than quinidine and, it is shown later (p. 434) that it can be eliminated from crude quinidine by recrystallisation from boiling alcohol, the first fractions being rejected and any traces that escape in this process being left in the mother-liquors in the final fractionation, cinchonine dihydrochloride being much more soluble in water than quinidine dihydrochloride.

Elimination of dihydro-bases. The second group of impurities consists of the naturally-occurring dihydro-alkaloids, each primary alkaloid being accompanied by its own reduction product. The vinyl group in each of the parent bases is readily oxidised to a carboxyl group by permanganate, while the reduced bases are relatively stable to this reagent, and this method has hitherto been used both for the isolation of these dihydro-bases and for their estimation. Since the dihydro-bases are only relatively stable to permanganate, such a method can merely give approximate results and for the detection and estimation of the dihydro-bases, the authors have used a new method, *viz.* catalytic hydrogenation in an apparatus designed for quantitative work [Paget and Solomon, 1932]. The errors in such a determination are estimated not to exceed ± 1 %, and a primary cinchona alkaloid has been regarded as free from its dihydro-base when it absorbed 99·5 to 100·5 % of the quantity of hydrogen required to convert the vinyl group into an ethyl group. Colloidal palladium on barium sulphate or Adams's platinic oxide has been used as a catalyst, the former being preferred as it is less liable to form colloidal solutions from which the reduction product is difficult to recover. For the estimations, 0·4 to 0·5 g. of the base or one of its salts dissolved in 10 ml. of $N/10$ sulphuric acid, containing 0·05 g. of palladised barium sulphate, is exposed to hydrogen with constant, vigorous mechanical agitation. Absorption is rapid during the first half hour and finishes in about 2 hours after which it is taken as complete if, during a further 2 hours, the change in volume of gas is not greater than 0·1 ml. If, after this point is reached, action is continued, there is a slow absorption of 0·2 to 0·5 ml. *per diem* due no doubt to the very slow formation of more highly hydrogenated bases, but there is no practical difficulty in determining the point at which rapid formation of the dihydro-base is complete and the second very slow absorption begins. The results for hydrogen absorption given below are corrected for hydrogen absorbed by the catalyst, which must be determined by blank experiments on each new batch of catalyst made.

The bulk samples of dihydro-bases used were made by catalytic hydrogenation in like manner of the purified primary alkaloids and were crystallised as bases or salts to constant optical rotation. As so much attention has been given to the permanganate test for the dihydro-bases, it was thought desirable to use this in a more or less quantitative form. For that purpose, 5 ml. of a 2 % solution of the dihydro-base in water containing 0·3 ml. of N sulphuric acid was

treated with 0·5 ml. of $N/50$ potassium permanganate. The colour disappears in not less than 10 minutes with all the dihydro-bases except dihydrocinchonine, which discharges it in 30 seconds. The specific rotations of the pure bases and their salts are recorded in Tables III and IV. It is well known that in this series the specific rotation varies with the nature of the solvent and the concentration of the solution. As the authors had to make extensive use of polarimetric observations to follow the course of crystallisation in the final purification of the alkaloids, they found it convenient to use $M/40$ solutions of any salt selected for this purpose, since the series of readings were then directly comparable without calculation. The figures recorded for $[\alpha]_D$ in Tables III and IV are in all cases for $M/40$ or $M/10$ solutions of the dry salt or dry base in water or $0{\cdot}1\,N$ acid, usually $0{\cdot}1\,N$ sulphuric acid.

Additive compounds of cinchona alkaloids with metallic salts. The elimination of the dihydro-bases from quinine and cinchonidine is easy, and from cinchonine and quinidine slow and laborious. In the hope of improving it numerous salts and derivatives were tried. Quinidine sulphate argentonitrate, $B_2.H_2SO_4,AgNO_3$, proved useless, because it could not be recrystallised without decomposition. The zincichlorides of the type $B.(HCl)_2,ZnCl_2$, can be recrystallised, are easily purified, and the alkaloids are readily recoverable from them, but they are formed with equal facility and are of similar solubility with both the parent and the reduced bases and are therefore of no value as a means of separation. When this work was almost completed, a paper was published [Cohen, 1933] describing the specific precipitation by cupric chloride of cinchona alkaloids containing the vinyl group, and making the statement that "no complex salts are precipitated when the corresponding dihydro-bases are treated with cupric chloride under exactly the same conditions, even after several weeks at $-4°$." As the cuprichlorides are represented by the formula $B.(HCl)_2,CuCl_2$, which is strictly analogous with that found for the zincichlorides, it seemed curious that this specificity should be exhibited by cupric chloride and not by zinc chloride. The behaviour of cupric chloride with commercial cinchonine and commercial quinidine was investigated carefully and it was found that the cuprichlorides did, in fact, effect a partial separation of the dihydro-bases. The resulting fractionation showed clearly that the dihydro-bases must form insoluble cuprichlorides and, on trying the experiment, no difficulty was experienced in preparing cuprichlorides of all four dihydro-bases, except in the case of dihydrocinchonidine, where the additive compound is readily soluble in conc. HCl at atmospheric temperatures and when prepared in small quantities is best manipulated in a cold room.

In the following account, the melting-points quoted are corrected; the combustion results are for material dried at 120° *in vacuo* unless otherwise stated and were obtained by micro-analysis.

