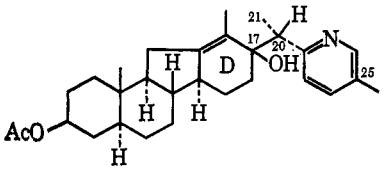
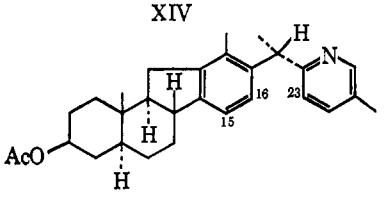
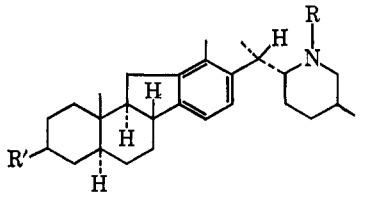


XIV



XV

authentic sample (melting point, ir, tlc, nmr) prepared from veratramine.[5]



XVI, R = H; R′ = OAc
XVII, R = Ac; R′ = OAc
XVIII, R = Ac; R′ = OH
XIX, R = Ac; R′ = O
XX, R = Ac; R′ = O; 4,5 double bond
XXI, R = Ac; R′ = OH; 5,6 double bond

Oxidation of N-acetyl-5α,6-dihydroverarine (XVIII) with Jones reagent yielded the 3-ketone XIX which was converted to the α,β-unsaturated ketone XX by the method of Evans, *et al.*[17] This compound (XX, ν_{max}^{CHCl} 1661 cm^{-1}; τ 4.22 (1 H, singlet, C_4–H)) was then converted into the β,γ-unsaturated alcohol XXI by the procedure of Dauben.[18] Compound XXI, N-acetylverarine, was identical with authentic material (melting point, ir, tlc, nmr) prepared as cited above.[5]

The final step in the sequence, removal of the N-acetyl group, was achieved with 10% potassium hydroxide in refluxing ethylene glycol. The resulting product was shown to be verarine (I), mp 174–176°, by comparison with authentic material (melting point, ir, tlc, nmr).[19]

It is now clear that, by utilization of the appropriately substituted pyridine derivative in the condensation reaction mentioned above, a route to veratramine and jervine is available. Results in this direction will be presented in future communications.

Acknowledgment. Financial aid from the National Research Council of Canada, Medical Research Council of Canada, President's Research Fund, University of British Columbia, and Smith, Miller and Patch, Inc., is gratefully acknowledged.

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Inhibition of Coenzyme Q Systems by Chloroquine and Other Antimalarials[1,2]

Sir:

Basic studies on the metabolism of malarial parasites (*Plasmodium*) have led to new data showing that chloroquine, primaquine, quinacrine, and a naphthoquinone antimalarial inhibit, *in vitro*, the mitochondrial oxidation of DPNH and succinate by coenzyme Q (CoQ). The inhibitory effects of chloroquine and the naphthoquinone are the most pronounced, and the effects of the four antimalarials differ considerably in nature and degree. While these inhibitions of electron transfer involving CoQ by certain antimalarials are newly evident, and the presence of CoQ in the metabolism of *Plasmodium* is now known, any correlation between such inhibition and antimalarial activity remains a subject for study. This specific correlation would support only one of many mechanisms for antimalarial activity.

Stemming largely from the research of Fieser and Leffler and their many respective coworkers,[3a] the two naphthoquinones (I, II, or M-1916 and M-2350) were found in the late 1940's to show antimalarial activity in man. Of M-1916, it was said "effect was not satisfactory, but enough to show that M-1916 has definite antimalarial activity in man."[3b] Of M-2350, it was stated "two patients with primary vivax infection were given 2 g ... for 4 days .... The results were dramatic. ...The patients left the hospital in perfect condition with no parasites in the blood. ... or without relapse."[3c] The chemical, biological, and medical research on naphthoquinones during the World War II period was prodigious and seemed to imply that vitamin K was intrinsic in the metabolism of *Plasmodium*. This was a reasonable implication at that time, since it was known that vitamin K is a product of microbial metabolism, and such antimalarial naphthoquinones produced hemorrhagic symptoms in rats which were prevented by vitamin K.[4] Nevertheless, there were no data during those years which proved the participation of vitamin K in the metabolism of the *Plasmodium*.

