chloride was prepared from 3.75 g (0.01 mol) of **9** by a procedure similar to that for **32**. This was dissolved in 30 mL of benzene and heated to a refluxing temperature. To the boiling mixture was added portionwise 1.72 g (0.013 mol) of 1,3-isopropylideneglycerol under efficient stirring. During the addition period, the benzene was distilled slowly to remove the vapor of HCl produced from the reaction mixture. After the addition was complete, the mixture was heated for an additional 2 h under slow distillation of the benzene and condensed to afford the crude **81**, which was used usually for the subsequent reactions. This crude product was purified by column chromatography on silica gel with $CHCl_3$ as eluent. The fraction which had R_f 0.5 (silica gel F_{254} Merck, with the same solvent) was collected and crystallized to afford pure **81** (Table VI). A mixture of the above crude **81**, 6.18 g (0.1 mol) of H_3BO_3, and 25 mL of 2-methoxyethanol was heated on a boiling water bath for 1 h. After cooling the mixture, about 50 mL of H_2O was added, and the precipitated solid products were collected, washed with H_2O, and crystallized to afford **97** (Table V): TLC [silica gel G impregnated with 10% H_3BO_3, $CHCl_3$–Me_2CO (9:1)] R_f 0.14; UV (EtOH) λ_{max} 296 nm (ϵ 44065); IR (KBr) 3350 (OH), 1698 (ester C=O); NMR ($CDCl_3$) 7.67 ppm (olefinic proton).

2-[*p*-(Myristyloxy)-α-methylcinnamoyl]-1,3-benzylideneglycerol (83) and 2-[*p*-(Myristyloxy)-α-methylcinnamoyl]-glycerol (106; Method H). To a solution of the acid chloride, prepared from 3.75 g (0.01 mol) of **9** in 25 mL of $CHCl_3$, were added 2.34 g (0.013 mol) of 1,3-benzylideneglycerol and 1.52 g (0.02 mol) of pyridine under cooling at 0–5 °C. The reaction mixture was allowed to stand for 12 h at room temperature and about 25 mL of $CHCl_3$ was added. The mixture was washed with H_2O and dried. The residue obtained by evaporation of the solvent was purified by column chromatography on silica gel with $CHCl_3$ as eluent and crystallized to afford **83** (Table VI). The benzylidene group of **83** was removed by treatment with H_3BO_3 in 2-methoxyethanol as described for the removal of the isopropylidene group (method G). The crude product was recrystallized to afford pure **106** (Table V).

Cis Isomer of 97 (98). Compound **97** (8.06 g, 0.018 mol) was dissolved in 500 mL of EtOH and irradiated with a xenon lamp for 40 h. After the solvent was evaporated, the residue was recrystallized several times, yielding 780 mg of **98** (Table V): TLC [silica gel G impregnated with 10% H_3BO_3, $CHCl_3$–Me_2CO (9:1)] R_f 0.22; UV (EtOH) λ_{max} 282 nm (ϵ 40990); IR (KBr) 3350 (OH), 1720 (ester C=O); NMR ($CDCl_3$) 6.75 ppm (olefinic proton).

Monopalmitate of 97 (108; Method I). To a mixture of 4.49 g (0.01 mol) of **97**, 1.58 g (0.02 mol) of pyridine, and 50 mL of $CHCl_3$ was added 2.75 g (0.02 mol) of palmitoyl chloride. After standing overnight, the solvent was evaporated and the residue was crystallized by adding a small amount of MeOH. These crude crystals were chromatographed on silica gel, $CHCl_3$–Me_2CO (9:1) as the eluent to yield **108** (Table VII): NMR ($CDCl_3$) 4.25 (m, 5 H protons attached to glycerol), 2.7 ppm (m, 1 H, OH).

Folate Analogues Altered in the C^9–N^{10} Bridge Region. 14. 11-Oxahomofolic Acid, a Potential Antitumor Agent

M. G. Nair,* Colleen Saunders, Shiang-Yuan Chen,

Department of Biochemistry, College of Medicine, University of South Alabama, Mobile, Alabama 36688

Roy L. Kisliuk, and Y. Gaumont

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111.
Received July 16, 1979

The chemical synthesis of 11-oxahomofolic acid (**2**) has been carried out using an unambiguous procedure. Reaction of methyl *p*-hydroxybenzoate with β-propiolactone gave 3-[*p*-(carbomethoxy)phenoxy]propionic acid (**7**), which was converted to 1-bromo-4-[*p*-(carbomethoxy)phenoxy]-2-butanone (**8**) by the Arndt–Eistert procedure. Protection of the carbonyl group of **8** as the oxime resulted in the formation of **10**, which on reaction with potassium phthalimide in the presence of crown-18 ether as a catalyst gave 1-phthalimido-4-[*p*-(carbomethoxy)phenoxy]-2-butanone oxime (**11**). Hydrazinolysis of **11** gave 1-amino-4-[*p*-(carbomethoxy)phenoxy]-2-butanone oxime (**4**), which was used as the key intermediate for the construction of 11-oxahomofolic acid (**2**) by modifications of the Boon and Leigh procedure. The dithionite reduction product of **2**, 7,8-dihydro-11-oxahomofolic acid, served as a substrate of *Lactobacillus casei* dihydrofolate reductase and exhibited a relative rate of 50% of the natural substrate under identical conditions. The catalytic reduction product of 11-oxahomofolic acid consisting of a mixture of diastereomers exhibited powerful antifolate activity against both MTX-sensitive and -resistant *L. casei* and *Streptococcus faecium*. The enzymatic reduction product of 7,8-dihydro-11-oxahomofolate having the "natural" configuration at C^6 exhibited good antifolate activity against both MTX-sensitive and -resistant strains of *L. casei* and *S. faecium*. This paper details the synthesis and preliminary biological evaluation of an antifol, which is a substrate of *L. casei* dihydrofolate reductase in its 7,8-dihydro form and the resulting enzymatic reduction product capable of inhibiting the growth of the same organism from which the enzyme was derived. Thus, 7,8-dihydro-11-oxahomofolic acid has been shown to be potentially capable of inducing a "lethal synthesis" in *L. casei*.

As early as 1961, Misra[1] and co-workers suggested the possibility of developing antitumor agents which might be capable of exhibiting selective toxicity against MTX-resistant tumor cells by exploiting the high levels of dihydrofolate reductase present in these cell lines. Ideally, the potential drug should be a substrate of this enzyme and the product thus formed should be capable of interfering with tetrahydrofolate utilization in such a manner that this process will lead to cytotoxicity.[2] If this concept could be realized in vivo, then it appeared possible to generate a cytotoxic drug preferentially in the resistant tumor cells as opposed to the normal ones.

This hypothesis was first put to experimental test when DeGraw and co-workers[3] synthesized homofolic acid (**1**). The 7,8-dihydro derivative of homofolic acid served as an

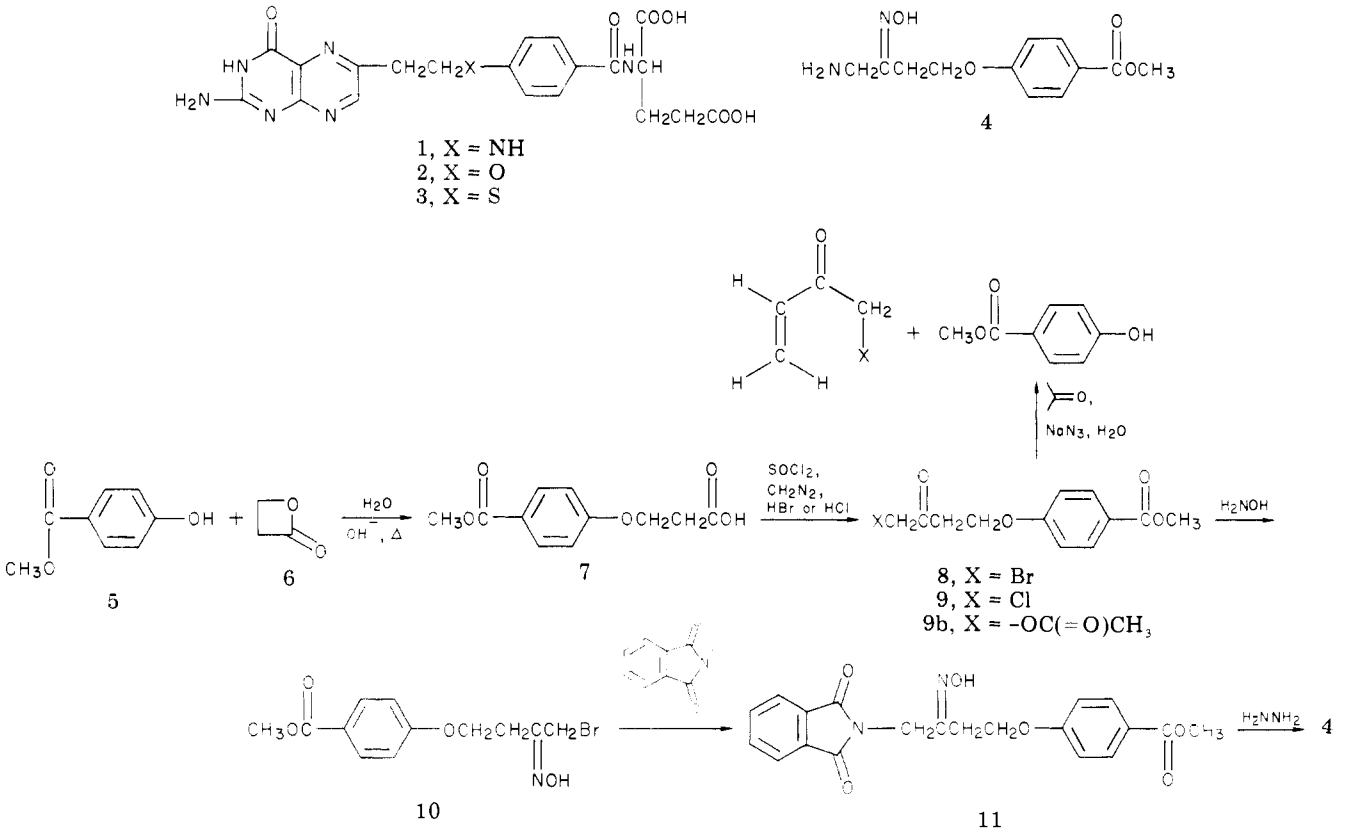
(1) Misra, D. K.; Humphreys, S. R.; Friedkin, M.; Goldin, A.; Crawford, E. J. *Nature (London)* **1961**, *189*, 39.

(2) Friedkin, M.; Crawford, E. J.; Plante, L. T. *Ann. N.Y. Acad. Sci.* **1971**, *186*, 209.

(3) DeGraw, J. I.; Marsh, J. P., Jr.; Acton, E. M.; Crews, O. P.; Mosher, C. W.; Fjuiwara, A. N.; Goodman, L. *J. Org. Chem.* **1965**, *30*, 3404.

0022-2623/80/1823-0059$01.00/0

Scheme I



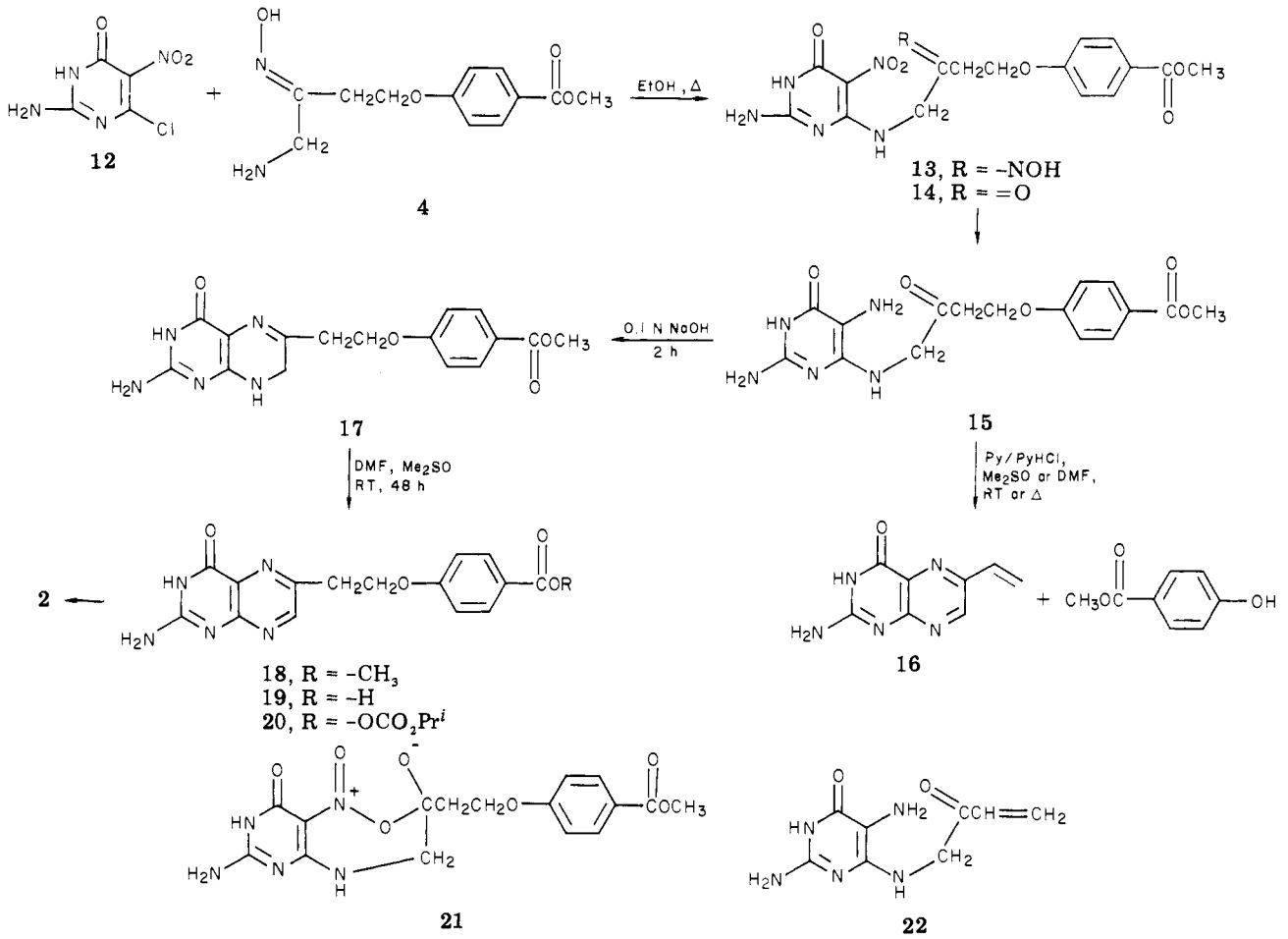
excellent substrate of dihydrofolate reductase. The catalytic reduction product of homofolic acid, consisting of a mixture of diastereomers, antagonized the growth of *L. casei* and *S. faecium* and showed antitumor activity against MTX-resistant L1210/FR8 leukemia in mice.[4,5] The enzymatic reduction product of 7,8-dihydrohomofolic acid possessing the natural[6] configuration at C^6, although it inhibited thymidylate synthetase derived from *Escherichia coli*,[7] unfortunately served as a pseudocofactor in the thymidylate synthetase reaction in *L. casei* and *S. faecium*.[8,9] Since *L. casei* DHFR was used to prepare the optically pure tetrahydro derivative, homofolic acid narrowly missed the properties of a potential folate analogue which could induce an in vivo lethal synthesis in an organism. The antifolate activity of the catalytic reduction product of homofolic acid for *L. casei* was due to the presence of the "unnatural" isomer in the reaction product.[8,9] The pseudocofactor activity of tetrahydrohomofolate has been attributed to its ability to be converted to N^5,N^{11}-methylenetetrahydrohomofolate. We reasoned that if the formation of this methylene bridge could be prevented in a close analogue of homofolic acid, the enzymatically reduced tetrahydro derivative of this analogue might interfere with tetrahydrofolate utilization. The first experimental test of this hypothesis was conducted with 11-thiohomofolic acid,[10] which showed some encouraging results. As part of a continuing program[10-16] aimed at developing such analogues of folic acid, we detail the synthesis and preliminary biological evaluation of 11-oxahomofolic acid in this paper.

Chemistry. Reaction of methyl *p*-hydroxybenzoate (**5**) with β-propiolactone (**6**) in the presence of base gave an acceptable yield of 3-[*p*-(carbomethoxy)phenoxy]propionic acid (**7**). Alternative attempts to prepare this compound by reacting methyl *p*-hydroxybenzoate with either 3-bromo- or 3-iodopropionic acid under varying conditions, including the use of cesium bicarbonate,[11,12] were unsuccessful. Subsequent elaborations of **7** to the bromomethyl ketone **8** and the chloromethyl ketone **9** were accomplished in good yield by the use of the Arndt–Eistert procedure.

In an attempt to prepare the chloromethyl ketone **9** more directly, the Michael addition reactions of methyl *p*-hydroxybenzoate with chloromethyl, hydroxymethyl and

(4) Mean, J. A. R.; Goldin, A.; Kisliuk, R. L.; Friedkin, M.; Plante, L.; Crawford, E. J.; Kwok, G. *Cancer Res.* **1966**, *26*, 2374.
(5) Mishra, L. C.; Parmar, A. S.; Mead, J. A. R. *Proc. Am. Assoc. Cancer Res.* **1970**, *11*, 57.
(6) Fontecillia-Camps, J. C.; Bugg, C. E.; Temple, C., Jr.; Rose, J. D.; Montgomery, J. A.; Kisliuk, R. L. In "Chemistry and Biology of Pteridines"; Kisliuk, R. L.; Brown, G. M., Eds.; Elsevier/North Holland: Amsterdam, 1979; p 235.
(7) Goodman, L.; DeGraw, J. I.; Kisliuk, R. L.; Friedkin, M.; Pastore, E. J.; Crawford, E. J.; Plante, T. L.; Nahas, A.; Morningstar, J. F., Jr.; Kwok, G.; Wilson, L.; Donovan, E. F.; Ratzan, J. *J. Am. Chem. Soc.* **1964**, *86*, 308.
(8) Kisliuk, R. L.; Gaumont, Y. "Chemistry and Biology of Pteridines"; International Acadmic Printing Co., Ltd.: Tokyo, 1970.
(9) Kisliuk, R. L.; Gaumont, Y. *Ann. N.Y. Acad. Sci.* **1971**, *186*, 438.
(10) For the previous paper in this series, see Nair, M. G.; Chen, S. Y.; Kisliuk, R. L.; Gaumont, Y.; Strumpf, D. *J. Med. Chem.* **1979**, *22*, 850.
(11) Nair, M. G.; O'Neal, P. C.; Baugh, C. M.; Kisliuk, R. L.; Gaumont, Y.; Rodman, M. *J. Med. Chem.* **1978**, *21*, 673.
(12) Nair, M. G.; Campbell, P. T. *J. Med. Chem.* **1976**, *19*, 825.
(13) Nair, M. G.; Campbell, P. T.; Braverman, E.; Baugh, C. M. *Tetrahedron Lett.* **1975**, *31*, 2645.
(14) Nair, M. G.; Campbell, P. T.; Baugh, C. M. *J. Org. Chem.* **1975**, *40*, 1745.
(15) Nair, M. G.; