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Naphthoquinone Antimalarials. I. General Survey[1,2]

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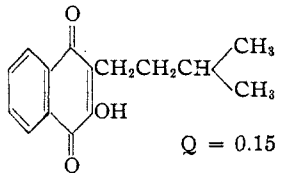
In 1942 Abbott Laboratories contributed to the antimalarial research sponsored by the Committee on Medical Research by collecting university research samples for screening assays against *P. lophurae* in ducks conducted at the University of Tennessee in Memphis under the direction of A. P. Richardson. In April, 1943, Dr. Richardson reported that among several hundred random samples he had found three substances from Harvard to possess antimalarial activity. These were hydrolapachol and two related, less active, quinones (M-1537 and M-1538) derived from the collection of samples bequeathed by the late Samuel C. Hooker[3] to L. F. Fieser. The indication of chemotherapeutic activity in substances of simple structure containing neither nitrogen nor sulfur seemed of particular significance. Additional samples from the Hooker collection and from naphthoquinone researches at Bryn Mawr and Harvard were at once submitted for assay,



Hydrolapachol (M-1523, SN-2527)

and the synthesis of additional compounds was initiated, particularly by use of a diacyl peroxide alkylation reaction reported in 1942. Some of the new quinones proved to be as much as one hundred times as active against avian malaria as the substances that had given the clue to the series. That the feeble activity of hydrolapachol was recognized in the first assays attests the efficiency of the screening procedure. In this and in further biological and medical phases, the investigation was materially advanced by the active participation of several groups operating under coördination by the Committee on Medical Research,[4] and we gratefully acknowledge our indebtedness for the many instances of coöperation.

Activity.—Paper II presents a summary and analysis of the assay data for some three hundred compounds classified into various series, and it constitutes an index to papers reporting the syntheses. The compounds are listed by the M numbers by which they were coded in the two laboratories[5]; SN-numbers[4] are not available for several of the compounds but are given in this

(1) The work described in this series of papers was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. The work at Harvard has also been aided by grants from the Rockefeller Foundation both during and since the war.

(2) Papers in press: Fieser, Chang, Dauben, Heidelberger, Heymann and Seligman, XVIII, Metabolic Oxidation Products; Heymann and Fieser, XIX, Antirespiratory Study of Protein Binding; Fieser, Heymann and Seligmann, XX, Metabolic Degradation (*J. Pharmacol. Exp. Therap.*); Heymann and Fieser, XXI, Antisuccinate Oxidase Activity; Fieser and Heymann, XXII, Relative Antirespiratory Activities (*P. lophurae*) (*J. Biol. Chem.*).

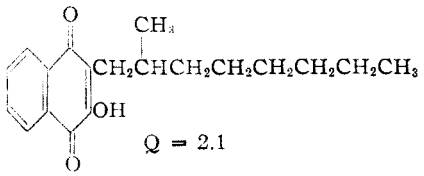
(3) A collection of Hooker's papers in a Memorial Volume "The Constitution and Properties of Lapachol, Lomatiol, and other Hydroxynaphthoquinone Derivatives," Mack Printing Co., Easton, Pa., 1936; is obtainable from the Kresge–Hooker Library at Wayne University.

(4) Wiselogle, "Survey of Antimalarial Drugs," 1946.

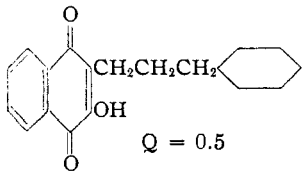
(5) The M (for Malaria) numbers are arbitrary and non-consecutive; compounds synthesized or supplied by the Abbott and Harvard groups are identified by numbers in three and in four digits, respectively.

paper for the ones most fully documented by CMR.