Recent recognition of the resistance of certain strains of *Plasmodium* to otherwise effective drugs, and the world implications of this resistance, prompted our research for basic knowledge on the electron-transfer mechanisms of *Plasmodium*. Not only was a specific search made for the presence of vitamin K in *Plasmodium*, but a search for CoQ was also made, since the latter closely related quinone was discovered subsequent to the World War II antimalarial program. It was surprisingly discovered[5,6] that CoQ_8 and possibly CoQ_9

(1) Coenzyme Q. CVIII.

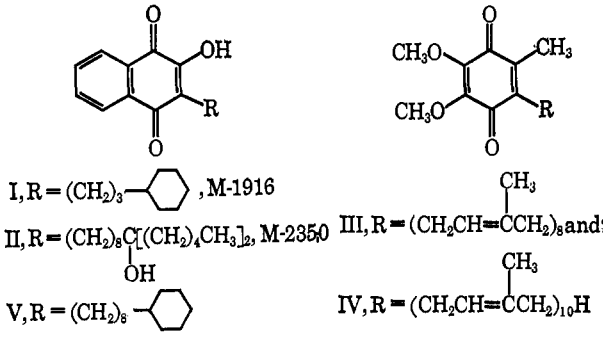
(2) This investigation was partially supported by U. S. Army Medical Research and Development Command Contract DA-49-193-MD-2784. This is Contribution No. 421 from the Army Research Program on malaria.

(3) (a) L. F. Fieser, E. Berliner, F. J. Bondhus, F. C. Chang, W. C. Dauben, M. G. Ettlinger, G. Fawaz, M. Fields, M. Fieser, C. Heidelberger, H. Heymann, A. M. Seligmann, W. R. Vaughan, A. G. Wilson, E. Wilson, M. Wu, M. T. Leffler, K. E. Hamlin, R. J. Hathaway, E. J. Matson, E. E. Moore, M. B. Moore, R. T. Rapala, and H. E. Zaugg, *J. Am. Chem. Soc.*, 70, 3151 (1948); (b) *ibid.*, 70, 3154 (1948); (c) *ibid.*, 70, 3155 (1948).

(4) C. C. Smith, R. Fradkin, and M. D. Lackey, *Proc. Soc. Exptl. Biol. Med.*, 61, 398 (1946); 64, 45 (1947).

(5) P. J. Rietz, F. S. Skelton, and K. Folkers, Abstracts, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967, No. M-23.

(III) are present in duck blood parasitized by *Plasmodium lophurae*, in addition to CoQ_{10} (IV), which is the sole CoQ of the duck. Evidently CoQ_8 and CoQ_9 are biosynthesized by *P. lophurae*. In contrast, vitamin K could not be detected and, if present at all, its concentration was less than $^1/_{50}$ that of CoQ; consequently vitamin K does not seem to be present in *P. lophurae* at a level compatible with a role as one of the components of the electron-transfer mechanism.[6]

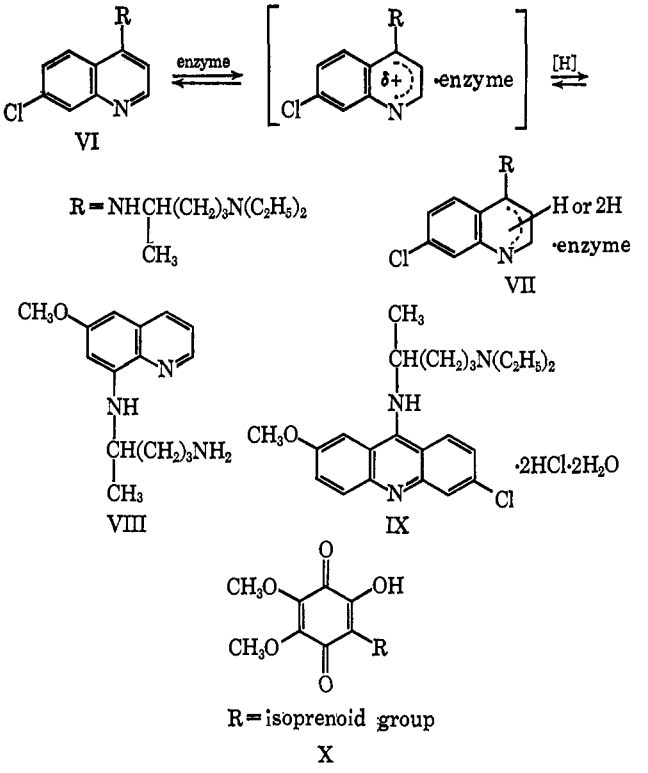


The presence of CoQ and the apparent absence of vitamin K in *P. lophurae* and probably in other species,[7] and the very close structural relationship between CoQ and vitamin K, suggest that such naphthoquinones as M-1916 and M-2350 may exert at least a part of their antimalarial activity through interference with the biosynthesis or the function of CoQ in the metabolism of the *Plasmodium*.

