Experimental Section

Chemicals.—Phenoxyacetic acid and the corresponding acid chloride were prepared as described by Mameli, *et al.*[14] *o*-Hydroxyphenoxyacetic acid lactone (**2**) was prepared from the corresponding acid as described by Ludewig.[15] *p*-Chlorophenoxyacetic acid and the corresponding acid chloride were prepared according to Minton and Stephen.[16] 1,2-Epoxy-3-(*o*-toloxy)propane (**4**) was prepared as indicated by Chizhevskaya, *et al.*,[17] and was purified by distillation under reduced pressure, bp 132–135° (10 mm). All other starting materials were commercially available products, purified by crystallization to constant melting point.

Procedures for the Preparation of the Esters. A.—Compounds **6**, **8–11**, and **16** were prepared from **4** and the appropriate acid, following the procedure given by Petrow, *et al.*,[18] for the preparation of mephenesin benzoate.

B.—Compounds **6**, **8**, **13**, **15**, and **16** were prepared by treatment of mephenesin (**3**) with the appropriate acid chloride. Treatment of **3** with *o*- or *p*-nitrobenzoyl chloride gave only the bisester **15** and **13**, respectively, even when the reactants were used in equimolar amounts. As an example, the preparation of **8** is reported. A mixture of **3** (5.0 g, 27 mmoles), *p*-chlorophenoxyacetyl chloride (5.6 g, 27 mmoles), and anhydrous pyridine (10 ml) was heated 1 hr at 100°, then was poured into cold water. The mixture was extracted with ether, the ethereal extract was washed with water, 10% Na_2CO_3, and water, dried ($MgSO_4$), and evaporated to give an oil which crystallized from benzene–petroleum ether to afford pure **8** in 40% yield.

C.—The ester **7** was prepared by treatment of **3** with the lactone **2** as follows. A mixture of **3** (9.1 g, 0.05 mole) and **2** (7.5 g, 0.05 mole) was heated at 130° for 24 hr. The resulting syrupy material afforded, on crystallization from benzene–petroleum ether (bp 60–80°), 5.9 g (28%) of ester, which was purified by further crystallization from the same solvent mixture.

D.—Compounds **12** and **14** were obtained from **11** and **13**, respectively, by catalytic reduction over PtO_2 in dioxane, as follows. A solution of the compound (5.0 g) in anhydrous dioxane (80 ml) was hydrogenated at normal pressure until the theoretical amount of H_2 had been adsorbed. The catalyst was filtered off and the solvent was evaporated at reduced pressure; the oily residue was crystallized from anhydrous ether.

Oxidation of 6 and 11.—The oxidation of **6** and **11** to the corresponding 1-hydroxy-3-(*o*-toloxy)propan-2-one derivatives **17** and **18** was carried out as follows. To a solution of the compound (3.0 mmoles) in acetone (10 ml, previously distilled over $KMnO_4$) was added dropwise an 8 *N* solution of CrO_3 in H_2SO_4 (1.5 ml),[19] while stirring and cooling at 5°. The mixture was then diluted with H_2O, and the solid which separated was collected, washed with 10% Na_2CO_3 and H_2O, dried, and crystallized from EtOH.

Acid-Catalyzed Hydrolysis of 13 to 11.—A solution of **13** (1.0 g) in 95% EtOH (10 ml) was treated with several drops of concentrated HCl, then was heated 1 hr under reflux and poured into H_2O. The solid which separated gave on crystallization from C_6H_6 0.41 g (60%) of pure **11**,[20] mp 98–99°.

Kinetic Experiments. Hydrolysis of 6–11 in Aqueous Acetone.—The kinetic experiments were performed with an electrically controlled oil bath (100 ± 0.01°), using analytical grade acetone, purified by reflux over $KMnO_4$, desiccation over K_2CO_3, and fractionation.