Quinine. The material used consisted of base prepared from commercial quinine sulphate. It had $[\alpha]_D^{15°}$ −280·7° ($c = M/40$ in $N/10$ H_2SO_4) and hydrogen absorption 94·7 %. It is known that cinchonidine can be eliminated from quinine by repeated crystallisation of the acid sulphate [Tutin, 1909]. The authors have found that quinine purified in this way is also free from dihydroquinine, as indicated by its absorptive capacity for hydrogen. Purification can also be effected by crystallisation of the dihydrobromide [Emde, 1932]. The quinine acid sulphate was prepared by repeated crystallisation (five or six times is usually necessary) from hot water (1 in 7 to 8) until the rotation became constant. It was then recrystallised in three fractions, for which $[\alpha]_D^{15°}$ showed the narrow range −216·1° to −217·0° ($c = M/40$ in water); the hydrogen absorption, for which the middle fraction was used, was 100·1 %. The dihydrobromide was prepared by recrystallisation from hot (43°) water (1 in 3 to 4) with the same precautions as the acid sulphate: the range of specific rotation for the three final fractions was −189° to −189·6°, and the hydrogen

absorptions of the middle fractions were 100·8, 101·5 and 101·6 % (hydrogen absorptions by quinine dihydrobromide were generally high). The neutral hydrochloride and sulphate, made by neutralising pure quinine base with the appropriate acid, were recrystallised from hot water containing a trace of the appropriate acid to avoid any risk of inclusion of free base, which accounts for the high rotation shown by some specimens of neutral quinine salts.

Constants for the pure base and salts are given in Table III.

Dihydroquinine. The base, prepared by hydrogenation of pure quinine was crystallised from hot benzene, or as the neutral sulphate or the dihydrobromide from hot water. Constants for these three preparations are given in Table III.

Dihydroquinine cuprichloride. Dihydroquinine (5 g.) was dissolved in conc. HCl (8 ml.) and cupric chloride (5 g.) in conc. HCl (8 ml.) added: the mixture was kept at 7° for 24 hours, but nothing separated. The side of the beaker just under the surface of the liquid was then rubbed vigorously with a glass rod for 5 minutes. A yellow streak appeared and in 20 minutes the liquid had set to a semi-solid, crystalline magma. The crystals were filtered out on Whatman filter-paper No. 54, washed sparingly with conc. HCl and dried in a vacuous desiccator over soda lime; wt. 7·0 g. The product was recrystallised from 14 ml. hot conc. HCl; wt. 3·9 g. It crystallised on the sides of the container in silky, cushion-like masses of golden-brown needles, M.P. 204° (decomp.). (Found: C, 44·61; H, 5·22; N, 5·26; Cl, 26·60; Cu, 11·68 %. $C_{20}H_{26}O_2N_2.2HCl, CuCl_2$ requires: C, 44·98; H, 5·28; N, 5·25; Cl, 26·57; Cu, 11·9 %.)

Dihydroquinine zincichloride. $C_{20}H_{26}O_2N_2.2HCl, ZnCl_2$. The base (5 g.) dissolved in conc. HCl (8 ml.) was mixed with zinc chloride (12·5 ml. of 40 % solution in conc. HCl). Crystallisation began almost immediately, and the product was isolated in the same way as the cuprichloride. Yield 6·9 g. It crystallises from hot water (1 in 5) but shows a tendency to separate as an oil, which can be corrected by the addition of a little alcohol; also from hot 50 % alcohol or from hot conc. HCl in masses of colourless, flattened prisms, M.P. 257°; froths 262°. (Found: C, 45·12; H, 5·43; N, 5·28; Cl, 26·35; Zn, 12·20 %. Calc.: C, 44·82; H, 5·27; N, 5·23; Cl, 26·48; Zn, 12·22 %.)

Quinidine. The quinidine base used had $[\alpha]_D^{15°}$ +324·3° ($c = M/40$ in $N/10$ H_2SO_4) and hydrogen absorption 77·9 % indicating the presence of 22·1 % of reduced bases, chiefly dihydroquinidine. After recrystallisation from boiling alcohol, the first fraction (about 10 %) being rejected, it had $[\alpha]_D^{15°}$ +325·5° and hydrogen absorption 76·4 %; these slight changes are accounted for by removal of cinchonine. The hydrogen absorption could not be raised by crystallisation of either the base from alcohol, or the dihydrobromide from water, but the dihydrochloride (1 in 1 in hot water) proved suitable for this purpose, the less soluble fractions being richest in quinidine, as indicated by increasing rotation and hydrogen absorption. Three such crystallisations give a yield of about 33 % by weight of the starting material, and raise the hydrogen absorption to 90 %. This procedure answers well until a product having hydrogen absorption 98·0 to 98·5 % is reached. At this stage crystallisation from weaker solutions (1 in 3) ensures more rapid purification. At the first point at which approximate purity is reached, the yields are about 2·75 % of material with hydrogen absorption 100·6 % and about 7·5 % showing hydrogen absorption from 98 to 99 %.

In the course of attempts to find a derivative of quinidine, which would expedite the tedious fractional crystallisation necessary especially in the last stages of purification by means of the dihydrochloride, addition compounds of the alkaloid with silver nitrate and with zinc chloride were examined.

Quinidine argentonitrates. Two silver nitrate addition products of quinidine are known, one represented by the formula $(C_{20}H_{24}O_2N_2)_2, AgNO_3$ [Stenhouse, 1864], which cannot be recrystallised without decomposition, and a second, obtained as a crystalline precipitate when silver nitrate is added to dilute solutions of quinidine sulphate. Though used as a test for quinidine, the origin of this latter reaction has not been traced, and the nature of the precipitate appears to be unknown. It was prepared by adding silver nitrate solution (5 ml. of 50 %) to a boiling, aqueous, faintly acid solution of quinidine sulphate (5 g.). As the solution cooled, colourless needles of quinidine sulphate argentonitrate separated. Yield 6·2 g. The substance is sparingly soluble in boiling water, but can be crystallised with some decomposition from boiling 50 % alcohol in colourless needles containing $2H_2O$. Loss at 120° *in vacuo* 3·9: calc. 3·7 %.) M.P. 213° (decomp.). (Found: C, 51·3; H, 5·98; N, 7·72; MeO, 6·32; Ag, 11·74; S, 3·51 %. $(C_{20}H_{24}O_2N_2)_2.H_2SO_4, AgNO_3$ requires: C, 52·3; H, 5·4; N, 7·6; MeO, 6·7; Ag, 11·7; S, 3·49 %.) The carbon determinations were per-

sistently low in recrystallised specimens, owing to the slight decomposition observed on recrystallisation, but there can be little doubt that the substance is correctly represented by the formula given. The "quinidine" recovered from the silver nitrate compound absorbed 81·1 % of the calculated amount of hydrogen, which is only a slight improvement on the original figure of 78 %.