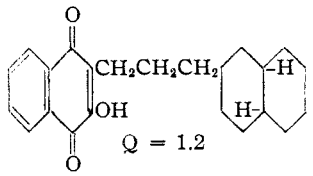
As the isoalkyl side chain of hydrolapachol is lengthened by the insertion of more methylene groups the activity against *P. lophurae* in ducks at first increases, reaches a maximum with a C_9-side chain, and then falls off. Much the same relationship and the same peak of activity is found in the *n*-alkyl series, and among quinones with branched side-chains of various structural types, although a particular structure may have a slight special advantage or disadvantage; for example, M-285 is nearly twice as potent as the straight-chain and isoalkyl isomers. When the naphthoquinone side chain contains one alicyclic ring (M-1916) the peak of activity is shifted to a C_{10} or C_{11} chain (M-1916 is not at the peak), and if two rings are present (M-297, M-2279, or M-2293) it is shifted to a C_{12} or C_{13} group. An aryl substituent in the side chain produces a still greater shift. The steric arrangement is important, as can be seen from a comparison of M-297 and M-2279 (mixtures rich in *trans*- and *cis*-decalyl derivatives). M-2293 (*trans*) is thirteen times as potent as the *cis* isomer; the direct attachment of the alicyclic ring to the naphthoquinone nucleus is a favorable feature and M-2293 is the most potent compound encountered. The quinonoid hydroxyl group seems indispensable, for all activity is lost if it is replaced by OCH_3, H, CH_3, Cl, $NHCOCH_3$, or SH. Substitutions in the benzenoid ring (CH_3, Br, OCH_3) result in complete or extensive loss in activity. Substitution in the hydrocarbon side-chain of halogen oxygen, nitrogen or a double bond, invariably results in a drop in activity.



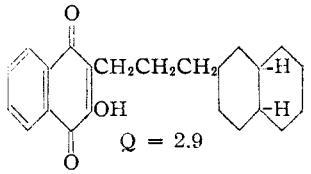
M-285 (SN-5949)



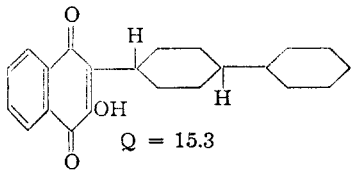
M-1916 (SN-5090)



M-297 (SN-8557)



M-2279 (SN-12320)



M-2293 (SN-13336)

Properties.—The typical hydroxynaphthoquinone with a C_9–C_{13} chain is a crystalline yellow solid, m. p. about 70 to 150°, moderately soluble in alcohol or ligroin and more soluble in benzene, ether or acetic acid, but very slightly soluble in water. Electrometric titrations in acetone–water mixtures (Table I) indicate a degree of acidity about the same as found for lapachol and phthiocol (pK^{30} 5.02 and 5.08[6]); the substances are about 1 pK unit weaker than 2-hydroxy-1,4-naphthoquinone (pK^{25} 3.98).[7] The oxidation–reduction potentials can be inferred[5,8] to be close to E_0^{30} (H_2O) = −0.300 v. The various members do not differ in absorption spectrum; the curves for M-1916 (Fig. 1) are typical (compare phthiocol[9]). The most active members resemble the higher fatty acids in acidic strength and molecular weight, and also in solubility and distribution characteristics. A typical compound dissolves readily in aqueous–alcoholic alkali to give a deep red solution of the sodium salt but is only sparingly soluble in aqueous alkali. On the other hand, the sodium salt dissolves freely in ether or in isoamyl alcohol and may be extracted from aqueous media by these solvents in the presence of excess sodium ion.

TABLE I

pK VALUES AT 25°

	50% Acetone	10% Acetone	Water
Acetic acid	5.85	4.84	4.72 (known)
Lapachol	6.32	4.94	5.02 (Ball,[6] 30°)
M-1916	6.67		
M-285	6.77		
M-266 (2-Hydroxy-3-cyclohexyl-1,4-naphthoquinone)	6.72		

Colorimetric Determination (See Paper XIX).—Aqueous and non-aqueous solutions of the red sodium salts can be analyzed by direct colorimetry in the region of optimum absorption (490 mμ). For the analysis of plasma, a sample is shaken with aqueous sodium hydroxide–sodium chloride and isoamyl alcohol and the color density of the alcoholic extract of sodium salt is measured;

