THE STRUCTURE OF A NEW METABOLIC DERIVATIVE OF QUININE*

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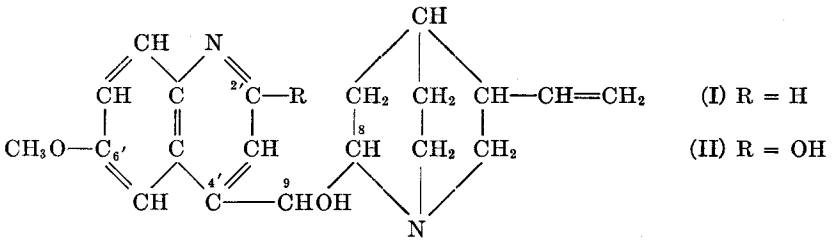
The isolation and crystallization of a product obtained by the *in vitro* action of rabbit liver on quinine (I) have recently been described by Kelsey, Geiling, Oldham, and Dearborn (1). The work reported here was undertaken with the object of obtaining information, as rapidly as possible, as to the possible structure of this product, which might prove useful in other malarial investigations. Thus no attempt has been made to obtain or present a rigid proof of structure of the metabolic product, which, for convenience, will be referred to as QDP (quinine-derived product). Evidence will be presented which suggests that QDP is levorotatory 2′-hydroxy-6′-methoxy-3-vinylruban-9-ol (II), according to the notation introduced by Rabe (2).



(I) R = H

(II) R = OH

EXPERIMENTAL

QDP is soluble in the alcohols, slightly soluble in acetone, ether, or chloroform, and insoluble in water and the hydrocarbon solvents. It exhibits a dark blue fluorescence in all its solutions and in contrast to quinine the fluorescence is not depressed by chloride ion.

QDP in chloroform solution was adsorbed on a column of calcium carbonate (Merck, heavy powder) and the chromatogram developed with chloroform containing 2 per cent ethanol. In ultraviolet light only a single fluorescing zone appeared on the column, which during further develop-

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ment migrated slowly, without differentiation, into the chromatographic filtrate.

QDP crystallizes in long colorless needles when its methanol solution is diluted with water almost to the point of precipitation and is then allowed to stand in a desiccator over calcium chloride. In the melting block, the crystals show a distinct change in crystal structure at 150° (1), finally melting to a clear dark brown liquid at 247.5–248.5° (corrected). The chemical properties appeared unchanged by heating at 160° for several hours. QDP is optically active, a solution of 0.0165 gm. in 2 ml. of ethanol in a 2 dm. tube having a rotation of $-0.54°$; $[\alpha]_D^{25} = -65.5° \pm 0.5°$.

QDP is soluble in dilute mineral acids, from which it can be precipitated by dilute NH_4OH. It is insoluble in NH_4OH or in N alkali hydroxides but dissolves quite slowly, even on heating, in 2 to 3 N aqueous NaOH or KOH. When a suspension of QDP in 6 N NaOH or KOH is warmed, an insoluble oil is formed, which on dilution of the alkali to about 3 N readily dissolves. QDP cannot be extracted from a 3 N NaOH solution with a mixture of ether and butanol, but may be recovered unchanged, even after prolonged boiling, by acidification with dilute acid and neutralization of the acid solution with NH_4OH.

Despite this apparent acidity, QDP does not appear to have a titratable acid group and it does not react with diazomethane or give a color reaction with ferric chloride.

Analyses of QDP—A sample of QDP was purified for analysis by repeated recrystallization from methanol and water. The final sample was dried at 110° *in vacuo* but proved to be somewhat hygroscopic after drying. The individual samples were therefore dried to a constant weight and weighed separately in a closed system for analysis.

$C_{20}H_{24}O_3N_2$.	Calculated.	C 70.56, H 7.11, N 8.23, MeO 9.11
340.4	Found.	" 70.28, " 7.16, " 8.51, " 9.08
	"	" 70.41, " 7.01, " 8.53, " 9.06
	"	" 70.40, " 7.09, " 8.32, " 9.08

Molecular weight determinations by the Rast method proved unsatisfactory; the QDP sample darkened somewhat in the camphor melt (180°). The results obtained varied between 475 and 532.

Potentiometric Titration—50 ml. of a 90 per cent ethanol solution containing 4.4×10^{-4} mole of quinine and 50 ml. of a 90 per cent ethanol solution containing 4.3×10^{-4} mole of QDP were titrated with 0.0466 N HCl. The pH measurements were made with a Beckman pH meter after each addition of 1 to 2 ml. of acid. The graphical point from a plot of pH against the volume of acid added which corresponded to half neutralization of the first basic group was taken as the ionization constant, K_{b_1}; this point for both quinine and QDP was pH 7.45.

