spectively. VIII was prepared also by the demethylation of VII using concentrated HBr under reflux.

The various substituted derivatives of 3-amino-3,4-dihydrocarbostyril thus prepared were isolated as their HCl salts. These compounds decomposed at their melting points and gave a positive ninhydrin reaction. Unlike the lactams VII and VIII, the cyclic hydroxamates III and IV gave the characteristic violet color with $FeCl_3$.

The microbiological activities of III, IV, VII, and VIII compared to that of the previously reported 3-amino-3,4-dihydro-1-hydroxycarbostyril[4] using *E. coli* 9723 and *L. dextranicum* 8086 as the test organisms are shown in Table I. In each case, the 1-hydroxy substituted compound is completely inhibitory to the growth of both microorganisms at a concentration level of 2 μg/ml. Of the other 3-amino-3,4-dihydrocarbostyrils tested, only the 7-methoxy derivative showed appreciable biological activity and inhibited the growth of *E. coli* and *L. dextranicum* at concentration levels of 60 and 200 μg/ml, respectively.

TABLE I
RELATIVE MICROBIOLOGICAL ACTIVITIES OF SOME SUBSTITUTED 3-AMINO-3,4-DIHYDROCARBOSTYRILS

Substituted 3-amino-3,4-dihydrocarbostyrils	Microorganism, μg/ml[a]	
	E. coli	*L. dextranicum*
3-Amino-3,4-dihydro-1-hydroxycarbostyril	2	2
III	2	2
IV	2	2
VII	60	200
VIII	>200[b]	>200[b]

[a] Minimal concentration required for complete inhibition of growth. [b] Maximum concentration at which compound was tested in the assay medium.

This preliminary study of the microbial activity of the carbostyril compounds in two microorganisms showed that the more inhibitory derivatives were uniformly those containing the cyclic hydroxamate linkage, –N(OH)–CO–.

Experimental Section[6]

3-Amino-3,4-dihydro-1-hydroxy-7-methoxycarbostyril·HCl (III).—A solution of 500 mg of I[5] in 75 ml of 50% aq MeOH was acidified to pH 1.0 by the dropwise addition of concd HCl and then treated with an aq slurry of 50 mg of Pt black. The resulting mixture was agitated under 3.18 kg/cm² of H_2 for 30 min at room temperature. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to cause precipitation. The solid was filtered to yield 300 mg (58%) of III, mp 246° dec. The product gave an intense purple color with 10% $FeCl_3$ solution and R_f values of 0.38 and 0.89 in 1-BuOH–AcOH–H_2O (3:1:1) and 65% pyridine, respectively; major ir absorption bands, 3.3–3.5 (broad), 6.0, 6.7–6.8, 7.8, and 9.7 μ. *Anal.* ($C_{10}H_{12}N_2O_3$·HCl) C, H, N.

3-Amino-3,4-dihydro-7-methoxycarbostyril·HCl (VII).—A 500-mg sample of V[5] was suspended in 30 ml of H_2O, and solution was effected by adjusting the pH to 1 by the dropwise addition of 2 *N* HCl. After the solution was heated for 20 min, powdered charcoal was added and the mixture was filtered. On concentrating the filtrate to 10 ml by evaporation of the solvent *in vacuo*, a white solid precipitated. There was obtained 340 mg (63%) of VII, mp 296–297°; R_f values 0.38 and 0.88 in 1-BuOH–AcOH–H_2O (3:1:1) and 65% pyridine, respectively; major ir absorption bands, 3.5, 5.9, 6.7, 6.9, 7.8, 8.6, 9.7, and 11.6 μ. *Anal.* ($C_9H_{12}N_2O_2$·HCl) C, H, N.

3-Amino-3,4-dihydro-1,7-dihydroxycarbostyril·HCl (IV).—To a solution of 4.1 g of II·HCl[5] in a minimal amount of hot 50% aq MeOH was added an aqueous slurry of 50 mg of Pt black and 0.5 ml of concd HCl. The mixture was shaken under 3.18 kg/cm² of H_2 for 1.5 hr at room temperature. After removal of the catalyst by filtration, the product was recovered from the filtrate by precipitating with the addition of an equal vol of concd HCl to yield 2.8 g (75%) of IV, dec at 260–272°. This compound gave an intense purple color with 10% $FeCl_3$ solution and R_f values of 0.27 and 0.90 in 1-BuOH–AcOH–H_2O (3:1:1) and 65% pyridine, respectively; major ir absorption bands, 3.0–3.5 (broad), 6.0, 6.2, 6.8, and 7.7 μ. *Anal.* ($C_9H_{10}N_2O_3$·HCl) C, H, N.