(6) Ball, *J. Biol. Chem.*, **106**, 515 (1934); **114**, 649 (1936).
(7) Fieser and Fieser, THIS JOURNAL, **56**, 1565 (1934).
(8) Fieser, *ibid.*, **50**, 439 (1928).
(9) Crowe, *J. Biol. Chem.*, **115**, 479 (1936).

acetic acid is added to convert the red salt to the weakly absorbing yellow acid, a second reading is made, and the concentration is calculated from the known difference in absorptive power of the red and yellow forms. The results are independent of the presence of variable amounts of carotenoid pigments.

Distribution Characteristics (Paper XV).—An accurately determinable constant, *p*E, characterizing the hydrophilic–lipophilic properties of a hydroxyalkylnaphthoquinone is defined as the *p*H of an aqueous buffer capable of extracting 1/101-part of pigment from an equal volume of ether. In each series *p*E increases with increasing molecular weight, and the curves for the different series are approximately parallel. No difference is discernible between *n*- and iso-alkyl derivatives, but the effect of the introduction of one or two alicyclic rings or of an aryl group is to produce progressive shifts in the direction of increased hydrophilic character. These shifts follow closely the displacements in the peaks of antimalarial activity from series to series; for highest potency a naphthoquinone must have a balance between hydrophilic and lipophilic character represented by a *p*E in the range 10–12.

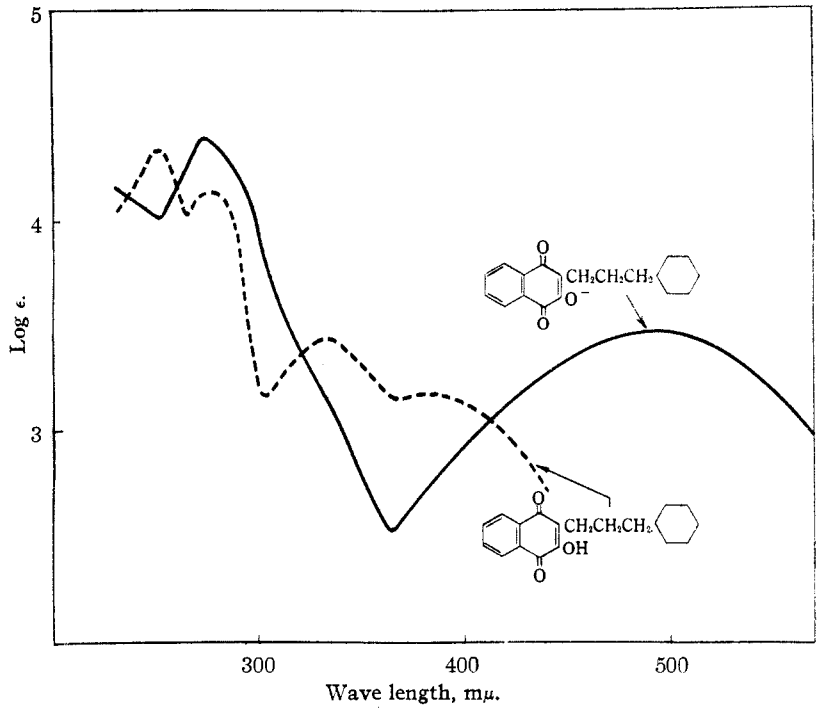


Fig. 1.—Absorption spectra of M-1916 and its anion in methanol, values for $\lambda_{max.}$ (and log ϵ values); free acid: 252(4.35), 279(4.15), 333(3.44), 382(3.18); anion: 274(4.41), 490(3.47).

