Synthesis of Potential Antimalarials: Primaquine Analogs

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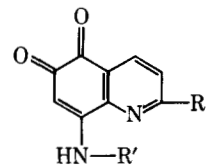
Abstract □ A series of 8-aminoquinolines, analogous to primaquine and carrying a substituent in position two, have been synthesized by standard methods and tested for activity against *Plasmodium berghei* in mice. The results of preliminary biological tests are reported.

Keyphrases □ 8-Aminoquinolines—synthesis □ Antimalarial activity—8-aminoquinolines □ TLC—separation □ IR spectrophotometry—identity

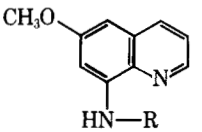
Quinoline quinones may offer a lead in the search for better antimalarials, since the mechanism of action for the important 6-methoxy-8-aminoquinolines appears to involve their *in vivo* conversion to the quinoline-*o*-quinone (1, 2). Pamaquine-5,6-quinone (I) is apparently identical with a product isolated from the feces of chickens which have been fed the drug (3, 4), and this metabolite has been reported to be 16 times as active as the parent *in vitro* (5, 6). It is likely, therefore, that the antiplasmodial activity (*Plasmodium gallinaceum*) was associated with the metabolic intermediate. Thus, the high *in vitro* antimalarial activities of 6-hydroxypentaquine, pentaquine-5,6-quinone, and pamaquine-5,6-quinone, in contrast to the *in vitro* inactivity of pentaquine (II) and pamaquine (III), lend support to Schonhofer's theory (7–9) that the *in vivo* action of the 8-aminoquinolines upon the erythrocytic or tissue stages of malaria plasmodia is due to the quinonoid products to which these drugs are converted by the host.

Much of the early work directed toward the synthesis of various 5,6-quinones was apparently abandoned because of compound instability. However, a clue to a technique for stabilizing the quinoline-*o*-quinone may be found in a paper by Holmes (10), involving introduction of a 2-hydroxyl function into the quinoline nucleus. Furthermore, the introduction of such a group suggests a modification which might be made in the 8-aminoquinolines in order to lower toxicity. Holmes (10) speculated that the oxidative detoxication of quinine by rabbit liver, far from indicating, as usually assumed, the advantage of blocking the 2-position of the quinoline antimalarials, might equally well be construed as an argument in favor of deliberately introducing an oxygen at this position. Specifically, it appears possible that substances which are structurally related to the 8-aminoquinoline drugs, and which are both quinoline quinones and carbostyrils, might exhibit a desirable combination of low toxicity and high antimalarial activity.

This report describes experiments directed toward the synthesis of substituted 8-aminocarbostyril-5,6-quinones (IV) with particular attention given to the synthesis of analogs of primaquine (V), as this agent is among the more active 8-aminoquinoline antimalarials. The target compounds (IV) were not obtained, but a series of 2-benzyloxy- or 2-hydroxy-6-alkoxy-8-(aminoalkylamino)quinolines were successfully prepared. These represent a new group of 8-aminoquinolines, which are the immediate precursor of the carbostyril-*o*-quinones.



I; R = H, R′ = $-CH(CH_3)(CH_2)_3N(C_2H_5)_2$
IV; R = OH, R′ = $-CH(R'')(CH_2)_nNH_2$
R″ = H, CH_3 $n = 1–4$



II; R = $-(CH_2)_5NHCH(CH_3)_2$
III; R = $-CH(CH_3)(CH_2)_3N(C_2H_5)_2$
V; R = $-CH(CH_3)(CH_2)_3NH_2$

To achieve the synthesis of the desired carbostyrils, it was necessary to prepare 2-benzyloxy-6-alkoxy-8-aminoquinoline (VI) (11), followed by incorporation of an appropriate side chain at position-8 (VII, VIII) (11, 12) and cleavage of the ether (IX) (11) or ethers (X) (11, 13) (see Scheme I). Theoretically, oxidation (1, 10) of this latter product (IX or X) should give the carbostyril-*o*-quinone (XI). Present evidence (IR, elemental analysis, molecular weight, *etc.*) suggests that the quinoline nucleus was destroyed in attempts to prepare the carbostyril-5,6-quinones.