Solutions (0.1 *M*) of the compounds in acetone containing 40% H_2O by volume were heated at 100° in sealed 10-ml ampoules. The rates of reaction were measured by titration of successive ampoules, removed after appropriate intervals, with standard alkali (phenol red indicator). The hydrolysis of **7** was found to be first order in ester up to 90% completion; $K = 3.5 \times 10^{-5}$ sec^{-1}, half-life *ca.* 5.5 hr. All other esters were not appreciably hydrolyzed after 24 hr.

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Reduced Derivatives of Methotrexate[1]

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It has been reported that 5,6,7,8-tetrahydromethotrexate (III) is a more potent folic acid antagonist than methotrexate (I) for *Streptococcus faecalis*,[4] *Pediococcus cerevisiae*,[4] mice,[5] chicks,[6] and dogs.[7] When a method developed for separating dihydrofolate and tetrahydrofolate[8] was applied to III it was observed that the material was actually a mixture of dihydromethotrexate (II) and III. Some properties of the purified derivatives are reported here.

The reduced material showed two major peaks on diethylaminoethylcellulose chromatography. It was shown spectrally that the peak eluted first was III and the second II. They accounted for 39 and 52% of the total absorbing material, respectively. The absorption maxima are shifted 10 mμ toward longer wavelengths as compared with the corresponding aminopterin derivatives.[9] The extinction coefficients at maximum absorption were assumed to be the same as for aminopterin derivatives.[9]

III and II are less potent than I as inhibitors of dihydrofolate reductase but more potent as inhibitors of thymidylate synthetase (Table I). In every system tested II was more inhibitory than III. III is most likely a mixture of diastereoisomers resulting from the addition of hydrogen to carbon 6. The contribution of each diastereoisomer to the inhibition is not known.

Experimental Section

Compound I, provided by Lederle Laboratories, Pearl River, N. Y., was purified by diethylaminoethylcellulose chromatography as described for aminopterin.[9] Hydrogenation was carried out in AcOH using PtO_2 catalyst.[10] The reduced material was filtered under H_2 and washed with ether.[11]

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TABLE I
EFFECT OF METHOTREXATE DERIVATIVES ON ENZYMES AND BACTERIAL GROWTH

Cmpd	Dihydrofolate reductase	Thymidylate synthetase	Concn for 50% inhib, mμg/ml *S. faecalis*	*P. cerevisiae*	*L. casei*
Methotrexate	9 (1)[a]	45,000 (1)	0.15 (1)	60 (1)	0.01 (1)
Dihydromethotrexate	16 (0.56)	1,125 (40)	0.011 (14)	24 (2.5)	0.008 (1.3)
Tetrahydromethotrexate	46 (0.20)	2,250 (20)	0.047 (3.4)	68 (1)	0.056 (0.18)

[a] Numbers in parentheses indicate potency relative to methotrexate.

Thymidylate synthetase from *Escherichia coli* B[12] was provided by Dr. M. Friedkin and Miss E. Donovan. Dihydrofolate reductase was obtained from a mouse tumor L1210-C95.[9] The enzymes were assayed as described.[9]

Fractions to be assayed microbiologically were diluted in potassium ascorbate (6 mg/ml, pH 6.0) and added aseptically to the assay medium.[13] The final concentration of ascorbate in the assay was 0.6 mg/ml. *Lactobacillus casei* (ATCC 7469), *Streptococcus faecalis* (ATCC 8043), and *Pediococcus cerevisiae* (ATCC 8081) were grown on the corresponding Difco assay media for 24 hr at 37°. The *L. casei* and *S. faecalis* media contained 1 mμg of folate/ml and the *P. cerevisiae* medium contained 1 mμg/ml of calcium *dl*-1,5-formyltetrahydrofolate. Growth was determined turbidimetrically.