At the calculated half neutralization point of the second basic group of quinine, 14.15 ml. of acid had been added and the observed pH was 4.27. At the same point for QDP, 13.85 ml. of acid had been added and the pH was 2.80. In the calculation of the constant K_{b_2} for the second basic group

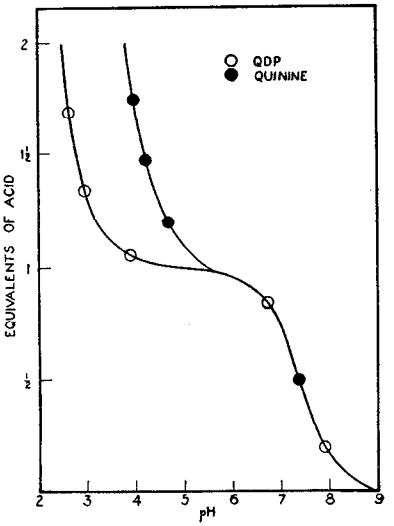

FIG. 1. Potentiometric titration curves of a quinine-derived product and quinine

of quinine or QDP, a correction was applied for the error due to hydrolysis by the use of the following equation.

$$K_b = \frac{K_w}{(H^+)_m} \frac{\frac{C}{2} - (H^+)_m}{\frac{C}{2} + (H^+)_m}$$

where K_b is the basic dissociation constant, C is the total concentration of the base at the half neutralization point, and $(H^+)_m$ is the observed hydrogen ion activity at that point. When the effect of ethanol is disregarded, the calculated dissociation constants at 25° are as follows: quinine, $K_{b_1} = 2.8 \times 10^{-7}$ (82 per cent ethanol), $K_{b_2} = 1.8 \times 10^{-10}$ (70 per cent ethanol); QDP, $K_{b_1} = 2.8 \times 10^{-7}$ (82 per cent ethanol), $K_{b_2} = 2.3 \times 10^{-12}$ (70 per cent ethanol). The titration curves are given in Fig. 1.

Absorption Spectra—The absorption curves of quinine, QDP, and 2-hydroxy-6-methoxy-4-methylquinoline[1] (6-methoxy-4-methylcarbostyril) were investigated with a Beckman quartz spectrophotometer in 0.0002 M

[1] Prepared by Mr. Alf Reims; compare Ainley and King (3).

ethanol solutions from 250 to 380 mμ. The hydrogen lamp was used to 320 mμ and a filter added to 380 mμ. The latter values were checked with a tungsten lamp.

With a cell thickness of 1 cm. and at a temperature of about 26°, quinine showed maxima at 279 and 333 mμ and minima at 225 and 301 mμ. Under the same experimental conditions, QDP showed a maximum at 352 mμ and a minimum at 297 mμ, compared with a maximum of 350 mμ and a minimum of 294 mμ for 2-hydroxy-6-methoxy-4-methylquinoline. For purposes of comparison the three curves are plotted in Fig. 2.

Hydrogenation of QDP—At 22° and 741 mm., an ethanol solution containing Adams' catalyst and 9.021 mg. of QDP absorbed 0.667 ml. of

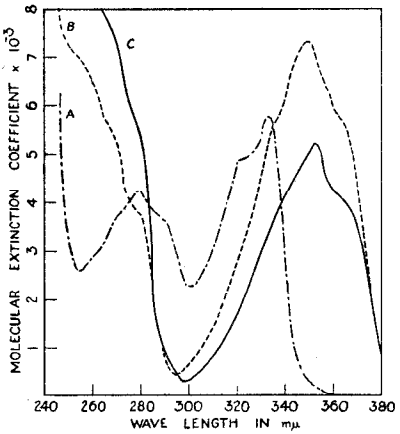

FIG. 2. Absorption spectra of quinine (Curve A), a quinine-derived product (Curve B), and 2-hydroxy-6-methoxy-4-methylquinoline (Curve C).

hydrogen.[2] Assuming one olefinic linkage to have been hydrogenated, the molecular weight of QDP is calculated from this to be 336.

Ozonization of QDP—A suspension of 0.2828 gm. of QDP in 20 ml. of purified chloroform at 0° was treated with a stream of 5 to 6 per cent ozone at 100 ml. per minute for 4 hours (4). The chloroform was evaporated at room temperature and the colorless residue was warmed with 25 ml. of water on the water bath for 1 hour. 20 ml. of this solution were distilled into 5 ml. of ice water and then treated with 0.28 gm. of medon. When the mixture was warmed for 5 minutes on the water bath, a precipitate appeared which, after filtration and recrystallization from ethanol, melted at 188–190° (corrected) and gave a mixed melting point of 188–190°

[2] The authors are indebted to Dr. G. Oppenheimer for carrying out this determination.

(corrected) with a known sample of formaldehyde dimedon. The recrystallized product weighed 0.0384 gm. or 16 per cent of the theory. Quinine, under the same conditions, gave a corresponding amount of formaldehyde dimedon.