Quinidine zincichloride. This was made by the usual method (p. 434) using quinidine 1 in 5 and zinc chloride 1 in 3 of conc. HCl. It crystallises from hot water or 50 % alcohol but best from hot conc. HCl (1 in 3) when it forms much-twinned colourless prisms, M.P. 242° (decomp.). In the following analyses the two figures for each constituent are for material crystallised from conc. HCl and water respectively and dried at 120° *in vacuo*. (Found: C, 44·98, 45·0; H, 5·13, 5·10; N, 5·32, 5·49; MeO, 6·02, 6·23; Cl, 25·9, 25·48; Zn, 12·5, 12·4 %. $C_{20}H_{24}O_2N_2.2HCl, ZnCl_2$ requires: C, 44·9; H, 4·87; N, 5·25; MeO, 5·8; Cl, 26·5; Zn, 12·2 %.) This is the formula adopted by Stenhouse for what was undoubtedly the same substance and the foregoing confirms and extends his description.

Quinidine cuprichloride. In view of the claim [Cohen, 1933] that the vinyl-containing cinchona alkaloids form solid cuprichlorides whilst the dihydro-bases do not, this method was tried on quinidine. The "quinidine" was not recovered by Cohen's method of removing copper as the sulphide but by the simpler process of dissolving and thereby decomposing the cuprichloride in cold water and pouring the filtered solution into excess of ammonia solution, when the base is precipitated and is readily filtered and washed free from the ammoniacal copper solution. The following is a typical result of a close imitation on a larger scale of one of Cohen's experiments with the modification just referred to, starting with a "quinidine" fraction having a hydrogen absorption of 51·6 %: Cohen used a mixture, quinidine-dihydroquinidine (1:1).

The "quinidine" (11 g.) was dissolved in conc. HCl (40 ml.) and cupric chloride (11 g.) in 36 ml. hot conc. HCl added. On cooling, the orange-coloured crystalline cuprichloride separated and was collected, dried overnight on a porous tile and finally in a vacuous desiccator over soda lime for 48 hours. Yield 11 g. A filtered aqueous solution of the cuprichloride (3 g. in water 30 ml.) was run into solution of ammonia (10 ml. conc. NH_4OH with water 30 ml.) and the precipitated base collected, thoroughly washed and dried in a vacuous desiccator. Yield 1·97 g. It absorbed 67·5 % of the calculated amount of hydrogen, *i.e.* an improvement of 15·9 %. In other trials with varying amounts of hydrochloric acid as solvent such results as the following were obtained; the figures are the hydrogen absorption values % of (*a*) the "quinidine" used, and (*b*) the recovered "quinidine."

(*a*)	77·9	77·9	86·6	78·6	80·4	88·1	95·8
(*b*)	91·0	88·0	88·7	80·4	88·1	95·8	98·0

The method has obvious possibilities and by combining it with the present authors' method—recrystallisation of the dihydrochloride—it is possible to obtain the latter in a pure state. The remainder of the "quinidine" cuprichloride (8 g.) prepared from the 51·6 % "quinidine" referred to above, was recrystallised from hot conc. HCl (1 g. in 3 ml.) and the whole of the crop (4·6 g.) ground in a mortar with water (9 ml.). Under these conditions the cuprichloride decomposed, and part of the quinidine dihydrochloride formed crystallised out. This was collected and without washing or drying was converted into base as already described. Yield 2·15 g. This absorbed 85·2 % of the calculated amount of hydrogen, an improvement of nearly 34 %. Still better quinidine but in poorer yield can be obtained by the use of more water (3 or 4 ml. instead of 2 ml. for 1 g. of cuprichloride). The base recovered from the dihydrochloride mother-liquors is of poorer quality.

Starting with "quinidine" showing hydrogen absorption 77·9 % and using conc. HCl (5 ml.) for each g. of quinidine or cupric chloride employed, it is possible by one treatment by this modified process, to obtain 1/3 of the original amount as a fraction showing hydrogen absorption about 95·4 % and by repeating the operation to obtain about 1/9 of the original amount having hydrogen absorption 99 to 100 %.

The quinidine used in determining the constants of the base, acid sulphate and dihydrobromide recorded in Table III was made by both processes. No figures are given for quinidine dihydrochloride as this substance is not completely dehydrated at 120° *in vacuo*, and between 120° and 130° the loss is irregular owing to incipient decomposition.

Dihydroquinidine. This base, prepared by catalytic hydrogenation of pure quinidine was crystallised from boiling alcohol, or as the dihydrobromide from water.

Dihydroquinidine cuprichloride was prepared by mixing the base (2 g.) with cupric chloride (2 g.) each having been dissolved separately in conc. HCl (5 ml.). Nothing separated on standing for 24 hours except a few crystals of cupric chloride, but on rubbing the side of the beaker, yellow granules appeared and the whole liquid quickly became semi-solid. Wt. 1·71 g. It was recrystallised from hot conc. HCl (1 in 1·5 ml.) from which it separated in clusters of thin, dark orange, rectangular prisms, M.P. 222° (decomp.). Yield 0·9 g. (Found: C, 44·94; H, 5·37; N, 5·17; Cl, 26·55; Cu, 11·92 %. $C_{20}H_{26}O_2N_2.2HCl, CuCl_2$ requires: C, 44·98; H, 5·28; N, 5·25; Cl, 26·57; Cu, 11·91 %.)

