50% H_2O–EtOH, dried, and recrystallized; characteristic ir absorptions, compounds **1–4**: 3160–3200 (NH); 1635 (C=N and NH_2); 1530 (CNH); 1125 (NCN); 810–830 (C=S) cm^{-1}.

Preparation for Dithiocarbohydrazones (Table II).—A mixture of thiocarbohydrazide (0.01 mol) and aldehyde or ketone (<0.04 mol) was heated from 0.5 to 1 hr at 100°, diluted with hexane, then cooled. The precipitate that formed was collected by filtration, washed with cold hexane, dried, and recrystallized; characteristic ir absorptions, compounds **5–15**: 3120–3200 (NH); 1600–1645 (C=N); 1535–1560 (CNH); 1120–1145 (NCN); 810–850 (C=S) cm^{-1}.

The fungistatic properties of the mono- and dithiocarbohydrazones were evaluated by the tube dilution method,[9] using the tests organisms *C. globosum* (strain USDA 1042.4) and *A. niger* (strain USDA 215-5373.16). Concentrations of the compounds being tested of 10, 100, and 1000 ppm were tried. The criterion of effectiveness was taken to be the absence of fungal growth after a 2-week (*C. globosum*) or a 48-hr (*A. niger*) incubation period.

A typical thiocarbohydrazone, di-2-octanone thiocarbohydrazone, was applied to a 10-oz bleached cotton duck[12] from dioxane or EtOH solution containing a wetting agent. Either dioctyl sodium sulfosuccinate (Alrowet D-65, Geigy) or a linear aliphatic ethoxylate (Igepal A, General Aniline & Film Corp.) were used at a level of 1–10 wt % of the fungicide. Suitable pieces of the treated fabric as well as control pieces were subjected to the plate-disk method[9] and the soil burial method.[13] Breaking strengths of fabric evaluated by the latter method were measured at 21.1° and 65% RH with the Instron tensile tester (type TT-C) using 152.4 × 25.4 mm ravelled strips, with the warp in the long direction. The number of warp yarns in the test samples was always the same, and the average of ten breaking strength values was used in each case.

Acknowledgments.—Thanks are due Miss G. Colin for evaluating the fungicidal effectiveness of the thiocarbohydrazones and R. Ironside and V. Boyko for recording the infrared spectra.

Antiprotozoal 4-Aryloxy-2-aminoquinolines and Related Compounds

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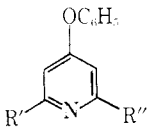
Recently we reported the inhibition of growth of *Tetrahymena pyriformis* and *Crithidia fasciculata* by ethers of 2,6-diamino-4-pyridone.[1] These compounds were shown to interfere with biopterin and/or with enzymes involved in fat metabolism. Since they have an N–C–N–C–N atomic sequence similar to the one in biopterin and other biologically active pteridines we wished to test whether or not this structural feature was necessary for inhibition of the organisms. We therefore synthesized 4-phenoxy-2-aminopyridine for comparison with 4-phenoxy-2,6-diaminopyridine which was the most active ether of the earlier series.

Since quinoline derivatives approximate pteridines in size more than do pyridine derivatives we also prepared and tested 4-phenoxy-2-aminoquinoline. A report of *in vitro* amebicidal activity of 4-propoxy-2-aminoquinoline and its homologs[2] also suggested testing of that compound. Although 4-phenoxy-2-aminoquinoline was a poor inhibitor of *C. fasciculata* it was found to inhibit *T. pyriformis* at considerably lower concentration than 4-phenoxy-2,6-diaminopyridine. In view of these results we prepared several analogs of that compound with substituents in the benzene ring in the hope of finding highly specific inhibitors which could be used as tools in the study of the intermediary metabolism of these organisms, since they (especially *T. pyriformis*) have been widely used in screening programs for antineoplastic compounds.[3]

Curtius degradation of the corresponding esters gave the desired amines. The prerequisite esters were obtained by reaction of ethyl 4-chloroquinaldate with appropriate phenoxides (or thiophenol[4]) and by phenylation of the Na salts of ethyl 4-pyridone-2-carboxylate and ethyl 4-quinolone-2-carboxylate with diphenyliodonium chloride.[1]

The growth of both *T. pyriformis* and *C. fasciculata* is inhibited by 4-phenoxy-2-aminopyridine (culture methods for biological tests were essentially the same as the ones reported previously[5]), but only at concentrations an order of magnitude greater than that of the diamine (Table I). The inhibition of *Crithidia* is not reversed by biopterin as is that caused by the diamine.