Reaction with Methyl Iodide—An excess of methyl iodide was added to a solution consisting of 0.3 gm. of QDP which had been dissolved in 5 ml. of chloroform with the aid of a little methanol. After the solution was warmed, a heavy precipitate appeared which was filtered off, washed with chloroform, and dried to yield 0.42 gm. of crystalline solid. This was recrystallized from ethanol to give colorless needles, m.p. 276–277° (with decomposition; corrected).

$C_{21}H_{27}O_3N_2I$.	Calculated.	C 52.28, H 5.64, N 5.81, I 26.31, MeO 6.4
482.4	Found.	" 52.21, " 5.63, " 5.52, "26.37, " 6.7

This would indicate that QDP forms a monomethiodide which dissolves in dilute mineral acids and in bases, including NH_4OH.

When the reaction of QDP with methyl iodide was carried out in a sealed tube with methanolic KOH, a product could not be isolated. If a sample of the methiodide was refluxed in methanol with methyl iodide, the solution became acidic and the solid went into solution. A definite compound could not be isolated from this reaction mixture.

Reaction with Benzenesulfonyl Chloride—0.3 gm. of QDP was suspended in 3 ml. of 6 N KOH and warmed on the water bath; an oil formed which dissolved on dilution with 10 ml. of water. To this solution there was added 0.3 ml. of benzenesulfonyl chloride, the mixture was shaken for 1 hour, and an additional 0.1 ml. of benzenesulfonyl chloride was added. After another hour of shaking and stirring the mixture was warmed to decompose excess acid chloride. An oily precipitate, which had gradually formed and then solidified, was filtered off, washed with water, and dried. After one crystallization from ethanol and two from water the product melted at 179–180°, but still contained ash and gave a flame test for potassium. It was therefore recrystallized from water with the addition of a drop of HCl just at the point of crystallization. After three more crystallizations from water, long sheaves of colorless needles were obtained; m.p. 180–181° (corrected). A sample for analysis was unchanged after heating for 15 hours at 140° *in vacuo*.

$C_{56}H_{66}O_{16}N_4S_3$.	Calculated.	C 59.36, H 5.84, N 4.78, S 8.20, MeO 5.29
1173.3	Found.	" 59.44, " 5.83, " 4.78, "8.22, " 5.35

This compound is soluble in ethanol and hot water and insoluble in hydrocarbon solvents. The possibility that it is a salt of benzenesulfonic acid is excluded by the finding that QDP can be recovered unchanged from

a solution containing a corresponding amount of benzenesulfonic acid. It is insoluble in dilute bases and slowly soluble in dilute mineral acids; from the latter solution, after 2 hours refluxing, QDP was recovered by neutralization with NH_4OH.

Miscellaneous Reactions of QDP—When heated for 3 hours in a sealed tube with water at 150°, or with 2 N HCl at 150°, QDP dissolved but was recovered unchanged on cooling or on neutralization of the acid solution. Moreover it was recovered unchanged after refluxing 45 hours with dilute acetic acid (compare von Miller and Rohde (5)).

QDP did not react with *p*-nitrophenylhydrazine, 2,4-dinitrophenylhydrazine, or hydroxylamine and was recovered unchanged from its absolute ethanolic sodium ethylate solution through which methyl nitrite had been passed (compare (5)).

With nitrous acid in dilute HCl, QDP gave a precipitate which gave the diphenylamine test and the Liebermann phenol test for nitrosamines. With nitrous acid in acetic acid solution it gave a deep yellow color. With ceric nitrate reagent QDP gave a deep red color; this color is given by carbostyril (2-hydroxyquinoline) but not by quinine.

In chloroform-methanol solution QDP took up bromine rapidly, forming a yellow precipitate which melted at about 280°.

Unsuccessful attempts were made to obtain a recognizable fragment of the QDP molecule after oxidation with a number of agents and under a variety of conditions. Thus, no quininic acid could be obtained after oxidation with chromic acid, although parallel experiments with quinine led to the isolation of good yields of quininic acid (6). In the case of the oxidation of QDP with dilute HNO_3 a crystalline product was obtained which proved to be a nitrate. This compound on treatment with dilute NH_4OH gave a red crystalline material which did not melt below 300°. Preliminary analyses of this compound indicated that degradation of the QDP molecule had not been effected; therefore further attempts at oxidation with HNO_3 were abandoned.

DISCUSSION

The following discussion summarizes the experimental evidence and the reasons for assigning the structure (II) to QDP.

The homogeneity of QDP is evidenced by its constant rotation and melting point after successive crystallizations and by its behavior when chromatographed.

The analyses are in excellent agreement for an empirical formula containing 1 more oxygen atom than that of quinine. It was therefore assumed that no deep seated structural change had taken place in the quinine molecule.