A new naphthoquinone (V) was synthesized by Fieser and Archer and their respective associates.[8] Dr. David P. Jacobus of the Walter Reed Army Institute of Research kindly informed us of the considerable current interest in the antimalarial activity of this new naphthoquinone (V) and provided a sample. The naphthoquinone V has substantial interest to us and has been tested in the DPNH-oxidase and succinoxidase systems which are known as two sites[9] for the participation of CoQ. The use of CoQ_{10} rather than CoQ_8 in these *in vitro* studies makes no fundamental difference, since both have the same activity in DPNH-oxidase and succinoxidase systems.[10]

We have found that in the presence of an additional 100 mμmol of CoQ_{10}, 1, 10, and 25 mμmol, respectively, of 2-(ω-cyclohexyloctyl)-3-hydroxy-1,4-naphthoquinone (V) depressed enzyme activity to 83, 12, and 11% of the CoQ_{10}-treated control in the *intact* DPNH-oxidase system,[11] as described by Szarkowska. In the pentane-extracted DPNH-oxidase system,[10,11] the same levels of the naphthoquinone depressed enzyme activity to 92, 59, and 11% of the CoQ_{10}-treated (100 mμmol) control. The naphthoquinone depressed the succinoxidase activity in both *intact* and *extracted* systems almost completely to 7% at a level of 100 mμmol.

4-Aminoquinolines, exemplified by chloroquine (VI), could conceivably be reduced *in vivo* by a one- or two-step biochemical electron transfer. This could occur at



one of the sites of reduction of a quinoid precursor of CoQ or perhaps more likely at one of the sites of participation of CoQ and in competition with CoQ. A two-electron reduction could conceivably give a product such as VII on the basis of the organic chemistry of dihydropyridine derivatives.[12] An 8-aminoquinoline derivative such as primaquine (VIII) would not be expected to participate in biochemical reduction in the same manner as chloroquine. Both chloroquine and primaquine have been tested, *in vitro*, in the CoQ-deficient DPNH-oxidase and succinoxidase systems.

Chloroquine diphosphate depressed DPNH-oxidase activity to 70, 60, 50, and 17% when levels of 25, 50, 100, and 1000 mμmol, respectively, were added to the *intact* system supplemented with 100 mμmol of CoQ_{10}. An amount of 25 mμmol of chloroquine alone, without added CoQ_{10}, depressed activity to 42% of the CoQ_{10}-supplemented control in the *intact* system. When the antimalarial concentration was maintained at 25 mμmol and the CoQ_{10} concentration increased to 500 and 750 mμmol, activity was restored to 80 and 86%, respectively, in the *intact* system. Equimolar amounts of primaquine diphosphate, in the presence of CoQ_{10}, did not depress the enzyme activity of either the *intact* or the *extracted* system, but 1 μmol of the antimalarial, in the presence of 100 mμmol of CoQ_{10}, reduced oxygen uptake to about 45%. Both chloroquine diphosphate and primaquine diphosphate depressed enzyme activity in both the *intact* and *extracted* succinoxidase systems to only 80–90% of the CoQ_{10}-supplemented control. Quinacrine hydrochloride (IX) showed no inhibition of the *extracted* DPNH-oxidase system at a level of 750 mμmol in the presence of 100 mμmol of CoQ_{10}. The antimalarial activity of quinacrine hydrochloride probably depends on a mechanism that is not concerned with CoQ.

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While the mechanism of the significant inhibition by chloroquine and the naphthoquinone V of CoQ-enzymic systems remains a subject for further study, it is evident that this inhibition could be directly related to their antimalarial activity. However, the antimalarial activity need not be solely related to interference with the biosynthesis or with the function of CoQ but may involve binding to enzymes directly related to CoQ. However, partial reversal of *in vitro* inhibition is observed by CoQ. Chloroquine appears to have other modes of action in the body. Schueler and Cantrell[13] report that ferrihemic acid complexes with chloroquine, and that the complex is an antagonist of the antimalarial action of chloroquine. Hahn, *et al.*,[14] have evidence that chloroquine inhibits nucleic acid biosynthesis and that the mechanism of action is the formation of a molecular complex with DNA. However, complexes of chloroquine with DNA and ferrihemic acid are not known to be directly related to the antimalarial activity.

