Racemic 5-Ethyl-5-(3-hydroxy-1-methylbutyl)barbituric Acid (II).—Upon standing for a few days in a refrigerator the filtrate from the above procedure deposited 250 mg of light brown long blunt needles, mp 170–181°. After decolorization with charcoal in EtOH, repeated precipitation by heptane from Me_2CO, and recrystallization (H_2O), the product melted at 188–189°, $[\alpha]^{28}D$ 0 ± 0.1° (1.5, AcOH, $\lambda_{max}^{0.5NNaOH}$ 255 mμ (ε 6600). *Anal.* C, H, N.

The compound displayed a positive iodoform test.[8] Its ir spectrum was identical with that of the *d* alcohol[3] but different from that of the *l* alcohol.[3] It depressed the melting points of both optically active alcohols but not that of synthetic 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid.[2]

Chromatography.—The ω-1 metabolites of pentobarbital were examined in four systems (Table I). The dried paper chromatograms were sprayed with 0.5 *N* NaOH and inspected in uv light. The dried thin layer plates were sprayed with a saturated solution of $HgNO_3$ to reveal the barbiturates as shiny, grayish white spots.[9] In all systems mixtures of the *d* and racemic alcohols yielded only one spot, whereas mixtures involving the *l* alcohol yielded two spots.

TABLE I
R_f VALUES OF
5-ETHYL-5-(3-HYDROXY-1-METHYLBUTYL)BARBITURIC ACID

Metabolite		System[a]			
Mp, °C	$[\alpha]^{28}D$, deg	A	B	C	D
209–210	+26.6	0.63	0.58	0.63	0.44
152–153	−5.6	0.58	0.54	0.58	0.41
188–189	0	0.63	0.58	0.63	0.44

[a] A, *i*-PrOH–28% aqueous NH_3 (4:1), Whatman No. 1; B, *n*-BuOH saturated with 0.5% aqueous NH_3, Whatman No. 1; C, *i*-PrOH–28% aqueous NH_3 (4:1), thin layer silica gel G; D, $CHCl_3$–Me_2CO (1:1), thin layer silica gel G.

Racemic 5-Ethyl-5-(3-acetoxy-1-methylbutyl)barbituric acid was prepared and purified in the usual manner[3] and melted at 136–137°. *Anal.* C, H, N.

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Potential Antimalarial Agents. Derivatives of 2-Chloro-1,4-naphthoquinone

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The chemotherapy of malaria has been stimulated by the use of several classes of effective compounds such as quinolines, naphthoquinones, and sulfones, but it has been limited appreciably by the toxicity of the various chemicals. Boldt and Goodwine[1] reported extensive studies with chloroquine as an antimalarial agent. Ter Horst and Felix[2] demonstrated the high fungistatic activity of 2,3-dichloro-1,4-naphthoquinone; Fosdick, *et al.*,[3] found that 1,4-naphthoquinones were useful inhibitors of acid formation by oral bacteria, and Fieser[4] reported naphthoquinones as potential antimalarials. DeGowin, *et al.*,[5] then showed that 4,4′-diaminodiphenyl sulfone possessed high antimalarial activity. For a number of years, we have also synthesized and studied the effects of a number of naphthoquinone derivatives as potential chemotherapeutic agents. The results have indicated that certain amine derivatives of 2-chloro-1,4-naphthoquinone may possess significant antibacterial activity *in vitro*. This study stimulated our interest in the possibility of these amine derivatives as potential antimalarial agents. The chemical structures of the amine derivatives are shown in Figure 1. The present report includes the synthesis and evaluation of 64 compounds, with analyses and tests for acute toxicity in mice for *in vivo* antimalarial activity against *Plasmodium berghei* infection in mice.

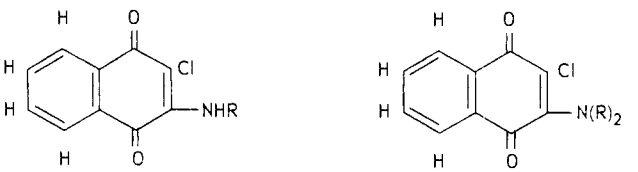

Figure 1.—Amine derivatives of 2-chloro-1,4-naphthoquinone: I, primary amine-substituted derivative, RNH = component added; II, secondary amine-substituted derivative, $(R)_2N$ = component added.

Experimental Section

General Procedure.—All the amine compounds employed were commercial preparations. The new amine derivatives were prepared by condensation of 2,3-dichloro-1,4-naphthoquinone with various primary and secondary amine compounds as follows. A 0.1-mole amount of 2,3-dichloro-1,4-naphthoquinone suspended in warm 95% EtOH (200 ml) was mixed with excess amine (0.2 mole) in EtOH (50 ml) and refluxed gently. Excess amine was employed in the system to neutralize the liberated HCl. The mixtures turned red. The basic aliphatic amines condensed readily on refluxing for 30 min. In most instances, the red crystalline product precipitated from the warm reaction mixture. Equimolecular quantities of the sulfones, amino acids, and pyridine compounds with the 2,3-dichloro-1,4-naphthoquinone had to be refluxed 15–18 hr for condensation to take place. After cooling, the insoluble condensation products were filtered and crystallized (70% EtOH). The products were fine shiny crystalline compounds obtained in yields from 70–95%. See Table I.