Dihydroquinidine zincichloride was prepared by mixing the base (2 g.) in conc. HCl (5 ml.) with zinc chloride (5 ml. of 40 % solution in conc. HCl). Nothing crystallised out until the inside of the beaker was rubbed vigorously with a glass rod. Wt. 1·7 g. It can be recrystallised from water, 50 % alcohol or hot conc. HCl (1 g. in 2 ml.) and separates from the latter in colourless, spheroidal masses of minute silky needles, M.P. 249–252° (decomp.). (Found: C, 44·6; H, 5·31; N, 5·19; Cl, 26·45; Zn, 12·00 %. $C_{20}H_{26}O_2N_2.2HCl, ZnCl_2$ requires: C, 44·82; H, 5·27; N, 5·23; Cl, 26·48; Zn, 12·22 %.)

Constants for dihydroquinidine and its salts are given in Table III.

Table III. *Constants of pure cinchona alkaloids.*

Methoxy-bases: quinine, dihydroquinine; quinidine, dihydroquinidine.

Substance	α_D	c*	Solvent	$[\alpha]_D^{15°}$ calc. for Salt	$[\alpha]_D^{15°}$ calc. for Base	Additional constants
Quinine (Q):						
Base, M.P. 173·5°	− 4·611°	*M*/40	*N*/10 H_2SO_4	—	−284·5°	H.A.† =99·93
Acid sulphate, $Q.H_2SO_4, 7H_2O$	−17·875	*M*/10	Water	−211·7°	−275·9	H.A. =100·1: p_H =2·86
,, ,,	− 4·555	*M*/40	,,	−216·1	−281·4	p_H =3·08
,, ,,	− 4·607	*M*/40	*N*/10 H_2SO_4	−218·3	−284·3	—
Neutral sulphate, $Q_2.H_2SO_4, 7H_2O$	− 4·608	*M*/40	*N*/10 H_2SO_4	−247·1	−284·4	—
Dihydrobromide, $Q.2HBr, 3H_2O$	−17·890	*M*/10	Water	−184·1	−276·2	H.A. =100·8: p_H =2·61
	− 4·608	*M*/40	,,	−189·6	−284·4	p_H =2·94
Neutral hydrochloride, $Q.HCl, 2H_2O$	− 4·597	*M*/40	*N*/10 H_2SO_4	−255·1	−283·8	—
	− 4·582	*M*/40	*N*/10 HCl	−254·3	−282·9	—
Dihydroquinine (HQ):						
Base, M.P. 173·5°	− 3·842	*M*/40	*N*/10 H_2SO_4	—	−235·7	—
Dihydrobromide, $HQ.2HBr, 3H_2O$	−14·880	*M*/10	Water	−152·5	−228·3	—
	− 3·845	*M*/40	,,	−157·5	−235·8	—
Neutral sulphate, $(HQ)_2.H_2SO_4, 6H_2O$	− 3·838	*M*/40	*N*/10 H_2SO_4	−204·6	−235·4	—
Quinidine (Qd):						
Base, M.P. 173·5°	+ 5·415	*M*/40	*N*/10 H_2SO_4	—	+334·2	H.A. =99·8 %
Acid sulphate, $Qd.H_2SO_4, 4H_2O$	+20·915	*M*/10	Water	+247·8	+322·7	—
	+ 5·409	*M*/40	*N*/10 H_2SO_4	+256·4	+333·9	—
Dihydrobromide, $Qd.2HBr, 3H_2O$	+21·315	*M*/10	Water	+219·3	+328·9	—
	+ 5·420	*M*/40	,,	+223·0	+334·5	—
Dihydroquinidine (HQd):						
Base, M.P. 169·5°	+ 4·875	*M*/40	*N*/10 H_2SO_4	—	+299	—
Dihydrobromide, $HQd.2HBr, 3H_2O$	+19·178	*M*/10	Water	+196·5	+294·2	—
	+ 4·890	*M*/40	,,	+200·4	+300	—

* c is expressed as dry substance.
† H.A. =hydrogen absorption: % of calculated value (p. 432).

Cinchonidine. Commercial cinchonidine is stated to contain quinine 10 % and dihydrocinchonidine 8 % [Dawson and Garbade, 1930]. The base, used as a starting material, showed strong fluorescence on solution in dilute sulphuric acid, had $[\alpha]_D^{15°}$ −183·2° ($c = M/40$ in $N/10$ H_2SO_4) hydrogen absorption 97·9 % and contained methoxyl 0·66 %. These results indicate the presence of quinine (about 6·9 %) and dihydrocinchonidine (about 2 %).

It was found that crystallisation three times from boiling benzene (1 in 30), the first fractions only being collected, yielded about 78 % of a product, which passed the fluorescence test (p. 431) and in which no methoxyl could be detected. This, on fractional crystallisation from alcohol

(1 in 6), yielded first and third fractions having hydrogen absorptions of 98 to 99 % and a middle fraction with hydrogen absorption 101·2 %, the yield of the latter being about 25 % of the original material. In some cases it was necessary to crystallise the quinine-free product twice from alcohol, in order to reach this degree of purity. The constants for the base, acid sulphate and dihydrobromide are recorded in Table IV.

Dihydrocinchonidine. The base prepared by hydrogenation of pure cinchonidine was recrystallised from boiling benzene and finally from alcohol, or as the dihydrobromide from water. The constants are given in Table IV. The *cuprichloride* was prepared by mixing the base (3 g.) in conc. HCl (4 ml.) with cupric chloride (3 g.) in conc. HCl (5 ml.). Even after vigorous rubbing of the cooled liquid with a glass rod no crystals appeared, but after 24 hours at 7°, a crop of minute pale, canary-yellow needles separated, wt. 2·6 g. It was recrystallised in the same way from warm conc. HCl (3 ml.), wt. 1·5 g., and was dried on a porous tile. It sinters at 120–140°, melts at 166° and froths at 183°. (Found on substance dried at 90° *in vacuo*: C, 44·52; H, 5·29; N, 5·5; Cl, 28·15; Cu, 12·83 %. $C_{19}H_{24}ON_2.2HCl$, $CuCl_2$ requires: C, 45·27; H, 5·20; N, 5·56; Cl, 28·16; Cu, 12·62 %.)