In addition to the 6-methoxy derivatives, the authors have prepared several 6-ethoxy derivatives, since reports (13) in the literature indicate that ethyl ethers are more readily cleaved to the hydroxyl compound. The nature of the substituent on the 8-amino function was varied by incorporating different homologs of the alkylamino moiety (VIII), as well as some arylalkyl amino moieties (XII) (see Scheme II).

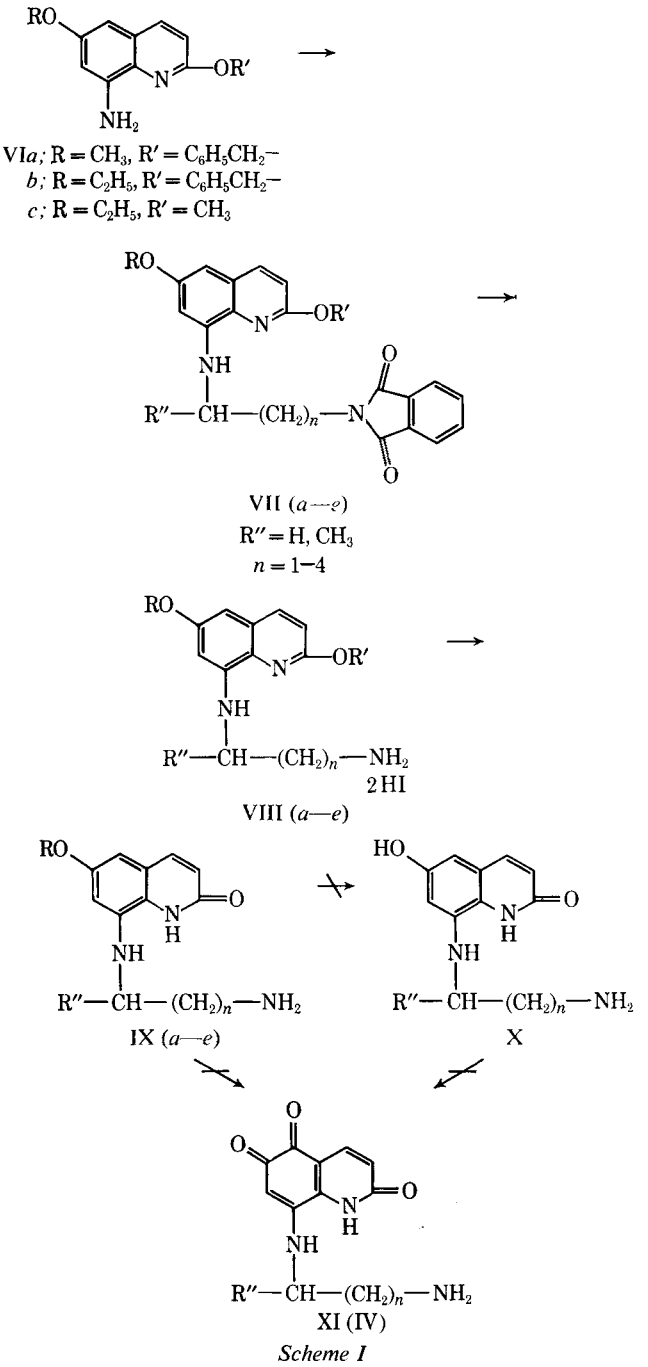
As cleavage of the 2-benzyloxy analogs produced erratic results during attempted hydrogenolysis to the 2-hydroxy analogs (IX*a–e*) (11),[1] a 2-methoxy analog (VIII*d*) was prepared in hope of achieving simultaneous acid cleavage of the 2,6-dialkoxy moieties (X). Preliminary experiments for this latter reaction have not been successful.