Antirespiratory Activity (Papers XIX–XXII).—W. B. Wendel[10] discovered that typical hydroxyalkylnaphthoquinones are powerful inhibitors of respiratory systems. At a concentration in the order of 1×10^{-6} *M*, M-1916 is able to effect 50% inhibition of the respiration of parasitized red blood cells drawn from a duck infected with *P. lophurae*. Determinations of the relative antirespiratory activities of 76 naphthoquinones in Memphis and of 82 in Cambridge show that the *in vitro* test can be safely used for screening. The compounds interfere with carbohydrate metabolism and cause accumulation of lactic acid in the organisms.[10]

Action against Avian Infections.—The first indication that the naphthoquinones are endowed with more than the ability to suppress malarial infections in birds was the discovery in 1943 by J. H. Bauer of the Rockefeller Foundation that the substances are effective prophylactic agents.[4,11] Administration in adequate dosage prior to and for about five days after the inoculation of chickens with *P. gallinaceum* sporozoites provides complete protection. In 1944, W. G. Downes, Delphine H. Clarke, and Max Theiler of the Rockefeller Foundation[11] discovered that the naphthoquinone drugs effectively destroy the exoerythrocytic forms of malaria parasites found in the reticulo-endothelial cells of chickens infected with *P. gallinaceum*. Quinine and atabrine effectively destroy the trophozoites of the red blood cells but exert no control over the exoerythrocytic forms of parasites. Positive indications of curative action have been observed in three avian infections. Walker and Richardson[12] found that M-2279 potentiates the curative action of Pamaquine. The naphthoquinone is from one tenth to one twentieth as active as the 8-aminoquinoline in both supression and cure of *P. cathemerium* in ducks. A combination of one tenth the 100% curative dose of M-2279 plus one tenth the 100% curative dose of Pamaquine resulted in 100% cures. In suppressive experiments the combined effect of these agents is purely additive.

Other Actions.—Richardson tested some of the compounds for activity against relapsing fever (*P. novyi* in mice), *T. equiperdum* in mice, and *hemolytic streptococci* and found no activity in the highest dose used. Dr. R. J. Dubos found the naphthoquinones distinctly effective in inhibiting the growth of human tubercle bacilli H37Rv *in*

(10) Wendel, *Federation Proc.*, **5**, 406 (1946).

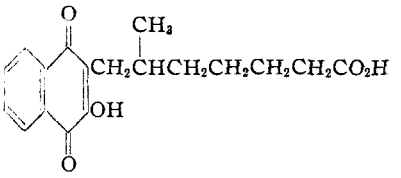
(11) Clarke and Theiler, *J. Infectious Diseases*, **82**, 138 (1948); Whitman, *ibid.*, **82**, 251 (1948).

(12) Walker and Richardson, *J. Nat. Mal. Soc.*, **7**, 4 (1948); Walker, Stauber and Richardson, *J. Infectious Diseases*, **82**, 187 (1948). Dr. W. Gingrich (private communication) has observed potentiation of Pentaquine by N-297 for cure of *P. cathemarium* in canaries.

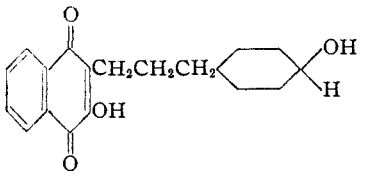
vitro; the effect is strongly counteracted by the presence of bovine albumin in the culture medium. C. C. Smith[13] observed that oral administration to rats produces a hemorrhagic syndrome; this is prevented to some extent by 2-methyl-1,4-naphthoquinone and more effectively prevented by vitamin K_1.

Clinical Trials.—For trials conducted in 1943 at the Goldwater Memorial Hospital on syphilitic patients undergoing malaria therapy,[4] M-285 and M-1916 were selected merely because they were the best available at the time at each coöperating laboratory. M-285 is four times as potent in the duck assay as M-1916 but proved to be completely inactive in five patients (blood-induced *P. vivax* and *P. falciparum*), whereas M-1916 produced a temporary suppression of fever extending for an average 9.5 days in eight cases. The effect was not satisfactory but enough to show that M-1916 has definite antimalarial activity in man. A clue to the reversal in activity was provided by an observation made by Dr. B. B. Brodie[4] in determining the plasma drug concentrations in the clinical subjects: whereas M-1916 is extracted from ligroin by alkali but not by bicarbonate, a considerable part of the red plasma pigment is extracted by bicarbonate.