Cinchonine. The commercial cinchonine used was markedly fluorescent in dilute sulphuric acid, contained methoxyl 0·25 %, corresponding to quinidine 2·6 % and had $[\alpha]_D^{17°}$ +264·5° ($c = M/40$ in $N/10$ H_2SO_4) and hydrogen absorption 86·2 % indicating the presence of 13·8 % of dihydrocinchonine. After three crystallisations from boiling alcohol (1 in 30) it passed the fluorescence test (p. 431) and the figure for hydrogen absorption fell to 84·9 owing to removal of quinidine. This partially purified base was converted into dihydrobromide and recrystallised from water (1 in 1). The first crop, which is rich in dihydrocinchonine is rejected and successive crops are obtained by slow concentration of the mother-liquor in a vacuous desiccator over sulphuric acid. When a conveniently large crop has accumulated in this way, it is scraped down into the mother-liquor, dissolved by warming and left to crystallise out again. The fourth crop so obtained had hydrogen absorption 93–94 %. When a hydrogen absorption about 95 % is reached, crystallisation is continued from stronger solutions (1:0·75). When material having hydrogen absorption over 99 % is first reached, the yields of the three best fractions are approximately as follows:

Hydrogen absorption, %	96 to 97	99·3	99·7
Yield, % of starting material	22·7	4·1	2·4

Purification of cinchonine by crystallisation of the dihydrobromide is slow and tedious and, in the hope of expediting the work, other derivatives were tried including the silver nitrate and the zinc chloride compounds, but neither of these was found effective as a means of eliminating dihydrocinchonine.

Cinchonine sulphate argentonitrate was prepared by adding silver nitrate solution (1 ml. of 50 %) to cinchonine sulphate (1 g. in 26 ml. water). On standing overnight a crop of colourless needles separated; wt. 0·984 g. This, on boiling with water (15 ml.), showed slight decomposition, but on filtration through charcoal gave a clear, faintly brown solution, which on cooling deposited nearly colourless needles (0·4 g.) containing $2H_2O$. (Loss at 130° *in vacuo* 4·26: calc. 4·27 %.) M.P. 199° (decomp.). (Found: C, 51·38; H, 6·01; N, 7·82; Ag, 12·88; S, 3·87 %. $(C_{19}H_{22}ON_2)_2.H_2SO_4$, $AgNO_3$ requires: C, 53·2; H, 5·41; N, 8·18; Ag, 12·5; S, 3·74 %.) As in the case of quinidine sulphate argentonitrate (p. 434), the carbon determinations were persistently low in the recrystallised material, owing no doubt to the slight decomposition already referred to.

Cinchonine zincichlorides. When a zinc chloride solution (4 ml. of 50 % in water) is added to cinchonine sulphate (1 g. in 26 ml. water) an oil is precipitated, which dissolves on addition of alcohol (10 ml.). On standing, a crop (0·9 g.) of colourless needles separates, which from solution in boiling water (20 ml.) deposits on cooling a crop of cinchonine hydrochloride. The mother-liquor on concentration in a vacuous desiccator deposits a *cinchonine hydrochloride zincichloride* represented by the formula $C_{19}H_{22}ON_2.HCl$, $ZnCl_2$. It can be recrystallised from boiling water or 50 % alcohol. M.P. 282° (decomp.). (Found: C, 48·69; H, 5·13; N, 6·39; Cl, 22·48; Zn, 14·4 %. Calc.: C, 48·8; H, 4·98; N, 6·0; Cl, 22·7; Zn, 14·0 %.) On solution in conc. HCl this substance is converted into a *zincichloride* of the usual type, $C_{19}H_{22}ON_2.2HCl$, $ZnCl_2$, which can also be formed in the usual manner by adding zinc chloride (2 g. in 3 ml. conc. HCl) to cinchonine (2 g.) in conc. HCl (10 ml.). This crystallises in anhydrous colourless, hair-like needles, from water, dilute alcohol

or conc. HCl (1 g. in 8 ml.). M.P. 263° (decomp.). (Found: C, 45·5; H, 5·08; N, 5·76; Cl, 28·04; Zn, 12·7 %. Calc.: C, 45·2; H, 4·80; N, 5·56; Cl, 28·1; Zn, 12·9 %.)

Two cinchonine zincichlorides have been described already [Gräzinghoff, 1865] to which the following formulae were assigned $(C_{19}H_{22}ON_2.2HCl)_2, ZnCl_2, 2H_2O$ and $(C_{19}H_{22}ON_2.3HCl)_2, ZnCl_2, H_2O$. It is suggested that these products were probably mixtures in which each of the two zincichlorides referred to above was still contaminated with cinchonine hydrochloride, which in the present instance separated as a first fraction in recrystallising the crude product.

The base recovered from the normal zincichloride made from crude cinchonine showed hydrogen absorption 84·0 % as against 86·2 % for the starting material, so that this derivative is of no value for purification purposes.