PHARMACOLOGICAL RESULTS

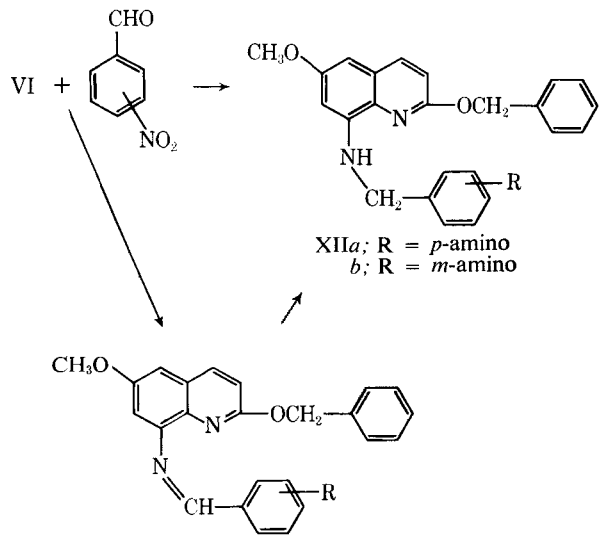
Test Methods—The compounds were tested against *P. berghei* in mice and *P. gallinaceum* in chicks and *P. gallinaceum* in mosquitos.[2]

[1] Only one of the 2-hydroxy-6-alkoxy-8-aminoquinoline derivatives was obtained by this procedure—*viz.*, IX*d*.

[2] These test data were supplied by the Walter Reed Army Institute of Research, Washington, D. C.



Scheme I



Scheme II

Mice were infected by two routes: (*a*) intraperitoneal injection of parasitized blood, and (*b*) intraperitoneal injection of a sporozoite suspension. The first test was conducted by Dr. L. Rane, University of Miami (14–16) and the other by Dr. Maurice King, Illinois Institute of Technology.[3]

The testing in chicks was also conducted by Dr. Rane[4] and the mosquito test by Dr. E. J. Gerberg, Insect Control and Research, Inc., Baltimore, Md. (17).

Testing Results—Table I summarizes the results of the Compounds VIII*a–e*. Chloroquine, quinine, and primaquine are included for comparison. The other compounds discussed in this paper are not included because they were found inactive in these test systems.

Compound VIII*a* was also tested as a prophylactic agent by Dr. King. It was found inactive at 480 mg./kg. while primaquine demonstrated activity at 90 mg./kg.

None of the compounds was active in the mosquito test.

EXPERIMENTAL

All melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected. The microanalyses and molecular weight determinations were by Galbraith Laboratories, Inc., Knoxville, Tennessee. IR spectra were determined with a Perkin-Elmer model 137 Infracord. TLC was performed on plates coated with silica gel G and Eastman chromatogram sheets, type 6060.

6-Methoxy-8-nitroquinoline and 6-Ethoxy-8-nitroquinoline (XIII)—These quinolines were prepared from 4-methoxy-2-nitroaniline and 4-ethoxy-2-nitroaniline by the procedure of Mosher *et al.* (18) or by the procedure of Yale *et al.* (19). (6-Methoxy-8-nitroquinoline was also purchased from Winthrop Laboratories, N. Y.)

Table I—Increase in Mean Survival Time (Days)

Compound	Dosage, mg./kg. *P. berghei* in Mice[a] 40	160	640	*P. gallinaceum* in Chicks[b] 120
VIII*a*	1.7	3.5	15.7[c]	6.4
	1.7	3.3	10.2[d]	
VIII*b*	—		Inactive	6.8
VIII*c*	—		Inactive	6.4[e]
VIII*d*	0.5	3.9 (2/5)[f]	(5/5)[f]	9.5 (3/5)[f]
VIII*e*	0.5	4.1	8.6[d]	7.6
Chloroquine	4.6	10.0	(5/5)[f]	
Quinine	1.2	3.4	6.5	
Primaquine phosphate	4.5	8.8 (2/5)[f]	(5/5)[f]	

[a] A compound is considered to be active if the mean survival time of the treated group is more than double the mean survival time of the control group (5 animals were used in a group). Controls normally live 6–7 days. [b] Controls normally live 3–3.5 days. [c] Curative for one animal. [d] Curative for two animals. [e] At 140 mg./kg. [f] Drug toxicity is considered the cause of death when treated animals die before controls; fractions in parentheses represent toxicity death over total animals in treated group.

[3] The detailed test method will be published elsewhere. Mice were treated with drug on Days -1, 0, and 1 days of infection. Blood smears for parasite determination are made on Days 6, 10, 14, and 21 after infection. Drug effectiveness was evaluated by comparing the mean parasitemia of the drug-treated group to that of the nondrug-treated controls at slide Day 14. A value of less than 0.25 of the control value was considered for activity. Primaquine was active under these conditions.