Although a direct molecular weight determination of QDP proved unsatisfactory, there is no evidence to suggest that the molecular weight is other than 340.4, as calculated from the simple empirical formula $C_{20}H_{24}O_3N_2$. On the contrary, the titration curve for QDP (Fig. 1) is not consistent with a double molecule. Secondly, ozonolysis indicated the presence of an expected vinyl group and quantitative hydrogenation confirmed an aliphatic double bond; from the hydrogenation data the molecular weight of QDP is calculated to be 336, based upon the reduction of one double bond, which is in good agreement with 340.4 for $C_{20}H_{24}O_3N_2$.

Evidence that the quinuclidine ring of quinine is retained unchanged in QDP is shown by the following: (*a*) the vinyl group is intact, as evidenced by ozonolysis, hydrogenation, and the addition of bromine; (*b*) QDP forms by addition a monomethiodide; (*c*) QDP does not react with methyl nitrite and therefore does not contain the grouping $—C^9O—C^8H_2—$ which would be present if the quinuclidine ring had been opened and a quinicine-like structure were involved; (*d*) the first basic dissociation constant, K_{b_1}, is the same for quinine and QDP.

Evidence that it is the quinoline portion of the molecule in quinine which has been altered in the formation of QDP is shown by the following: (*a*) quininic acid could not be obtained by oxidation of QDP; (*b*) in contrast to quinine, QDP is soluble in solutions of the hydroxides of the alkali metals; (*c*) QDP does not form a dimethiodide, as does quinine; (*d*) the second basic dissociation constant, K_{b_2}, for QDP is very small.

Consideration may now be given to the reasons for assigning to QDP the structure (II) containing an oxygen in the 2′ position of the quinoline ring. Since QDP is derived from quinine (I), the 4′ and 6′ positions must be occupied, the latter by the methoxyl group which has been shown to be present. The possibility of an amine oxide structure may be ruled out since QDP forms only a monomethiodide. The addition of an oxygen to the 3′, 5′, 7′, or 8′ position in quinine (I) would be expected to produce a phenolic compound of a sufficiently acidic nature to react with diazomethane and to give a color reaction with ferric chloride; QDP, however, does not react with either reagent. There remains therefore only the 2′ position to be occupied by the oxygen atom which must be accounted for in QDP; a hydroxyl group in this position, and *only* in this position, would account for acidic properties weaker than those shown by an ordinary phenolic hydroxyl group, as well as for the marked decrease in basic properties of the quinoline nitrogen.

Thus the structure (II) accounts very well for the properties of QDP and is in harmony with the experimental evidence. Such a structure may be looked upon as a 4,6-disubstituted carbostyril, and the absorption spectra shown in Fig. 2 are in excellent agreement with such a view. The acidic

properties of QDP are comparable to those of a substituted carbostyril, for, although carbostyril itself reacts with diazomethane to give an O-methyl ether, the acidity decreases with substitution, and 6-methoxy-4-methylcarbostyril does not react with diazomethane. Moreover carbostyrils are not smoothly oxidized under acid conditions (7), which accounts for the fact that attempts to obtain recognizable oxidation products of QDP were unsuccessful. The slight basicity of the carbostyril nitrogen in QDP explains the nitrosamine test as well as the fact that on prolonged treatment with methyl iodide a solution of QDP becomes acid, without doubt by liberation of HI, although the N-methyl ether was not isolated.

There remain two experimental observations for which a satisfactory explanation cannot be offered at this time, although neither observation necessarily conflicts with the structure (II) for QDP. The first of these is that QDP apparently does not isomerize to a quinicine-like structure as does quinine. This may be due to the altered quinoline ring in QDP, since any rearrangement involving the grouping —C^9HOH—C^8H—N—

might well be influenced by a change in the quinoline portion of the molecule from that of structure (I) to (II). The second is the formation of the compound with a melting point of 179–180° by the action of an excess of benzenesulfonyl chloride on QDP in alkali. The analytical results are in close agreement with the calculated values for the empirical formula $C_{58}H_{68}N_4O_{16}S_3$. This empirical formula could be accounted for on the assumption that the reactants have combined in any one of several proportions; *e.g.*, 2 moles of QDP plus 3 moles of benzenesulfonyl chloride plus 4 moles of water with loss of 3 moles of HCl, or 2 moles of QDP plus 3 moles of benzenesulfonic acid plus 1 mole of H_2O. However, it has not been possible to write a structure which properly accounts for any such combination. The significant fact is that the compound is not likely to involve an S—N linkage, since QDP was recovered quantitatively after mild acid hydrolysis.

SUMMARY

Evidence is presented that the crystalline metabolic product, m.p. 247.5–248.5°, derived from quinine, is levorotatory 2′-hydroxy-6′-methoxy-3-vinylruban-9-ol.

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