Cinchonine cuprichloride. The possibility of using the cuprichloride as a means of purifying cinchonine was investigated as described under quinidine (p. 435). After numerous trials, the best results were obtained by using equal quantities of cinchonine and cupric chloride dissolved separately to produce a final solution of solids (1 g.) in conc. HCl (8 ml.), recrystallising the resulting cuprichloride from hot conc. HCl (1 g. in 3 ml.), and recovering the base in the usual way from the recrystallised cuprichloride. Cinchonine dihydrochloride is very soluble in water so that no further fractionation can be effected by solution of the cuprichloride in a small quantity of water as in the case of quinidine (p. 435). The following are typical results:

	Hydrogen absorption, %			
Initial cinchonine	71·5	85·2	96·4	96 to 97
Recovered cinchonine	89·6	96·4	99·0	100·1
Yield, %	78·0	88·0	61·0	59·4

That is, starting with quinidine-free "cinchonine" showing hydrogen absorption of about 85 %, two passages through the cuprichloride are necessary to raise the hydrogen absorption to 99–100 %.

The cinchonine used for the final determination of the constants recorded in Table IV was prepared partly by fractionation of the dihydrobromide and partly by the cuprichloride method, and no difference could be detected between the two products.

Table IV. *Constants of pure cinchona alkaloids.*

Methoxyl-free bases: cinchonidine, dihydrocinchonidine; cinchonine, dihydrocinchonine.

Substance	α_D	c*	Solvent	$[\alpha]_D^{15°}$ calc. for Salt	$[\alpha]_D^{15°}$ calc. for Base	Additional constants
Cinchonidine (Cd):						
Base, M.P. 204·5°	− 2·617°	*M*/40	*N*/10 H_2SO_4	—	−178°	H.A.† = 101·2
Acid sulphate, $Cd.H_2SO_4, 5H_2O$	−10·498	*M*/10	Water	−133·9°	−178·5	H.A. = 100·2: p_H = 2·84
,, ,,	− 2·613	*M*/40	,,	−133·6	−178·1	p_H = 3·05
,, ,,	− 2·615	*M*/40	*N*/10 H_2SO_4	−133·4	−177·8	—
Dihydrobromide, $Cd.2HBr, 2H_2O$	−10·112	*M*/10	Water	−110·9	−172·0	p_H = 2·52
	− 2·607	*M*/40	,,	−114·3	−177·3	p_H = 2·89
	− 2·604	*M*/40	*N*/10 H_2SO_4	−114·2	−177·2	—
Dihydrocinchonidine (HCd):						
Base, M.P. 232°	− 2·140	*M*/40	*N*/10 H_2SO_4	—	−144·6	—
Acid sulphate, $HCd.H_2SO_4, 5H_2O$	− 8·417	*M*/10	Water	−106·8	−142·2	—
,, ,,	− 2·132	*M*/40	*N*/10 H_2SO_4	−108·3	−144·1	—
Dihydrobromide, $HCd.2HBr, 2H_2O$	− 8·149	*M*/10	Water	− 89·0	−137·7	—
,, ,,	− 2·128	*M*/40	,,	− 92·9	−143·7	—
Cinchonine (Cn):						
Base, M.P. 254°	+ 3·876	*M*/40	*N*/10 H_2SO_4	—	+263·7	H.A. = 100·1 %
Dihydrobromide, Cn.2HBr	+15·238	*M*/10	Water	+167	+259·2	H.A. = 99·74 %
	+ 3·867	*M*/40	,,	+169·6	+263·1	—
Dihydrocinchonine (HCn):						
Base, M.P. 267°	+ 3·342	*M*/40	*N*/10 H_2SO_4	—	+225·8	—
Dihydrobromide, HCn.2HBr	+13·154	*M*/10	Water	+143·6	+222·2	—
,, ,,	+ 3·347	*M*/40	,,	+146·2	+226·1	—

* c is expressed on dry substance.
† H.A. = hydrogen absorption: % of calculated value (p. 432).

Dihydrocinchonine. The base prepared by catalytic hydrogenation of pure cinchonine was finally purified by crystallisation from boiling alcohol, and from this the dihydrobromide was prepared in the usual way. Dihydrocinchonine differs from the three other dihydro-cinchona bases dealt with in this paper by decolorising permanganate in the standard test (p. 432) in 30 seconds. The *cuprichloride* was prepared by adding the base dissolved in conc. HCl (1 g. in 2 ml.) to cupric chloride in the same solvent (1 g. in 3 ml.). The yield was 0·9 g., which was reduced to 0·45 g. by recrystallisation from 1 ml. hot conc. HCl. From hot concentrated solutions it separates at once in golden-brown needles, but if crystallised slowly, it forms transparent much-twinned garnet-coloured prisms M.P. 212° (both forms). (Found: C, 45·20; H, 5·25; N, 5·54; Cl, 28·21; Cu, 12·31 %. $C_{19}H_{24}ON_2.2HCl, CuCl_2$ requires: C, 45·27; H, 5·16; N, 5·56; Cl, 28·16; Cu. 12·62 %.)

The constants found for the base and dihydrobromide are given in Table IV.

Biological tests[1].

Antimalarial activity. The comparison of the activity of the alkaloids was made with canaries infected with a strain of bird malaria kindly supplied by Sir Rickard Christophers. A mixture of the blood from infected birds diluted with 5–10 parts of saline, containing sodium citrate to prevent clotting, was injected under the pectoral muscles of a number of normal birds. The infected birds were bled by cutting off their heads and the feathers excluded by filtering the blood through glass wool. The blood and saline mixture was kept warmed at 37° during the period of the injections, as it was found that the latent period before the appearance of the parasites was considerably longer for the birds which were inoculated last, if this were not done. In the case of warmed blood the latent periods of the birds inoculated first and last do not differ by more than a day if all the injections can be completed in 15–25 minutes. To obviate any systematic error on this score the birds were arranged so that the average time of standing of the inoculum was constant for each group of birds used for the different drugs. The drugs were given orally by the method of Roehl through a thin piece of rubber tubing. The first dose was given 4 hours after the injection of the infecting material. Subsequent doses were given at daily intervals for the next 5 days, making 6 doses in all. The drugs were given in proportion to the weight of the bird, in the majority of cases, 5 or 2·5 mg. of base dissolved in 0·5 ml. per 20 g. weight of bird (most of the birds weighed 13–18 g.). The birds tolerate the material better if it is injected slowly. Six birds were usually used for each drug and the potency was estimated in comparison with that of quinine by taking the average time after infection at which parasites appeared in a particular group and comparing it with the average of the quinine group done at the same time. The authors consider this point of importance in the comparison of the activity of the drugs. The average time of appearance of parasites after inoculation and treatment with 6×5 mg. doses of quinine has varied from 16 to 23 days in these and other experiments not yet recorded. These differences show that unless in each batch of infected birds a group is treated with some drug to serve as a standard of curative activity, quantitative estimations of relative activity will suffer from a larger and almost incalculable error. The error of such comparisons has been very roughly calculated, and a preliminary estimate suggests that with two groups of six birds a difference in mean time of appearance of parasites of 1 day is significant and for two groups of three birds, a difference of 1·5 to 2 days.