TABLE I
INHIBITORY EFFECTS OF 4-PHENOXYAMINOPYRIDINES AND 4-PHENOXYPYRIDINECARBOXYLIC ACID HYDRAZIDES



R'	R''	μmol/ml for half-max inhib. *T. pyriformis*	*C. fasciculata*
NH_2	H	0.085 (3)[a]	0.245 (3)
$CONHNH_2$	H	0.053 (2)	0.393 (1)
$CONHNH_2$	$CONHNH_2$	0.5 (2)	0.123 (5)
NH_2	NH_2	0.0084[b]	0.028[b]

[a] Number in parentheses is the number of experiments. [b] See ref 1.

The intermediate 4-phenoxypicolinic acid hydrazide was also tested and found to be active against *T. pyriformis* but not against *C. fasciculata*. In contrast, 4-phenoxydipicolinic acid dihydrazide[1] inhibited significantly only the latter organism (Table I). The substituted quinaldic acid hydrazides like the monohydrazide of the pyridine series inhibited *T. pyriformis* but were only slightly active against *C. fasciculata*.

The substituted aminoquinolines were significantly more active against *T. pyriformis* but not against *C. fasciculata* (Table II). The inhibitory effects of none of the quinoline derivatives was susceptible to reversal by biopterin or folic acid. A few preliminary tests with crude materials have shown that liver fraction L (Nutritional Biochemicals Corp.) contains a sub-

(1) D. G. Markees, V. C. Dewey, and G. W. Kidder, *J. Med. Chem.*, **11**, 126 (1968).

(2) R. Hardman and M. W. Partridge, *J. Chem. Soc.*, 510 (1955).

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(4) D. G. Markees, *J. Org. Chem.*, **28**, 2530 (1963).

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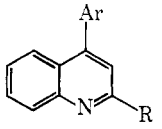
TABLE II
BIOLOGICAL ACTIVITY OF SUBSTITUTED QUINOLINES



Ar	R	μmol/ml for half-max inhib—*Tetrahymena*	*Crithidia*
C_6H_5O	$CONHNH_2$	0.0262 (4)[a]	0.254 (1)
C_6H_5S	$CONHNH_2$	0.0645 (2)	0.34 (1)
p-$CH_3C_6H_4O$	$CONHNH_2$	0.0224 (3)	0.213 (1)
p-ClC_6H_4O	$CONHNH_2$	0.177 (2)	0.32 (1)
p-$CH_3OC_6H_4O$	$CONHNH_2$	0.032 (3)	0.32 (2)
β-$C_{10}H_7O$	$CONHNH_2$	0.0095 (2)	*b*
C_6H_5O	NH_2	0.0072 (8)	0.377 (3)
C_6H_5S	NH_2	0.0025 (4)	0.265 (1)
p-$CH_3C_6H_4O$	NH_2	0.0038 (3)	0.120 (1)
p-ClC_6H_4O	NH_2	0.0028 (4)	*c*
p-$CH_3OC_6H_4O$	NH_2	0.0022 (2)	0.222 (1)
β-$C_{10}H_7O$	NH_2	0.0091 (2)	*b*

[a] Number in parentheses indicates number of experiments. [b] Not tested. [c] Not sufficiently soluble to test.

stance(s) capable of reversing the inhibition produced by aminoquinolines but not that by quinaldic acid hydrazides.

Experimental Section[6]

Ethyl 4-Phenoxyquinaldate.—Na (1.3 g) dissolved in 25 ml of absolute EtOH was added to a solution of 12 g of ethyl 4-quinolone-2-carboxylate[7] in 120 ml of absolute EtOH. The solvent was removed *in vacuo* and replaced with 100 ml of DMF. Diphenyliodonium chloride (17.0 g) was added and the mixture was refluxed for 3.5 hr. All volatile material was then removed at reduced pressure, the residue was mixed with H_2O, and the product taken up with Et_2O. The Et_2O solution was washed with 5% NaOH and H_2O and dried (Na_2SO_4), and the solvent removed. The crude product (9.5 g, 58%) was collected and recrystallized from EtOH, mp 125–126°. *Anal.* ($C_{18}H_{15}NO_3$) C, H.