[4] Chicks (9–12 days old) were infected with a uniform disease fatal to 100% of untreated controls within 3–4 days. Compounds under test were dissolved or suspended in peanut oil and administered subcutaneously or *per os* immediately after infection of the chicks. An increase of 100% in survival time was considered to be the minimum effective response to the antimalarial activity of a drug. Chicks that survived for 30 days were recorded as cured.

Table II—Substituted 8-(Phthalimidoalkylamino)quinolines (VII)

Structure (VII)	R	R′	R″	*n*	M.p.	Yield, %	Anal., % Calcd.	Found
a $C_{30}H_{29}N_3O_4$	CH_3	$C_6H_5CH_2$-	CH_3	3	121–123	25–37	C, 72.73 H, 5.86 N, 8.47	C, 72.48 H, 5.97 N, 8.58
b $C_{29}H_{27}N_3O_4$	C_2H_5	$C_6H_5CH_2$-	H	2	117	46.5	C, 72.04 H, 6.00 N, 8.69[a]	C, 71.95 H, 5.82 N, 8.54
c $C_{29}H_{27}N_3O_4$	CH_3	$C_6H_5CH_2$-	H	3	139–140	42	C, 72.30 H, 5.60 N, 8.75[b]	C, 72.41 H, 5.86 N, 5.75
d $C_{23}H_{23}N_3O_4$	C_2H_5	CH_3-	H	2	101–102	50[c]		
e $C_{30}H_{29}N_3O_4$	CH_3	$C_6H_5CH_2$-	H	4	65–68	38[c]		

[a] Molecular weight calculated for 483; found 947. (Dimer calculated mol./wt. = 966.) [b] Molecular weight calculated for 481; found 493. [c] This intermediate not analyzed at this step.

6-Methoxy-8-nitroquinoline and 6-Ethoxy-8-nitroquinoline Methiodides (XIV)—The quaternization procedure of Mislow and Koepfli (11) was utilized. The yield of the 6-methoxy derivative (XIV*a*) was 90–98% of theory, melting at 143–144° (lit. m.p. 149°). 6-Ethoxy-8-nitroquinoline methiodide (XIV*b*) was prepared in a crude yield of 98% and, after recrystallization from butanol, melted at 143–145°.

6-Methoxy-1-methyl-8-nitro-2-quinolone and 6-Ethoxy-1-methyl-8-nitro-2-quinolone (XV)—The crude methiodides (XIV) were oxidized by the procedure of Mislow and Koepfli (11). 6-Methoxy-1-methyl-8-nitro-2-quinolone (XV*a*), prepared in a 61.5% yield, melted at 190–191° (lit. m.p. 186–187°). 6-Ethoxy-1-methyl-8-nitro-2-quinolone (XV*b*) was recrystallized from methanol to give a 50% yield, m.p. 154–155°. (If pure methiodide was used, the yield could be raised to 87%.)

2-Chloro-6-methoxy-8-nitroquinoline and 2-Chloro-6-ethoxy-8-nitroquinoline (XVI)—Chlorination of the quinolone (XV) was carried out by the procedure of Mislow and Koepfli (11). The 2-chloro-6-methoxy derivative (XVI*a*) was obtained in a 71% yield, m.p. 224–226° (lit. m.p. 225–226°). 2-Chloro-6-ethoxy-8-nitroquinoline (XVI*b*) was prepared in a 77% yield, m.p. 162–162.5°.

Anal.—Calcd. for $C_{11}H_9ClN_2O_3$: C, 52.28; H, 3.56; N, 11.09. Found: C, 52.30; H, 3.60; N, 10.92.

6-Methoxy-8-nitrocarbostyril and 6-Ethoxy-8-nitrocarbostyril (XVII)—Hydrolysis of the chloro compounds (XVI) was accomplished using the procedure of Mislow and Koepfli (11). The 6-methoxy derivative (XVII*a*) melted at 209–211° (lit. m.p. 210–211°). The ethoxy derivative (XVII*b*) was obtained in a yield of 76% and melted at 162–164°.

Anal.—Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.39; H, 4.30; N, 11.87. Found: C, 56.18; H, 4.33; N, 11.90.