Emphasis can now be given to the organic synthesis of new antimalarials based on the interference with the fundamental role of CoQ in the electron-transfer process of *Plasmodium;* one group of new hydroxyquinones (X) which are apparent inhibitors of CoQ enzyme systems has been synthesized.[15]

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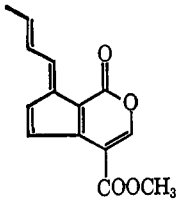
The Total Synthesis of Fulvoplumierin

Sir:

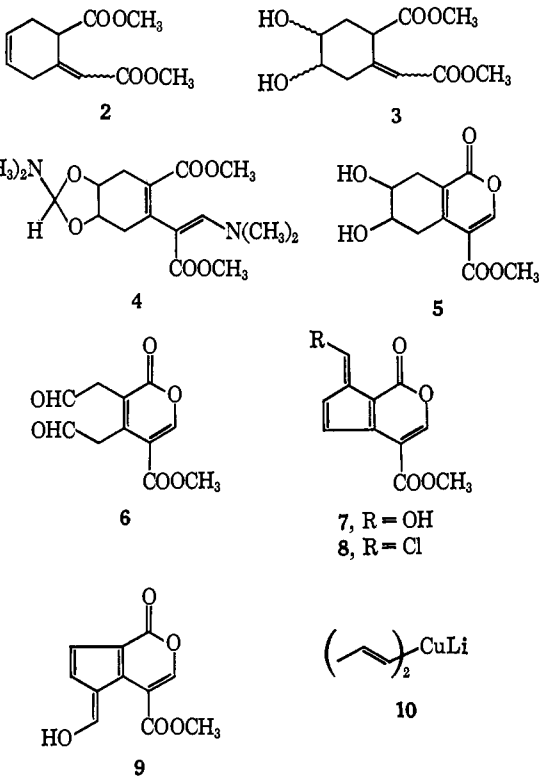
Fulvoplumierin (**1**), a constituent of *Plumeria acutifolia* with antibacterial activity, is a member of the small class of naturally occurring fulvenes.[1] Its structure was established by Schmid and coworkers,[2,3] and we now describe a total synthesis which confirms this structural assignment.



1

Condensation of dimethyl penta-2,3-dienedioate[4] with butadiene in benzene at 80° gave the adduct **2**, bp 78–80° (0.3 mm) (60%), which was oxidized in aqueous tetrahydrofuran at room temperature for 40 hr with potassium chlorate in the presence of a catalytic amount of osmium tetroxide[5] to a mixture of isomeric diols **3** (oil) (80%). Condensation with dimethylformamide dimethyl acetal[6] in dimethylformamide at 80° for 5 hr gave the dimethylaminomethylene compound **4** which, without purification, was hydrolyzed with dilute hydrochloric acid to the α-pyrone **5** (28%), mp 160–162°, ultraviolet absorptions at 253 (ϵ 7730) and 285 mμ (ϵ 4650) in ethanol and 251 mμ (ϵ 8850) in ethanol–NaOH. The latter was transformed with sodium periodate in aqueous methanol to the dialdehyde **6** and thence by cyclization with Amberlite IR 120 in dimethoxyethane at 78° for 4 hr to the yellow hydroxyfulvene **7** (61%), mp 162–163°, ultraviolet absorptions at 357 mμ (ϵ 7320) in ethanol and 304 (ϵ 9270) and 378 mμ (ϵ 24,100) in ethanol–NaOH.

The isomeric hydroxyfulvene **9** cannot be constructed with molecular scale models due to severe steric interaction between the hydroxymethylene and the carbomethoxy groups, and we consequently felt confident that the cyclization of the dialdehyde **6** had proceeded in the desired sense. Contrary to 6-hydroxyfulvene which is less stable than the tautomeric cyclopentadienaldehydes,[7] compound **7** exists entirely in the hydroxyfulvene form, and its nmr spectrum in $CDCl_3$ is characterized by two doublets, J = 14 Hz at δ 14 and 7.65, caused by the enolic and the adjacent vinylic hydrogen atoms, respectively.



2 **3** **4** **5** **6** **7**, R = OH **8**, R = Cl **9** **10**

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