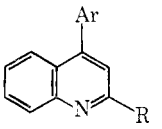
Ethyl 4-phenoxypicolinate was prepared similarly in absolute EtOH solution from ethyl 4-pyridone-2-carboxylate[8] and diphenyliodonium chloride in the presence of NaOEt. The ester was not further purified. Saponification gave 4-phenoxypicolinic acid, mp 178–179° dec (lit.[9] mp 178–179°).

4-Phenoxyquinaldic Acid.—Saponification of ethyl 4-phenoxyquinaldate[10] in aqueous-alcoholic NaOH gave the acid in 99% yield, mp 178–180.5° dec (from MeOH). *Anal.* ($C_{16}H_{11}NO_3$) C, H, N.

Ethyl 4-Phenylthioquinaldate.—Reflux of 10.0 g of ethyl 4-chloroquinaldate[10] with 5 g of PhSH in 50 ml of EtOH for 3 hr gave this ester. A first batch (4.7 g) crystallized after concentration of the reaction mixture to one-third of its original volume. More material was obtained upon removal of the remaining solvent. The two crops were combined, dissolved in MeOH, treated with charcoal, and reprecipitated with H_2O. The yield was 10.5 g (81%), mp 118–119° (from EtOH). *Anal.* ($C_{18}H_{15}NO_2S$) C, H.

Ethyl 4-*p*-Tolyloxyquinaldate.—A mixture of 10.0 g of ethyl 4-chloroquinaldate and 6 g of sodium cresoxide (prepared from 5.0 of *p*-cresol and 1.0 g of Na in 25 ml of absolute EtOH) and 35 ml of DMF was refluxed for 4 hr. The mixture was poured into H_2O and the product taken up with Et_2O. The solvent was

TABLE III
SUBSTITUTED QUINOLINES



Ar	R	Formula	Anal	Mp, °C	Solvent	Yield, %
p-ClC_6H_4O	COOEt	$C_{18}H_{14}ClNO_3$	C, H	140	EtOH	85
p-$CH_3OC_6H_4O$	COOEt	$C_{19}H_{17}NO_4$	C, H	124–126	EtOH–H_2O	58
β-$C_{10}H_7O$	COOEt	$C_{22}H_{17}NO_3$	C, H	143–144	EtOH–H_2O	35
p-$CH_3C_6H_4O$	$CONHNH_2$	$C_{17}H_{15}N_3O_2$	N	168–169	EtOH–H_2O	73
p-ClC_6H_4O	$CONHNH_2$	$C_{16}H_{12}ClN_3O_2$	N	192–194	EtOH	84
p-$CH_3OC_6H_4O$	$CONHNH_2$	$C_{17}H_{15}N_3O_3$	N	191	EtOH–H_2O	72
β-$C_{10}H_7O$	$CONHNH_2$	$C_{20}H_{15}N_3O_2$	N	186–188	EtOH	65
4-Phenoxypicolinic acid hydrazide		$C_{12}H_{11}N_3O_2$	N	119	Heptane	90
C_6H_5S	$CONHNH_2$	$C_{16}H_{13}N_3O_2S$	N	197–199	EtOH	82
p-$CH_3C_6H_4O$	NHCOOEt	$C_{19}H_{18}N_2O_3$	N	122–123	EtOH–H_2O	49
p-ClC_6H_4O	NHCOOEt	$C_{18}H_{15}ClN_2O_3$	N	167.5–169	EtOH	69[a]
p-$CH_3OC_6H_4O$	NHCOOEt	$C_{19}H_{18}N_2O_4$	N	187–189	EtOH	67
β-$C_{10}H_7O$	NHCOOEt	$C_{22}H_{18}N_2O_3$	N	164–166	EtOH	64[a]
C_6H_5S	NHCOOEt	$C_{18}H_{16}N_2O_2S$	N	124–125	EtOH–H_2O	41[a]
2-Carbethoxyamido-4-phenoxypyridine		$C_{14}H_{14}N_2O_3$	N	161.5–163	EtOH	38
p-$CH_3C_6H_4O$	NH_2	$C_{16}H_{14}N_2O$	C,H,N	164–166	EtOH–H_2O	
p-ClC_6H_4O	NH_2	$C_{15}H_{11}ClN_2O$	C,H,N,Cl	192–193	EtOH–H_2O	71
p-$CH_3OC_6H_4O$	NH_2	$C_{16}H_{14}N_2O_2$	C,H,N	211–213	EtOH	85
β-$C_{10}H_7O$	NH_2	$C_{19}H_{14}N_2O$	C,H,N	135–136	Heptane	23
C_6H_5S	NH_2	$C_{15}H_{12}N_2S$	C,H,N,S	188	EtOH	64
2-Amino-4-phenoxypyridine		$C_{11}H_{10}N_2O$	C,H,N	123–124	Heptane	93