2-Benzyloxy-6-methoxy-8-nitroquinoline and 2-Benzyloxy-6-ethoxy-8-nitroquinoline (XVIII)—The carbostyrils (XVII) were benzylated by standard procedures (11). The 6-methoxy derivatives (XVIII*a*; 65–76% yield) melted at 137–139° (lit. m.p. 139–140°). 2-Benzyloxy-6-ethoxy-8-nitroquinoline (XVIII*b*), obtained in a 52% yield, melted at 125–126°.

Anal.—Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.93; N, 8.64. Found: C, 66.82; H, 4.83; N, 8.62.

2-Benzyloxy-6-methoxy-8-aminoquinoline and 2-Benzyloxy-6-ethoxy-8-aminoquinoline (VI)—Reduction of the nitro compound (XVIII) was accomplished by the procedure of Mislow and Koepfli (11). Yields of 40–60% were obtained for the 6-methoxy derivative (VI*a*), which melted at 84–85° (lit. m.p. 86–87°). 2-Benzyloxy-6-ethoxy-8-aminoquinoline, obtained as a gray powder (74%), melted at 120–122°.

Anal.—Calcd. for $C_{18}H_{18}N_2O_2$: C, 72.98; H, 6.08; N, 9.46. Found: C, 73.54; H, 6.37; N, 9.27.

Sample revealed only one spot on TLC (silica gel G plate eluted with chloroform–ethanol, 15:1).

Preparation of 2-Methoxy-6-ethoxy-8-nitroquinoline (XIX)—To a solution of 23.85 g. of 6-ethoxy-8-nitrocarbostyril (XVII*b*) in 500 ml. of hot 2.5 *N* sodium hydroxide was added 200 ml. of dimethylsulfate in 25-ml. portions, the solution being basified after each addition. On cooling, light-yellow needles crystallized out. Recrystallization from ethanol gave a product which had three spots on TLC. The material was placed on an alumina column and eluted with benzene–chloroform, chloroform–ethanol, and ethanol. Fraction I (benzene–chloroform) gave 12.8 g. of shining yellow plates, m.p. 128–129°. Fraction II (chloroform–ethanol) gave 4.4 g. of yellow needles, m.p. 155–156°. The melting point and IR spectrum revealed that the product from Fraction II was identical to 1-methyl-6-ethoxy-8-nitro-2-quinolone (XV*b*). The last fraction gave a small amount of starting material, m.p. 164–165°. The product from Fraction I was recrystallized as the 2-methoxy-6-ethoxy-8-nitroquinoline. This product was homogeneous on TLC (benzene–chloroform, 15:3).

Anal.—Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.83; N, 11.29. Found: C, 57.95; H, 4.92; N, 11.15.

Table III—Substituted 8-(Aminoalkylamino)quinolines (VIII)

Structure (VIII)	R	R′	R″	*n*	M.p.	Yield	Anal., % Calcd.	Found
a $C_{22}H_{29}I_2N_3O_2$	CH_3	$C_6H_5CH_2$-	CH_3	3	125 dec.	74–92	C, 42.35 H, 4.67 N, 6.76	C, 42.51 H, 4.71 N, 6.66
b $C_{21}H_{27}I_2N_3O_2$	C_2H_5	$C_6H_5CH_2$-	H	2	119–120 dec.	85	C, 41.52 H, 4.45 N, 6.92	C, 41.17 H, 4.41 N, 6.71[a]
c $C_{21}H_{27}I_2N_3O_2$	CH_3	$C_6H_5CH_2$-	H	3	130 dec.	91	C, 41.51 H, 4.44 N, 6.91	C, 41.53 H, 4.65 N, 7.07[b]
d $C_{15}H_{23}I_2N_3O_2$	C_2H_5	CH_3-	H	2	127–128 dec.	64.4	C, 33.89 H, 4.33 N, 7.91	C, 33.63 H, 4.56 N, 7.80
e $C_{22}H_{29}I_2N_3O_2$	CH_3	$C_6H_5CH_2$-	H	4	92–95 dec.	79.1	C, 42.55 H, 4.71 N, 6.86	C, 42.27 H, 4.98 N, 6.56

[a] This compound (VIII*b*) was also prepared as the monohydrated diphosphate salt. Calcd. for $C_{21}H_{31}N_3O_{11}P_2$: C, 44.62; H, 5.84; N, 7.43. Found: C, 44.59; H, 6.29; N, 7.43. [b] Percent iodine: calcd., 41.68; found, 41.68.