Drug Degradation.—Studies at Harvard on plasma and urine extracts from non-malarial subjects given drug established that both M-285 and M-1916 are degraded very extensively and rapidly in the human organism (Paper XVIII). M-285 yields the carboxylic acid I, formed by the elimination of the terminal carbon atom and oxidation at the next position. The metabolite I is devoid of antirespiratory activity and several synthetic acids are inactive in this test and also in assays against *P. lophurae.* M-1916 is degraded to



I (M-285 metabolite)



II (M-1916 metabolite)

two isomeric secondary alcoholic derivatives, one of which has been identified by synthesis as the *trans*-4′-hydroxy derivative II. The hydroxyl group in the side chain causes a shift in *p*E from 9.45 to 6.40. These more hydrophilic substances are well tolerated by the organism and persist in the blood for several days. Early respiratory studies of the metabolites by Wendel failed to reveal activity, and assays of model compounds showed that a hydroxyl substituent markedly depresses antimalarial activity. The mild but definite therapeutic action of M-1916 in the clinical trials remained a mystery for two years, but continued to be the most promising clue to a solution of the problem. Efforts were made to isolate other metabolites that might be responsible for the therapeutic effect, analytical methods were sought for the detection of suspected products of desaturation, and the synthesis of possible metabolites was undertaken.

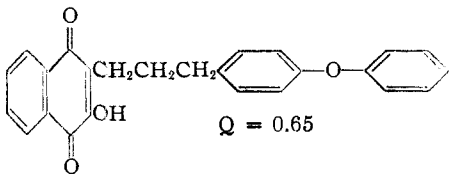
The metabolism of other members was also investigated in the hope of discovering a side chain more resistant to metabolic oxidation. The observation that hydrolapachol administered to humans is excreted abundantly in the urine in unchanged form accompanied by only a small amount of the γ-hydroxy derivative seemed at first to offer some hope, but the relative resistance of this compound of low molecular weight now seems attributable to its greater hydrophilic character. The more active higher homologs of the isoalkyl series were found to be metabolized largely to inactive carboxylic acids, and compounds with a normal alkyl group to suffer the same fate. The introduction of a terminal aryl group does not block degradation. These studies were conducted largely by the isolation and characterization of substances derived from urine extracts. When the decalylalkyl derivatives M-297 and M-2279 were recognized as substances of the M-1916 type but of higher potency against avian infections and were put through pharmacological processing and submitted to metabolism studies in human subjects, the procedure of isolation and chemical characterization of metabolites was recognized as inapplicable because only very minute amounts of naphthoquinone pigment are excreted in the urine. Consequently, in the spring of 1945 Wendel's procedure for the *in vitro* determination of drug activity was put into operation at Harvard for the more precise study of drug metabolism.

Determination of the Activity of Administered Drug (Paper XX).—The activity of a given pure drug relative to a standard (M-1916) is evaluated by determination in the Warburg apparatus of the concentration required to produce a 50% inhibition of the respiration of parasitized duck red blood cells. The course of the metabolic deactivation of a drug administered to a human subject either intravenously or orally is followed by drawing small blood samples from time to time, determining the naphthoquinone pigment, and determining the antirespiratory activity per colorimetric equivalent of pigment. A comparison of the activity of the plasma pigment with that of the drug administered gives an exact measure of the degree of degradation. A study of the activity of plasma extracts following intra-

(13) C. C. Smith, Fradkin and Lackey, *Proc. Soc. Exptl. Biol. Med.*, **61**, 398 (1946); C. C. Smith, *ibid.*, **64**, 45 (1947).