Occasionally it has not been possible to detect parasites in treated birds for 30 days after the infection. These birds have been reinjected with infected blood

[1] G. A. H. Buttle and J. W. Trevan are responsible for all the experiments on animals.

and if they have shown parasites after the infection, they are considered to have been complete cures.

The results are shown in Tables C and D.

Table C is a typical group treated with quinine. It shows the differences obtained in a group of birds treated in the same manner.

Table D shows the results obtained in different experiments.

Table C.

Bird No.	Drug	Proportion of red cells containing parasites (days after inoculation) 17	18	19	20	21	22	25
45	Quinine				(Died 5th day)			
46	6 × 5 mg.	—	VVF	—	1/15	—	1/50	—
47		—	—	—	—	—	—	—
48		—	VVF	—	1/15	—	1/15	—
49		—	VVF	—	1/10	—	1/10	—
50		—	—	—	1/120	1/100	—	1/40

VVF indicates one or two parasites in several fields.

Table D.

Date	Control. Average time of appearance of parasites in untreated birds (in days)	Drug (6 doses in each case)	Dose mg. per 20 g. bird weight	Average time of appearance of parasites in treated birds (in days)
13. i. 31	7·0	Dihydroquinine	5	21·6 + 2 cures
		Quinine	5	18·2 + 1 cure
		Dihydroquinidine	5	15·6
		Cinchonidine	5	14·0
		Dihydrocinchonine	5	11·0
		Dihydrocinchonidine	5	9·3
2. ii. 31	5·9	*Quinine*	5	17·6
		Dihydroquinine	3·5	16·3
		Dihydroquinidine	5	16·0
		Cinchonidine	5	14·6
		Quinine	2·5	14·6
		Quinine	1	14·0
		Dihydrocinchonine	5	12·0 (1st dose 10 mg.)
		Dihydrocinchonidine	5	11·2 (1st dose 10 mg.)
18. iii. 31	4·0	*Quinine*	5	16·0
		Quinidine	5	14·0* (2 birds only)
		Cinchonine	5	12·3
7. iv. 31	5·6	*Quinine*	5	19·0* (1 bird only)
		Quinidine	5	17·0* (1 bird only)
		Cinchonine	5	13·0

* Six birds were used for each group, usually some died during the test; if there were less than three survivors, it has been noted in the last column.

Toxicity. The toxicity of certain of these substances on intravenous injection was examined by intravenous injection into mice. The relevant results are given in Table E.

The average lethal doses are recorded in the last column. The figures represent the estimate of the dose necessary to kill 50 % of the mice. The differences between the different alkaloids are probably not statistically significant, with the possible exception of dihydroquinidine.

Table E. *Results of toxicity tests by intravenous injection into mice.*

Drug	Dose in mg. of base per 20 g. mouse	Mortality	Estimate of average lethal dose of base for 20 g.mouse mg.
Quinine acid sulphate	1·2	0/10	1·9
	1·8	4/10	
	2·1	8/10	
	2·4	23/25	
Quinine dihydrochloride	1·05	1/10	1·4
	1·5	6/10	
	1·9	5/5	
	2·25	10/10	
Dihydroquinine acid sulphate	1·0	1/10	1·9
	1·25	0/10	
	1·5	0/10	
	2·0	11/20	
	2·5	5/5	
	3·0	5/5	
Quinidine dihydrochloride	1·0	0/10	1·6
	1·5	5/10	
	2·0	11/15	
Dihydroquinidine acid sulphate	1·0	6/15	1·3
	1·25	2/10	
	1·5	7/10	
Cinchonine acid sulphate	1·0	1/10	1·75
	1·5	2/10	
	2·0	7/10	
Dihydrocinchonine acid sulphate	1·26	0/10	2·0
	1·9	0/10	
	2·2	10/10	
Cinchonidine acid sulphate	1·5	0/10	1·85
	1·75	3/10	
	2·0	17/20	
Dihydrocinchonidine acid sulphate	1·9	0/10	2·0
	2·2	10/10	
Cinchonine chloride hydrochloride	1·3	0/10	1·55
	1·6	7/10	
	1·8	9/10	

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on its conversion into —CO— (quininone), —CH_2— (desoxyquinine), :CH— (quinene) or —CH.OAc— (acetyldihydroquinine) [Giemsa and Oesterlin, 1933] and no change so far effected has left the activity unimpaired.

Table B.

Substance	Daily doses: No.	Daily doses: Quantity mg.	Retardation days
Ethylquitenine hydrochloride	6	5	5
iso-Amylquitenine dihydrochloride	6	5	6

The authors desire to place on record their indebtedness to Prof. van der Sleen of the Kina Bureau, Amsterdam, for information on a number of technical points connected with the analysis of cinchona alkaloids. They also owe thanks to Mr W. Solomon, B.Sc., for the preparation of the three cinchona alkaloid chlorides mentioned, to Messrs A. C. Camfield, E. M. Gibbs, and N. J. G. McLaren for much patient work on the fractionation and biological tests of the alkaloids, and to Messrs A. Bennett and H. C. Clarke for the micro-analyses.