3-Amino-3,4-dihydro-7-hydroxycarbostyril·HCl (VIII). Method A.—A mixture of 2.1 g of VI[5] in 50 ml of 95% EtOH and 50 ml of concd HCl was heated with stirring. The solution was evaporated to dryness by removal of the solvent under reduced pressure. The residue was dissolved in 95% EtOH, and powdered charcoal was added. After removal of the charcoal by filtration, the solvent was evaporated to 0.1 of its original vol and the product which separated was recovered to give 1.6 g (70%) of VIII, mp 297–298° dec. In 1-BuOH–AcOH–H_2O (3:1:1) and 65% pyridine, the R_f values were 0.27 and 0.88, respectively; major ir absorption bands, 2.9–3.5 (broad), 5.9, 6.7, 7.1, and 7.6 μ. *Anal.* ($C_9H_{10}N_2O_2$·HCl) C, H, N.

Method B.—A sample of 150 mg of VII was refluxed in 50 ml of 48% HBr for 12 hr. The reaction mixture was reduced to dryness by removal of the acid *in vacuo* leaving a residue which was dissolved in a minimal amount of H_2O. The resulting solution was neutralized by the addition of concentrated NH_4OH, and after chilling in the refrigerator, a solid formed. The solid was filtered, washed with cold H_2O, and dissolved in 2 ml of concd HCl. Upon cooling in the refrigerator, there was obtained 60 mg (35%) of VIII. The melting point, R_f values, and ir spectrum of this sample were identical with those of the product prepared by method A.

Microbiological Assays.—For *E. coli* 9723, a previously described inorganic salts medium[7] was employed, and the organism was incubated at 37° for about 16 hr. For *L. dextranicum* 8086, the same assay procedure was employed as previously reported.[4] The carbostyril derivatives were dissolved in sterile H_2O and added aseptically to the previously autoclaved tubes. In all assays the amount of growth was determined photometrically at 625 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set at zero absorbance.

(6) All melting points are corrected. The R_f values were determined using the ascending technique of paper chromatography in the solvents indicated, and ninhydrin reagent was used for development of the spots. Uv absorption spectra were obtained on a Bausch and Lomb Spectronic 505 recording spectrophotometer, at concentrations of 10 μg/ml in aq solutions at pH 2, 7, and 10 in the 200–350-mμ region. The pH of each sample was adjusted by the dropwise addition of concd HCl or 2 *N* NaOH. Ir spectra were determined using a Beckman IR-8 spectrophotometer (KBr). Elemental analyses were performed by the M-H-W Laboratories, Garden City, Mich. Where analyses are indicated only by symbols of the elements, analytical results obtained by those elements were within ±0.4% of the theoretical values.

(7) E. H. Anderson, *Proc. Nat. Acad. Sci. U. S.*, **30**, 120 (1946).

Antiprotozoal Quinones. IV. 2-Amino-1,4-naphthoquinone Imines as Potential Antimalarials[1]

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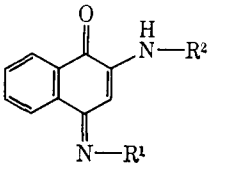
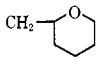
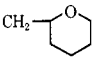
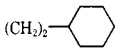
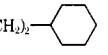
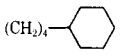
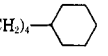
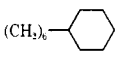
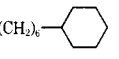
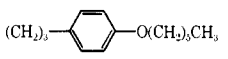
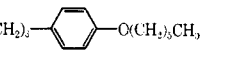
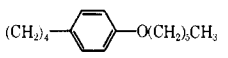
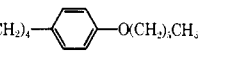
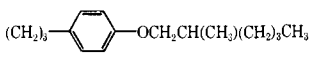
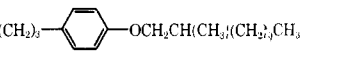
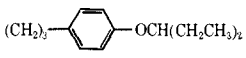
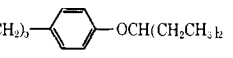
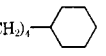
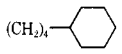
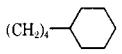
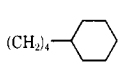
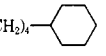
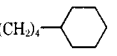
Received October 27, 1969

Investigation of the chemical and biological properties of the 2-amino-1,4-naphthoquinone imines (1) has been rather limited. Several of these quinones have

(1) This work, which was carried out under Contract DA-49-193-MD-2880 with the U. S. Army Medical Research and Development Command, was presented in part at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April, 1968. This paper is Contribution No. 326 from the Army Research Program on Malaria.

TABLE I
N²,N⁴-DISUBSTITUTED 2-AMINO-1,4-NAPHTHOQUINONE IMINES



No.	R¹	R²	Mp, °C	Yield (%)	Formula
3	CH_3	CH_3	132–133 dec	22	$C_{12}H_{12}N_2O$
4	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	114–115 dec	41	$C_{16}H_{20}N_2O$
5	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	93–93.5	24	$C_{18}H_{24}N_2O$
6	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	64–64.5	24	$C_{22}H_{32}N_2O$
7	$(CH_2)_{10}CH_3$	$(CH_2)_{10}CH_3$	53.5–54.5	24	$C_{32}H_{52}N_2O$
8	$(CH_2)_{11}CH_3$	$(CH_2)_{11}CH_3$	52.5–53	22	$C_{34}H_{56}N_2O$
9	$(CH_2)_{13}CH_3$	$(CH_2)_{13}CH_3$	53–53.5	27	$C_{38}H_{64}N_2O$
10	$CH(CH_3)(CH_2)_3CH(CH_3)_2$	$CH(CH_3)(CH_2)_3CH(CH_3)_2$	55–56	13	$C_{26}H_{40}N_2O$
11			116–117	31	$C_{22}H_{28}N_2O$
12			175–176	19	$C_{24}H_{27}N_2O$
13			75–76	39	$C_{22}H_{28}N_2O_3$
14			116.5–117	18	$C_{26}H_{36}N_2O$
15			85–86	30	$C_{30}H_{44}N_2O$
16			77–78	22	$C_{34}H_{52}N_2O$
17			73–74	21	$C_{40}H_{52}N_2O_3$
18			83–84	24	$C_{42}H_{56}N_2O_3$
19			61–62	33	$C_{46}H_{52}N_2O_3$
20			67–68	24	$C_{38}H_{49}N_2O_3$
21		$(CH_2)_5CH_3$	88.5–89	83	$C_{20}H_{26}N_2O$
22			103.5–104.5	54	$C_{26}H_{36}N_2O$
23		$(CH_2)_3CH_3$	80–81	56	$C_{24}H_{34}N_2O$
24		$(CH_2)_5CH_3$	74.5–75	67	$C_{26}H_{38}N_2O$
25	$(CH_2)_{10}CH_3$	$(CH_2)_5CH_3$	54–55	30	$C_{27}H_{42}N_2O$
26	$CH(CH_3)(CH_2)_3CH(CH_3)_2$	C_6H_5	123–124	63	$C_{24}H_{28}N_2O$
27		C_6H_5	103.5–104.5	46	$C_{26}H_{30}N_2O$
28	C_6H_5	$(CH_2)_2N(CH_2CH_3)_2$	82.5–83	40	$C_{22}H_{25}N_3O$
29	C_6H_5	$(CH_2)_3N(CH_3)$	85.5–86.5	27	$C_{21}H_{23}N_3O$
30	C_6H_5	$(CH_2)_5CH_3$	82.5–83.5	49	$C_{22}H_{24}N_2O$[a]
31	C_6H_5		103–103.5	64	$C_{26}H_{30}N_2O$
32	p-$CH_3OC_6H_4$		106.5–107.5	78	$C_{27}H_{32}N_2O_2$

[a] C: Calcd, 79.48; Found, 79.04.