Acute Toxicity.—Toxicity studies on the compounds were performed in the DBA strain of mice, as maintained at the National Institutes of Health, Bethesda, Md. The chemicals were suspended in 0.25% Methocel (Methylcellulose, Dow Chemical Co., Midland, Mich.) so that the dose per 20-g mouse was contained in 0.25 ml for subcutaneous injection and the results were judged by 72-hr survival. The tolerated dose of the various preparations ranged from 500 to 2000 mg/kg. Most of the compounds were of relatively low toxicity as compared with 2,3-dichloro-1,4-naphthoquinone. The highest dose of the 2,3-dichloro-1,4-naphthoquinone tolerated by DBA mice was 250 mg/kg.

Antimalarial Activity.—All of the derivatives of 2-chloro-1,4-naphthoquinone as well as the positive control chloroquine diphosphate were evaluated subcutaneously for antimalarial activity in *Plasmodium berghei* infected mice by Dr. Leo Rane of the University of Miami. The testing procedure employed has been described preciously.[6] Among the 64 compounds, bis[2-chloro-1,4-naphthoquinone-3,3′-sulfonylbis(*p*-phenylenimine)] (I) and N^4-(2-chloro-1,4-dihydro-1,4-dioxo-2-naphthyl)sulfanilamide (II) were found to possess high antimalarial activity against this parasite, as evidenced by the curative effect of I with survival of one, three, and two of five infected mice for 60 days in the mice that received doses of 40, 80, and 160 mg/kg, respectively. Higher doses of 320 and 640 mg/kg were toxic. Compound II was curative at 320, 640, and 1280 mg/kg with four, five, and

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TABLE I
CHEMICAL AND PHYSICAL PROPERTIES OF AMINE DERIVATIVES (RNH_2) OF 2-CHLORO-1,4-NAPHTHOQUINONE

Amine deriv	Mp, °C[a]	Formula[b]
Methyl	101	$C_{11}H_8ClNO_2$
Propyl	110–112	$C_{13}H_{12}ClNO_2$
Isopropyl	117	$C_{13}H_{12}ClNO_2$
Allyl	149–150	$C_{13}H_{10}ClNO_2$
Butyl	112	$C_{14}H_{14}ClNO_2$
Isobutyl	113	$C_{14}H_{14}ClNO_2$
sec-Butyl	82–83	$C_{14}H_{14}ClNO_2$[c]
t-Butyl	112–113	$C_{14}H_{14}ClNO_2$
Pentyl	97–98	$C_{15}H_{16}ClNO_2$
Heptyl	91	$C_{17}H_{20}ClNO_2$
n-Octyl	86	$C_{18}H_{22}ClNO_2$
Nonyl	96	$C_{19}H_{24}ClNO_2$
Dodecyl	89	$C_{22}H_{30}ClNO_2$
Hexadecyl	88–92	$C_{26}H_{38}ClNO_2$
Octadecyl	98	$C_{28}H_{42}ClNO_2$
Methoxyethyl	86	$C_{13}H_{12}ClNO_3$
Methoxypropyl	79	$C_{14}H_{14}ClNO_3$
Methoxyisopropyl	75–76	$C_{14}H_{14}ClNO_3$
3-*n*-Butoxypropyl	46–47	$C_{17}H_{20}ClNO_3$
$3\text{-}Me_2N(CH_2)_3$	55	$C_{15}H_{17}ClN_2O_2$
$H_2N(CH_2)_4NH$	99–101	$C_{23}H_{16}Cl_2N_2O_4$
$H_2N(CH_2)_6NH$	170–171	$C_{26}H_{22}Cl_2N_2O_4$
Ethoxyethoxyethyl	79–80	$C_{16}H_{18}ClNO_4$
Ethoxyethoxypropyl	45–46	$C_{17}H_{20}ClNO_4$
Ethoxyethoxyethoxypropyl	73	$C_{19}H_{24}ClNO_5$
Di(methoxyethyl)	92–95	$C_{16}H_{18}ClNO_4$
Di(ethoxyethyl)	51–53	$C_{18}H_{22}ClNO_4$
n-Aminopropyldiethanol	62–65	$C_{17}H_{23}ClN_2O_4$
Dicyclopentyl	65–68	$C_{26}H_{32}ClNO_2$
Di-*n*-hexyl	160–162	$C_{22}H_{38}ClNO_2$
Di-*n*-decyl	159	$C_{30}H_{46}ClNO_2$
Di-*n*-dodecyl	171	$C_{34}H_{54}ClNO_2$
Methyliminobispropyl	115	$C_{27}H_{25}Cl_2N_3O_4$
3,3'-Iminobispropyl	68	$C_{36}H_{36}Cl_2N_2O_6$
Benzyl	245–247	$C_{17}H_{12}ClNO_2$
p-Anisidine	205	$C_{17}H_{12}ClNO_3$
Phenetidine	239–241	$C_{18}H_{14}ClNO_3$
Thymyl	254	$C_{20}H_{18}ClNO_2$
p-Aminobenzenesulfonic acid	>300	$C_{16}H_{10}ClNO_5S$
Sulfanilamide	>300	$C_{16}H_{14}ClN_3O_4S$
Sulfapyridine	260	$C_{21}H_{17}ClN_4O_4S$
4,4'-Diaminodiphenyl sulfone	192–194	$C_{32}H_{18}Cl_2N_2O_8S$
n-Aminomorpholine	132	$C_{14}H_{13}ClN_2O_3$
n-Aminopropylmorpholine	159	$C_{17}H_{19}ClN_2O_3$
2,6-Dimethylmorpholine	126–129	$C_{18}H_{18}ClNO_3$
Piperazine	250	$C_{24}H_{16}Cl_2N_2O_4$
1-Methylpiperazine	220–225	$C_{15}H_{13}ClN_2O_2$
n-Aminoethylpiperazine	240	$C_{26}H_{22}Cl_2N_4O_4$
Bis(aminopropyl)piperazine	200–204	$C_{30}H_{30}Cl_2N_4O_4$
2-Hydrazino-5-nitropyridine	220	$C_{11}H_8ClN_5O_4$
2-Aminopyridine	275	$C_{15}H_9ClN_2O_2$
2-Aminothiazole	165–168	$C_{19}H_7ClN_2O_2S$
α-Naphthyl	170	$C_{20}H_{12}ClNO_2$
8-Aminoquinoline	289	$C_{19}H_{12}ClN_2O_2$
Furfuryl	134–136	$C_{15}H_{10}ClNO_3$
Imidazole	191–193	$C_{13}H_7ClN_2O_2$
2-Hydrazinobenzothiazole	204	$C_{17}H_{10}ClN_3O_2S$
Glutaric dihydrazide	170–173	$C_{23}H_{18}Cl_2N_4O_6$
1,4-Dihydrazinophthalazine	192–194	$C_{25}H_{16}Cl_2N_8O_4$
5-Aminoindazole	267–270	$C_{17}H_{10}ClN_3O_2$
6-Aminoindazole	252–255	$C_{17}H_{10}ClN_3O_2$
2-Aminoethylphosphonic acid	198	$C_{12}H_{10}ClNO_5P$
Glycine	174	$C_{12}H_8ClNO_4$
Hydrazine	>300	$C_{20}H_{16}Cl_2N_2O_4$

[a] All melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus. [b] All compounds were analyzed for C, H, N (±0.3% limit), except where indicated otherwise. [c] H: calcd, 5.35; found, 5.61.

TABLE II
ANTIMALARIAL ACTIVITY OF CERTAIN DERIVATIVES OF 2-CHLORO-1,4-NAPHTHOQUINONE AGAINST *Plasmodium berghei* IN MICE

Compd	Dose, mg/kg	Mean survival time, days[a]	Increase in survival time, days	Mortality	Remarks
I	20	14.4	7.1	5/5	
	40	24.8	17.5	4/5	1 mouse survived 60 days
	80	44.4	37.1	2/5	3 mice survived 60 days
	160			2/5	2 toxic deaths,[b] 1 mouse survived 22 days, 2 mice survived 60 days
	320			4/5	4 toxic deaths, 1 mouse survived 60 days
	640			4/5	4 toxic deaths, 1 mouse survived 60 days
II	40	13.4	7.2	5/5	
	80	15.8	9.6	5/5	
	160	16.4	10.2	5/5	
	320	14.0	7.8	1/5	4 mice survived 60 days
	640			0/5	5 mice survived 60 days
	1280			1/5	1 toxic death, 4 mice survived 60 days
Chloroquine diphosphate	40	11.0	4.0	5/5	
	80	12.8	5.8	5/5	
	160	17.0	10.0	5/5	
	320	24.0	17.0	5/5	2 toxic deaths
	640		0	5/5	5 toxic deaths

[a] Mean survival time of controls: 7.3 for I, 6.2 for II, and 7.0 for chloroquine diphosphate. [b] Deaths due to toxicity of drug occur in 3–5 days. Mice surviving 60 days are considered cures.

four mice, respectively, surviving in each group for 60 days with no evidence of toxicity. The results of the antimalarial activity of the two active compounds and of the control drug chloroquine diphosphate, supplied by Dr. David P. Jacobus of the Walter Reed Army Institute of Research, are summarized in Table II. Initial blood studies in normal white mice have indicated that the chemical structures of these complex molecules made them difficult to cleave, since no detectable amounts of the original components were found present in the blood.

Synthesis of Potential Antineoplastic Agents. XX. Compounds Related to the 3-*o*-Nitrophenylhydrazone of Isatin[1]

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In connection with other work in progress in this laboratory we synthesized the title compound (I) by the condensation of isatin and *o*-nitrophenylhydrazine.

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