Chemical section.

Crystallisable cinchona alkaloids. The impurities in commercial samples of the primary cinchona alkaloids are of two kinds. Each alkaloid contains its own reduction product, which in the case of quinidine is now shown to be present to the extent of 20 % and may be as high as 30 % [Dawson and Garbade, 1930], while cinchonine usually contains about 14 % of dihydrocinchonine. These remarks apply to well-crystallised specimens of these alkaloids, usually accepted by chemists and biologists as pure owing to their constancy of melting-point and optical rotation. The amounts of dihydro-bases in quinine and cinchonidine are much less but are not negligible. The other type of impurity is due to the difficulty of completely separating the components of each of the two similarly optically active pairs from each other, *i.e.* cinchonidine and quinine usually contaminate each other, as do quinidine and cinchonine, but components of the laevorotatory pair are not usually found in those of the dextrorotatory pair and the converse is also true.

The purification of any one of the four primary alkaloids thus involves two steps: (1) the removal of the second component of the similarly optically active pair, *e.g.* cinchonidine from quinine and *vice versa*, and (2) the elimination from each alkaloid of the dihydro-base, which naturally accompanies it.

Much work has been expended in attempts to prepare each of the primary cinchona alkaloids in a pure state, but only two of the numerous papers on this subject need be referred to here [Tutin, 1909; Emde, 1932]. Tutin prepared pure quinine by repeated crystallisation of the acid sulphate, but was unable to prove that it was free from dihydroquinine. This proof is now supplied. Emde set out to prepare all eight alkaloids in a pure state, specially recommending for this purpose crystallisation of the dihydrobromides, but he did not prove the absence of the dihydro-bases from his final specimens of the four primary alkaloids.

In the course of the present investigation, pure specimens of each of the primary alkaloids have been prepared, the chief criteria of purity adopted (apart from well-known specific tests, such as fluorescence, methoxyl determinations and the thalleioquin reaction) being (*a*) agreement of the "found" and "calculated" values for hydrogen absorbed, by the conversion of the vinyl group into

an ethyl group, under standard, quantitative conditions, as proof of the absence of dihydro-bases and (*b*) constancy of optical rotation of the base and its salts, when expressed on a uniform basis and taken under standard conditions. The chief effects of this purification are to remove quinine from "cinchonidine" and quinidine from "cinchonine," and to eliminate large quantities of dihydro-bases from what have hitherto been commonly regarded as pure cinchonine and pure quinidine. From the pure primary alkaloids the corresponding dihydro-bases have been made by catalytic hydrogenation.

The chief constants of the eight pure alkaloids so prepared are recorded in Tables III and IV.

Though numerous determinations of the specific rotations of the cinchona alkaloids are recorded, they are for such varied concentrations and solvents, that it is not practicable to use them for comparative purposes. The authors have however repeated their determinations for the dihydrobromides of all eight alkaloids, under the conditions used by Emde in order to establish a more exact comparison with the results of that author. These comparative results are given in Table II.

Table II. *Specific rotations of dihydrobromides of cinchona alkaloids.*

Base	Salt used	*c* (anhydrous salt)	Solvent	$[\alpha]_D$ recorded by Emde	$[\alpha]_D$ recorded by Present authors
Quinine	B.2HBr, $3H_2O$	3·5194	Water	−181·04°*	−184·4°
Dihydroquinine	B.2HBr, $3H_2O$	3·902	Water	−152·7	−153·2
Cinchonidine	B.2HBr, $2H_2O$	4·2575	Water	−112·5	−111·4
Dihydrocinchonidine	B.2HBr, $2H_2O$	2·0828	Water	− 91·9	− 90·6
Quinidine	B.2HBr, $3H_2O$	3·6163	Water	+209·5 to +210·7	+219·2
Dihydroquinidine	B.2HBr, $3H_2O$	3·6628†	Water + HBr	+196	+197·5
Cinchonine	B.2HBr	4·2355	Water	+163·2	+166·6
Dihydrocinchonine	B.2HBr	5·0233	Water	+142	+143·1

* Emde gives $[\alpha]_D^{25°}$ −183·6° for *c* 3·9363.

† Average of 7 determinations: *c* = 3·6491 to 3·6836 and $[\alpha]_D^{22 \text{ to } 27°}$ = 193·2 to 197·6°.

It will be seen that the two sets of figures are in good agreement so far as cinchonidine and the four dihydro-bases are concerned, the differences ranging from 0·5 to 1·5, which are not greater than might be expected from two workers, making observations on the same substance. With quinine the difference amounts to 3·36°. Emde purified his quinine, first as the acid sulphate and finally as the dihydrobromide and there can be no doubt as to its purity. It is suggested that his lower specific rotation may be due to incomplete drying. Thus, he describes quinine dihydrobromide as a dihydrate, but the present authors found that the loss on drying at 120° *in vacuo* in seven samples varied from 10·12 to 10·86 % (average 10·37 %). The loss calculated for a trihydrate is 10·0 % and for a dihydrate 6·9 %.

In the case of cinchonine the difference is 3·4°. In this instance the salt is anhydrous and the higher figure now recorded is doubtless due to the complete elimination of dihydrocinchonine. Assuming this, the lower figure recorded by Emde corresponds to a cinchonine containing 14·3 % of dihydrocinchonine, which is close to the figure 15·1 % now found (p. 437) for commercial cinchonine after recrystallisation from alcohol until free from quinidine.

The figures for quinidine show the largest difference 8·5 to 9·7°. Emde found quinidine dihydrobromide to be a dihydrate whereas the present authors found