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New Compounds

Synthesis of 6,8-Dibromo-3-substituted 2-(N,N-Dialkyl- (or N-Piperidino-) carboxamidomethylthio)-4(3H)-quinazolinones as Antimalarials

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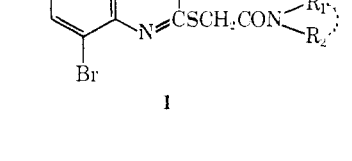
The antimalarial activity of febrifugine, an alkaloid of a 3-substituted 4(3H)-quinazolinone structure, has prompted the preparation and testing of a number of quinazolines,[1] and several patent claims have been made on quinazolines as intermediates for potential antimalarials.[2] Compounds having the side chain $-CH_2COCH_2R$ (where R = ω-N-morpholylpropyl or ω-N-piperidyl-*n*-butyl) at position 3 of the 4(3H)-quinazolinone nucleus were shown to have significant antimalarial activity.[3]

Since the activity of these compounds is influenced by various substituents and their positions, a number of derivatives have been synthesized in the course of our previous investigations[4] by introducing some new side chains into some 6,8-dibromo-substituted 2-mercapto-3-aryl- (or alkyl-) 4(3H)-quinazolones as antimalarials having the general structure **I**.

The standard tests for antimalarial activity in chicks infected with *Plasmodium gallinaceum* so far reported on these compounds indicate that they have no significant value pharmacologically.



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Experimental Section

6,8-Dibromo-3-phenyl-2-(N-piperidinocarboxamidomethylthio)-4(3H)-quinazolinone.—N-Chloroacetylpiperidine (2 ml) dissolved in EtOH, was added to a solution of 6,8-dibromo-2-thio-3-phenyl-2,4(1H,3H)-quinazolindione (4.5 g) in EtOH–NaOH. The resulting mixture was stirred thoroughly for 2 hr at 23–25°. On cooling the solution to 0° a crystalline product was formed. It was filtered and washed (H_2O, EtOH). Recrystallization of the product from EtOH–Me_2CO (1:2) gave a pure analytical sample.

Similarly various 6,8-dibromo-3-substituted 2-(N,N-dialkyl- (or N-piperidino-) carboxamidomethylthio)-4(3H)-quinazolinones have been prepared (see Tables I–V).

TABLE I
6,8-DIBROMO-3-SUBSTITUTED 2-(N,N-METHYLPHENYLCARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLINONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	58	87	$C_{23}H_{17}Br_2N_3O_2S$
o-$CH_3C_6H_4$	40	246	$C_{24}H_{19}Br_2N_3O_2S$
m-$CH_3C_6H_4$	50	83	$C_{24}H_{19}Br_2N_3O_2S$
p-$CH_3C_6H_4$	55	98	$C_{24}H_{19}Br_2N_3O_2S$
p-ClC_6H_4	50	95	$C_{23}H_{16}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	55	104	$C_{24}H_{19}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	60	218	$C_{25}H_{21}Br_2N_3O_3S$
n-C_4H_9	35	200	$C_{21}H_{21}Br_2N_3O_2S$
$C_6H_5CH_2$	53	221	$C_{24}H_{19}Br_2N_3O_2S$

[a] Crystallization solvent: EtOH. [b] All compounds were analyzed for N, S. The analytical results were within ±0.3% of the calculated values.

TABLE II
6,8-DIBROMO-3-SUBSTITUTED 2-(N,N-ETHYLPHENYLCARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLINONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	65	106	$C_{24}H_{19}Br_2N_3O_2S$
o-$CH_3C_6H_4$	50	105	$C_{25}H_{21}Br_2N_3O_2S$
m-$CH_3C_6H_4$	40	295 dec	$C_{25}H_{21}Br_2N_3O_2S$
p-$CH_3C_6H_4$	75	121	$C_{25}H_{21}Br_2N_3O_2S$
m-ClC_6H_4	45	248 dec	$C_{24}H_{18}Br_2ClN_3O_2S$
p-ClC_6H_4	65	110	$C_{24}H_{18}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	55	114	$C_{25}H_{21}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	70	104	$C_{26}H_{23}Br_2N_3O_3S$
$C_6H_5CH_2$	35	258 dec	$C_{25}H_{21}Br_2N_3O_2S$

[a] Crystallization solvent: EtOH. [b] All compounds were analyzed for Br, N. The analytical results were within ±0.3% of the calculated values.