If care was taken to ensure complete solution of the 6-ethoxy-8-nitrocarbostyril before addition of the dimethylsulfate, it was possible to raise the yield of desired product. In one case, tetrahydrofuran was employed to facilitate solution in the hot alkali solution (65–70°). With this modification, the procedure gave 86.6% 2-methoxy-6-ethoxy-8-nitroquinoline, m.p. 125–126°.

2-Methoxy-6-ethoxy-8-aminoquinoline (VI*c*)—A suspension of 2-methoxy-6-ethoxy-8-nitroquinoline was reduced on the Parr apparatus with platinum oxide. The product, which melted at 140–143° (72%), was used in the next step without elemental analysis (preparation of VIII*d*).

Preparation of *N*-(Bromoalkyl)phthalimides (XX)—The procedure of Elderfield *et al.* (20) was employed. [*N*-(3-Bromopropyl)-phthalimide was obtained commercially.]

Preparation of 2-Benzyloxy(or methoxy)-6-methoxy(or ethoxy)-8-(phthalimidoalkylamino)quinolines (VII*a–e*, Table II)—The procedure of Elderfield (20) was slightly modified for this condensation. A solution of 0.08 mole of 2-benzyloxy(or methoxy)-6-methoxy(or ethoxy)-8-aminoquinoline, 0.4 mole of sodium acetate, and 0.08 mole of the appropriate *N*-(bromoalkyl)phthalimide in 300 ml. of 66% ethanol was refluxed for 3 days. An additional quantity of the phthalimide (0.08 mole) was added on the second day and 0.4 mole of sodium acetate was added each day. The pH was maintained near 7–8. A dark-brown oil separated from the reaction. The reaction mixture was cooled, diluted with 600 ml. of water, saturated with potassium carbonate, and extracted with ether. (Any solid, which appeared, was collected with the ether fraction.) The ether extract was concentrated to one-half its original volume and chilled to give a yellow precipitate. [In some cases, it was necessary to dry the ether extract ($MgSO_4$), evaporate to dryness, and add anhydrous ether to achieve a precipitate.] The product which dissolved in the ether was found to be a mixture of starting materials. This procedure generally yielded a product of sufficient purity for the next step—TLC showed the product to be homogeneous. Analytical samples were prepared by recrystallization from 95% ethanol or aqueous DMF (see Table II for physical data).

Preparation of 2-Benzyloxy(or methoxy)-6-methoxy(or ethoxy)-8-(aminoalkylamino)quinoline Dihydriodides (VIII*a–e*, Table III)—The procedure of Mosher (12) was utilized. In some cases, the phthalimido product was not readily soluble in ethanol, and solution was achieved by the addition of chloroform. TLC showed the product to be homogeneous (methanol–diethylamine, 19:1) (see Table III for physical data).

Preparation of 6-Ethoxy-8-(3-aminopropylamino)carbostyril Monoacetate (IX*b*)—A solution of 2-benzyloxy-6-ethoxy-8-(3-aminopropylamino)quinoline was reduced on the Parr apparatus with palladium oxide. The product was isolated as the monoacetate and recrystallized from absolute ethanol to give light-yellow needles (47.7%), m.p. 183–184° (IR 3450, 1670, 1610, and 1550 $cm.^{-1}$).

Anal.—Calcd. for $C_{16}H_{23}N_3O_4$: C, 58.22; H, 7.16; N, 13.08. Found: C, 58.00; H, 7.33; N, 12.87.

2-Benzyloxy-6-methoxy-8-(4-amino-1-methylbutylamino)quinoline (VIII*a*) and 2-benzyloxy-6-methoxy-8-(4-aminobutylamino)quinoline (VIII*c*) were subjected to similar reaction conditions, but the analytical data were inconclusive and structural assignments have not been made at this time.

(VIII*a* to IX*a*) *Anal.*—Calcd. for $C_{15}H_{21}N_3O_2 \cdot CH_3COOH$ (IX*a*): C, 61.07; H, 7.18; N, 12.57; mol. wt. 334. Found: C, 57.29; H, 6.89; N, 13.35; mol. wt. 210 (CH_3OH).

(VIII*c* to IX*c*) *Anal.*—Calcd. for $C_{14}H_{19}N_3O_2 \cdot CH_3COOH$ (IX*c*): C, 60.00; H, 6.87; N, 13.12. Found: C, 55.06; H, 6.70; N, 13.49.

In one experiment, attempts were made to isolate the carbostyril as the hydriodide salt. It was not possible to obtain a product that was homogeneous on TLC.

Anal.—Calcd. for $C_{15}H_{21}N_3O_2 \cdot 2HI$: C, 33.96; H, 4.15; I, 48.21; N, 7.92. Found: C, 36.44; H, 4.54; I, 46.22; N, 7.10.

Attempted Preparation of 2,6-Dihydroxy-8-(3-aminopropylamino)quinoline (X)—A solution of 8 g. (0.022 mole) of 2-benzyloxy-6-ethoxy-8-(3-aminopropylamino)quinoline in 50 ml. of methyl cellosolve was heated and stirred with 80 ml. of 48% hydrobromic acid at 120–130° for 4 hr. The solvent was evaporated, *in vacuo*, to give the hydrobromide which was suspended in water and neutralized with sodium bicarbonate to give a product of unknown structure. This substance was recrystallized from pyridine–water to give yellow platelets, m.p. 202–203°. IR spectrum indicated ether cleavage, showing a band at 1680 $cm.^{-1}$, characteristic for carbonyl absorption. However, molecular weight and elemental analysis did not correlate with calculated values. TLC showed the product to be homogeneous (methanol–diethylamine, 15:2).

Anal.—Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.80; H, 6.43; N, 18.02; mol. wt., 233. Found: C, 70.90; H, 7.33; N, 11.83; mol. wt. (DMF), 480.

2-Benzyloxy-6-methoxy-8-(4-aminobenzylamino)quinoline Dihydrochloride (XII)—This procedure was adapted after that of Tipson and Clapp (21). Two grams (0.007 mole) of 2-benzyloxy-6-methoxy-8-aminoquinoline was mixed with 1.5 g. of *p*-nitrobenzaldehyde in 25 ml. of DMF and 5 drops of piperidine. This mixture was allowed to stand for 1 hr. with stirring after heating just briefly on a water bath. The solution was then shaken on the Parr apparatus, under hydrogen, with platinum oxide until 3 p.s.i. of pressure had been lost. Filtration of the catalyst and dilution of the filtrate with water gave a greenish-yellow product. This substance was dissolved in ether, dried, and treated with charcoal before removal of the drying agent. Hydrogen chloride was passed through the ethereal solution to give a product melting at 225–227° (78% yield). TLC revealed only one spot (methanol–diethylamine, 19:1) (IR 3400, 2850, 1610, and 1580 $cm.^{-1}$). Analytical sample recrystallized from ethanol–ether.

Anal.—Calcd. for $C_{24}H_{21}N_3O_2 \cdot 2HCl$: C, 62.88; H, 5.46; N, 9.17. Found: C, 62.76; H, 5.61; N, 9.20.

It was possible to isolate and characterize the intermediate, 2-benzyloxy-6-methoxy-8-(*p*-nitrobenzylideneamino)quinoline, m.p. 118–122° (XII*c*).

Anal.—Calcd. for $C_{24}H_{19}N_3O_4$: C, 69.73; H, 4.60; N, 10.16. Found: C, 69.65; H, 4.73; N, 9.92.

2-Benzyloxy-6-methoxy-8-(3-aminobenzylamino)quinoline dihydrochloride (XII*b*), m.p. 149–152°, was prepared by a similar procedure.

Anal.—Calcd. for $C_{24}H_{21}N_3O_2 \cdot 2HCl$ (XII*b*): C, 62.88; H, 5.46; N, 9.17. Found: C, 62.68; H, 5.32; N, 9.09.

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Odor Threshold and Gas-Chromatographic Assays of Vaginal Odors: Changes with Nitrofurazone Treatment

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Abstract □ Although vaginal disorders are frequently accompanied by malodors, the efficiency of drugs to reduce the malodors by reducing the infection has been difficult to estimate. Recently, techniques have become available for combining sensory and gas-chromatographic approaches to compare odor intensities of complex vapor mixtures. These techniques were applied to vaginal odors. An apparatus was devised to collect vaginal vapors in a form suitable for odor-revelant gas-chromatographic analyses, as well as for odor-threshold determinations. Odor changes in vaginal vapors upon treatment with nitrofurazone vaginal suppositories were studied on five patients with several types of disorders (hematuria, endometritis, and stress incontinence). The distribution of odorous components in the gas chromatograms reflected, through disappearance of many malodorous peaks, a significant reduction in the content of the malodorous volatile compounds in the vaginal vapors. Odor-threshold measurements were conducted in an apparatus where nonodorous methane tracer and a hydrogen-flame ionization detector were used to measure the degree of vapor dilution needed to reach the threshold, using the ASTM odor-threshold test design combined with ED_{50} statistics. These measurements similarly indicated that odors were reduced by the drug.

Keyphrases □ Vaginal odor threshold, intensity—analysis □ Odors, vaginal—threshold, intensity determination □ Nitrofurazone effect—vaginal odors □ GLC—analysis

It is usually noted that a reduction in bacterial population in suitable media is accompanied by a reduction in the odors. Odor reduction is often subjectively observed when the antibacterial agent nitrofurazone[1] is used in the treatment of vaginal disorders accompanied by malodor. In the absence of a satisfactory method for quantitative or precise qualitative assay of odors, such observations have remained in the form of testimonials.

Recently, techniques have become available for more detailed studies of the odors in complex mixtures. These techniques are based on combinations of psychophysical (sensory) and gas-chromatographic methods, the latter providing means for collecting and separating the components of odorous vapors. In the present work these techniques were adapted to assay the vaginal odors and their changes upon treatment with an antibacterial preparation. Odor thresholds of vaginal vapors were also determined.

[1] Furacin Vaginal Suppositories, Eaton Laboratories, Division of the Norwich Pharmacal Co.

PRINCIPLES OF ODOR MEASUREMENT

The odor results from interaction of vapors with the observer's chemoreceptors (primarily olfactory and to some extent trigeminal). Substances differ in odor thresholds, *i.e.*, in the lowest concentrations in air at which the odors of their vapors can be detected. Observers also differ in sensitivity (olfactory acuity), so that odor thresholds are represented not by sharp concentration levels but rather by concentration zones, within which the thresholds for different observers can vary severalfold. To a lesser extent, thresholds as estimated by the same observer may vary from time to time.

Above threshold levels, odor intensity increases in proportion to a fractional power (0.2–0.7) of the odorant concentration in air. These relations are expressed in Stevens' law (1, 2):

$$I = k\,(C - C_{\text{thr.}})^x \qquad \text{(Eq. 1)}$$

where I is psychophysical odor intensity, k is a coefficient (small for weak odors, large for strong odors), C is concentration of the odorant in air, $C_{\text{thr.}}$ is threshold concentration of the odorant, and x is an exponent. The values of k, $C_{\text{thr.}}$, and x are not necessarily related. Corollaries of this expression are: (*a*) odor intensity experienced by an observer does less than double when the odorant's concentration is doubled; *e.g.*, for $x = 0.5$, the concentration must increase by a factor of 4 to increase the intensity by a factor of 2; (*b*) if an odorant A has smaller values of k and x than another odorant B, an undiluted A can smell weaker than undiluted B, while diluted A can smell stronger than similarly diluted B; (*c*) since the highest possible concentration of an odorant is at its saturation pressure, odorants with low x, or high threshold and an average x, may never reach very high odor intensity.

Odor-threshold values, therefore, do not necessarily indicate the undiluted odor intensity; rather, the odor of a lower threshold odorant will be noticed farther from the source than will that of a higher threshold odorant. In addition, above the threshold, the character of the odor can be objectionable, as with the malodors, or acceptable, as with fragrances.

The composition of odorous vapors of biochemical origin is complex, with many odorants participating. Odorants present at subthreshold concentrations can summate (sometimes even synergistically) to reach threshold and can modify the character of odors of other substances which are present at suprathreshold levels. In odor assay, therefore, all those substances that are present at levels exceeding reasonable fractions, *e.g.*, one-tenth of their threshold concentrations, must be considered. Gas-chromatographic techniques permit the delivery of separated components for sensory