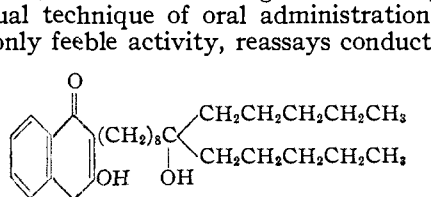
venous administration of M-1916 proved revealing. An extract taken one-half hour after the injection had only one-third the activity of the compound administered, and after four hours the activity reached a level of one-tenth that of M-1916 and then persisted at the same level for some twenty hours. The two hydroxylated metabolites that had been isolated were then found in repeated trials to possess antirespiratory activity one-tenth that of M-1916. It is concluded that the suppressive action of M-1916 observed in the clinical trials was due to the weakly active but persistent alcoholic metabolites. Degradation of the same type occurs in chickens and in rats, but much more slowly and to a lesser extent.

A hydroxyl group in the hydrocarbon side chain thus provides protection against metabolic attack. Although all information available at the time indicated that such substitution detracts very materially from the potency of the parent substances, the program of investigating all types of side chains eventually afforded results suggesting that the deactivating influence of oxygen substitution can be offset by an increase in the carbon content of the side chain. Thus the *p*-phenoxyphenylpropyl derivative M-2309, with a C_{15} side chain, possesses antimalarial activity in the practical range, and metabolism studies indicated that the substance is somewhat more resistant to metabolic deactivation than quinones with oxygen-free side chains. Compounds with very large hydroxylated



M-2309

side chains were then synthesized, and in the series with the side chains $-(CH_2)_8C(OH)R_2$ the antirespiratory activity was found to increase with increasing carbon content to a maximum of 2.2 times that of M-1916 in a compound with a C_{19} side chain, M-2350. Although initial assays by the usual technique of oral administration indicated only feeble activity, reassays conducted on



M-2350

the strength of the high antirespiratory activity disclosed that intramuscularly administered M-2350 possesses high potency. A quinone with an unoxygenated C_{19} alkyl side chain would be completely devoid of both antirespiratory and antimalarial activity, but is transformed into an active substance by hydroxyl substitution. The result can be attributed to the fact that the introduction of a hydroxyl group very greatly enhances the hydrophilic character of the molecule ($\Delta pE = 4$) and hence that a favorable distribution ratio is attained in the region of rather high molecular weight.

Metabolism studies in man and in various animals have shown that M-2350, the best of the oxygenated substances thus far investigated, is considerably more resistant to degradation than quinones with hydrocarbon side chains. The parenterally administered material affords satisfactory plasma levels of effective drug persisting for several hours, and hence M-2350 appears to have potentialities as a chemotherapeutic agent.

Nature of Drug Action (Papers XIX, XXII).—Plasma proteins, particularly albumin, bind the naphthoquinones in the form of dialysis-resistant complexes, and quinone so bound is less active in the inhibition of parasitized erythrocytes than free quinone. Differences found in susceptibility to antagonism by plasma proteins of different species probably account for species variations in the dosage required to produce a given response. A marked alternation in molar antirespiratory activity from homolog to homolog is noted in several of the series. Alternation also is found in susceptibility to human-protein antagonism, and the homologs of highest potency are the ones of greatest susceptibility. A competition for naphthoquinone appears to exist between plasma proteins and a respiratory enzyme, and the odd or even character of the side chain seems to promote firmness of binding to both proteins. The site of action is not clear, for no diffusion of naphthoquinone into the erythrocytes has been detected. Since hemoglobin does not exert an inhibitory action, the effect on respiration may be due to the diffusion of an amount of material too small for detection by the methods used.

NOTE ADDED TO PROOF, SEPTEMBER 23, 1948; Preliminary results of a clinical trial in progress at the American University of Beirut, Lebanon, are communicated by Dr. George Fawaz as follows: "Two patients with primary *vivax* infection were given 2 g. of M-2350 per day for four days by intravenous injection in gelatin solution. The results were dramatic. The fever stopped a few hours after the first injection and the patients left the hospital in perfect condition with no parasites in the blood. Both are still without relapses since discharge seven and eight weeks ago, respectively."

Summary

This first paper in a series of twenty-two is a general survey of the history, progress, and present status of the research as a whole. Since it constitutes an abstract of several of the individual researches, special summaries will be omitted from the papers in question.

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