been obtained incidental to other chemical studies,[2] while activity against *Mycobacterium tuberculosis* in mice has been claimed for a few others.[3] The structural relationship of **1** to the 2-hydroxy-1,4-naphthoquinone antimalarials[4] encouraged us to begin a systematic study of these quinone imines for possible antimalarial activity. In our recent chemical study of **1**[5] we de-

(2) *Cf.* T. Zincke and E. Kiegel, *Ber.*, **21**, 1039 (1888); L. F. Fieser and C. K. Bradsher, *J. Amer. Chem. Soc.*, **61**, 417 (1939); V. Calo and P. E. Todesco, *Chem. Commun.*, 571 (1968).

(3) J. P. English and R. C. Clapp, U. S. Patent 2,769,820 (1957), *Chem. Abstr.*, **51**, 8141h (1957); J. H. Clark and J. P. English, U. S. Patent 2,726,821 (1957), (*Chem. Abstr.*, **51**, 8141i (1957)).

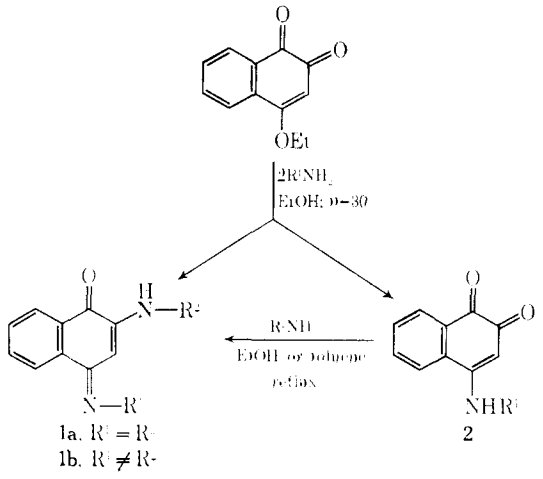
(4) L. F. Fieser, J. P. Schirmer, S. Archer, R. L. Lorenz, and P. I. Pfaffenbach, *J. Med. Chem.*, **10**, 513 (1967); L. F. Fieser, M. Z. Nazer, S. Archer, D. A. Berberian, and R. G. Slighter, *ibid.*, **10**, 517 (1967) and ref therein.

(5) F. J. Bullock, J. F. Tweedie, and D. D. McRitchie, *J. Chem. Soc. C*, 1799 (1969).

tailed several facile syntheses. We now wish to report the preparation and results of an evaluation of the series in Table I.

The synthetic routes used here are outlined in Scheme I. For the compounds **1a** the route from 4-ethoxy-1,2-naphthoquinone[6] was employed, which gives in addition the 4-alkylamino-1,2-naphthoquinone **2**.[7] For synthesis of **1b** the starting point was the appropriate 4-alkyl- or arylamino-1,2-naphthoquinone **2**. With this latter reaction limitations are imposed by the thermal instability of some compounds.

SCHEME I



The new compounds in Table I have been evaluated against *Plasmodium berghei* infected mice, *P. gallinaceum* infected chicks and against the sexual phase of *P. gallinaceum* in mosquitoes (*Aedes aegypti*).[8] None of the compounds evaluated showed any activity in chicks or mosquitoes, but unmistakable activity was found in mice. Compound **15** increased the mean survival time of infected mice 5.4 days at 640 mg/kg, 2.7 days at 160 mg/kg, and 1.7 days at 40 mg/kg with no toxicity; **22** increased the mean survival time an average of 3.2 days at 640 mg/kg, also with no toxicity. Quinones bearing hydrophilic groups (**13**, **28**, **29**) showed toxicity. The other quinone imines were essentially inactive and no clear structure-activity relationships emerged within this series except for the possibly coincidental fact that both **15** and **22** contain a cycloalkylalkyl chain reminiscent of that found in the side chain of the most active 2-hydroxy-1,4-naphthoquinones.[4]

Experimental Section

All melting points were obtained with a Fisher-Johns apparatus and are uncorrected. Ir (KBr pellets), uv, and nmr spectra were as expected for assigned structures. Nmr spectra were also discussed in our previous report.[5] All compounds were analyzed for C, H, N and were within ±0.4% of the theoretical values unless otherwise indicated. Analyses were carried out by Galbraith Laboratories, Knoxville, Tenn., or Dr. S. N. Nagy, Massachusetts Institute of Technology, Cambridge, Mass.

2-Amino-1,4-naphthoquinone Imines. The series **1a** was prepared by method A described in detail in the Experimental Section of our recent paper.[5] The series **1b** was prepared by method E of the same paper. All the compounds of Table I were recrystallized from EtOH except for quinones **26** and **27** where petroleum ether (60–90°) was used. The amines used here were commercial materials or prepared as described previously.[5]

Substituted *N*-Phenylanthranilic Acid Hydrazides as Potential Antimalarial and Antimicrobial Agents[1]

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Received November 29, 1969

In view of the current interest in antifolics as antimalarials[2] and because of the role of *p*-aminobenzoic acid (PAB) in folic acid synthesis,[3] we have prepared several of the title compounds, congeners of the *ortho* isomer, anthranilic acid, of PAB, for antimalarial evaluation. Since anthranilic acid is reported to be a metabolite of tryptophan for certain microorganisms,[4] (*e.g.*, *Escherichia coli*[4]) and in view of the numerous reports of antimicrobial activity of anthranilic acid derivatives[5] the title compounds were evaluated for microbial inhibition. The substituted anthranilic acids required as starting materials for these compounds were available from a previous investigation of substituted dibenz[*b*,*f*]azepines as potential antimalarials.[6] The anthranilic acids were esterified and subsequently treated with hydrazine to give the desired hydrazides as described in the Experimental Section.

The compounds listed in Table I were screened for antimalarial activity against *Plasmodium berghei* in mice by the method of Rane, *et al.*,[7] by the Walter Reed Army Institute of Research. The compounds were also tested against *P. gallinaceum* in mosquitoes.[8] Only slight activity is observed in the mouse screen. The longest increase in survival time noted, 1.3 days at a dose of 320 mg/kg, occurred when the animals were treated with **4**. Supression of oocysts as well as some toxicity was observed for most compounds in the mosquito screen. We are indebted to Drs. D. P.

(1) We acknowledge the U. S. Army Medical Research and Development Command under Contract No. DADA17-68-C-8035 for partial support of this work. This is Contribution No. 740 from the Army Research Program on Malaria.

(2) (a) B. S. Hurlbert, R. Ferone, T. A. Herrmann, G. H. Hitchings, M. Barnett, and S. R. M. Bushby, *J. Med. Chem.*, **11**, 711 (1968); (b) E. F. Elslager, Z. B. Gavrilis, A. A. Phillips, and D. F. Worth, *ibid.*, **12**, 357 (1969); (c) *cf.* P. B. Russell in "Medicinal Chemistry," 2nd ed, A. Burger, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, Chapter 42.

(3) A. White, P. Handler, and E. L. Smith, "Principles of Biochemistry" 4th ed, New York, N. Y., McGraw-Hill Co., 1968, p 1034.

(4) (a) C. Yanofsky, *J. Biol. Chem.*, **224**, 783 (1957); (b) ref 3, pp 574–575.

(5) N. D. Heindel, T. F. Lemke, S. M. Lemke and V. B. Fish, *J. Med. Chem.*, **11**, 369 (1968) and ref cited therein.

(6) R. S. Varma, L. K. Whisonant, and D. W. Boykin, Jr., *ibid.*, **12**, 913 (1969).

(7) T. S. Osdene, P. B. Russell, and L. Rane, *ibid.*, **10**, 431 (1967).

(8) E. J. Gerberg, L. T. Richard, and J. B. Poole, *Mosquito News*, **29**, 359 (1966).

(6) L. F. Fieser, *J. Amer. Chem. Soc.*, **48**, 2922 (1926).

(7) Antiprotozoal Quinones. II. F. J. Bullock, J. F. Tweedie, D. D. McRitchie, and M. A. Tucker, *J. Med. Chem.*, **13**, 97 (1970).

(8) Evaluation was carried out by the methods detailed in our previous paper, Antiprotozoal Quinones. III. F. J. Bullock, and J. F. Tweedie, *J. Med. Chem.*, **13**, 261 (1970).