[a] Reaction medium for preparation of azide consisted of 1.5 *M* HCl and DMF (1:1).

(6) Melting points were taken on a Mel-Temp apparatus and are not corrected. Microanalyses were performed by Mr. J. F. Alicino, Metuchen, N. J. Where analyses are indicated only by symbols of the elements, analytical results for those elements were within 0.4% of the theoretical values.

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removed after proper washing and drying and the crude ester (9.2 g, 71%) was collected, mp 135–136° (from EtOH). *Anal.* ($C_{21}H_{17}NO_3$) C, H.

Similar procedures carried out with the appropriate phenols gave the esters listed in Table III.

4-Phenoxyquinaldic Acid Hydrazide.—Crude 4-phenoxyquinaldate (7.5 g) in 50 ml of EtOH was refluxed with 4 ml of $H_2NNH_2 \cdot H_2O$ for 4 hr. After cooling, H_2O was added to complete the precipitation of the hydrazide (6.5 g, 91%), mp 182–184° (from EtOH). *Anal.* ($C_{16}H_{13}N_3O_2$) N.

Similar procedures were used to prepare the hydrazides listed in Table III.

2-Carbethoxyamido-4-phenoxyquinoline.—Crude 4-phenoxyquinaldic acid hydrazide (6.0 g) was dissolved in 50 ml of 1.5 *M* HCl. Insoluble gummy material was removed and the solution cooled to 10°. At approximately this temperature $NaNO_2$ (1.4 g) in a small amount of H_2O was added dropwise and with stirring. The solid which formed was collected (5.2 g), washed with H_2O, air-dried, and suspended in absolute EtOH. The mixture was refluxed for 3 hr until the evolution of N_2 ceased. On cooling 2.8 g was collected. An additional 1 g was obtained by concentrating the mother liquor. The analytical sample melted at 138–139° (from EtOH). *Anal.* ($C_{18}H_{18}N_2O_3$) N.

The carbamates listed in Table III were prepared essentially according to this method.

2-Amino-4-phenoxyquinoline.—A solution of 2.9 g of crude 2-carbethoxyamido-4-phenoxyquinoline and 2.0 g of KOH in 40 ml of EtOH was refluxed for 4 hr. Most of the solvent was removed and the residue diluted with H_2O and acidified with concentrated HCl. The mixture was boiled briefly, then cooled, and the crystalline material collected and dissolved in 100 ml of hot H_2O. The hot solution was filtered and made alkaline with 2.5 *M* NaOH. The precipitate was collected and recrystallized from C_6H_6–petroleum ether (bp 60–70°) (1.0 g, 45%), mp 135–136°. *Anal.* ($C_{13}H_{12}N_2O$) C, H, N.

Similar procedures were used to prepare the amines listed in Table III.

Acknowledgment.—The authors wish to thank Miss Jane A. Beach of Wells College for her valuable contributions to the synthetic work.

Antimalarials. 4,4′-Diaminodiphenyl Sulfone-Type Compounds

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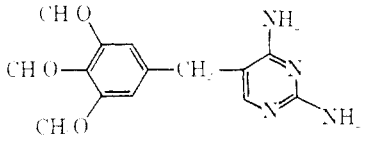
We have already reported[1,2] several derivatives of 4,4′-diaminodiphenyl sulfone (DDS) and their antimalarial activity. In view of the activity[3] of trimethoprim (I) against the resistant strains of malaria, greater interest was focussed on the structural features



I

of this drug. Compounds **3**, **8**, and **13** were prepared which incorporate the 3,4,5-trimethoxyphenyl group of trimethoprim on one side and the 4-aminophenyl group of DDS on the other. We had hoped to uncover a relationship of structure in folic acid inhibition to antimalarial activity.

Compounds **1**, **2**, **5**–**7**, and **9**–**12** were intermediates en route to the syntheses of **3**, **8**, and **13**. **4** was made as a derivative and **14** appeared as a by-product in the preparation of **1** and **5** through air oxidation of 3,4,5-trimethoxybenzenethiol.

Whereas it was easy to oxidize **1** with H_2O_2, the similar oxidation of **5** failed to give the sulfone. Some low melting substances were produced by fragmentation of the starting material, but further work was not pursued to characterize these materials. This difficulty was overcome by reducing **5** to **6** first, acetylating **6**, and then oxidizing with H_2O_2–AcOH.

The structure assigned to the triacetate **7** was rationalized on the consideration of steric hindrance prevailing at the 2 position which would therefore give only monoacetylation.

Biological Tests.—The compounds were tested for their antimalarial activity against *Plasmodium berghei* in mice by Dr. L. Rane according to the procedure already published.[4] None of them were found to be active.

Experimental Section[5]

4-Nitro-3′,4′,5′-trimethoxydiphenyl Sulfide (1).—A solution of 3,4,5-trimethoxyaniline (9.16 g, 0.05 mol) in 120 ml of H_2O and 20 ml of concentrated HCl was cooled to −5° and diazotized with 3.8 g of $NaNO_2$ in 20 ml of H_2O. Excess HCl was neutralized with 25 g of NaOAc maintaining the temperature at −5 to 0°. This solution was added, dropwise with vigorous stirring, to a hot (75–80°) solution of 16.0 g of ethyl potassium xanthate in 35 ml of H_2O. The mixture was then refluxed for 1 hr. After cooling to room temperature, it was extracted (Et_2O) and the extract dried (Na_2SO_4), filtered, and evaporated to leave a dark residue. This was mixed with 90% EtOH, 6.0 g of KOH, and 5 g of glucose and refluxed for 2 hr under N_2. Some solid separated at this stage which, on investigation, proved to be the disulfide **14**. This was removed by filtration and the filtrate evaporated to leave a dark brown residue which was mixed with some Zn dust and acidified, by cooling, with cold dilute H_2SO_4. The dark oil was extracted (Et_2O), dried (K_2CO_3), filtered, and concentrated to give 11.0 g of crude 3,4,5-trimethoxybenzenethiol. This was immediately mixed, under N_2, with 80 ml of MeOH, 20 ml of H_2O, 7.5 g of K_2CO_3, and *p*-fluoronitrobenzene (7.1 g, 0.05 mol), and the whole mixture refluxed for 2 hr. Solvent was removed under reduced pressure, the residue treated with H_2O, the solid removed by filtration and washed several times with cold MeOH.

4-Nitro-3′,4′,5′-trimethoxydiphenyl Sulfone (2).—A solution of **1** (9.64 g, 0.03 mol) in 140 ml of glacial AcOH and 60 ml of H_2O_2 was kept at 65° for 1.5 hr. On cooling to room temperature overnight 9.5 g of the product crystallized.

4-Amino-3′,4′,5′-trimethoxydiphenyl sulfone (3) was prepared by the reduction of **2** in 90% AcOH over Pt at room temperature and atmospheric pressure.

4-Diacetylamino-3′,4′,5′-trimethoxydiphenyl sulfone (4) was prepared by acetylating **3** in boiling Ac_2O.

2,4-Dinitro-3′,4′,5′-trimethoxydiphenyl sulfide (5) was prepared in the same way as **1**.

2,4-Diamino-3,4,5-trimethoxydiphenyl sulfide (6).—A mixture of **5** (18.32 g, 0.05 mol), $ZnCl_2 \cdot 2H_2O$ (78.75 g, 0.35 mol), 250 ml of concentrated HCl, and 1500 ml of EtOH was warmed on a steam bath to 80° till a clear solution was formed. EtOH was removed under reduced pressure and the residue was strongly basified with cold concentrated NaOH with cooling. The solid product was removed by filtration and washed free of alkali.

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(5) See Table I for experimental data.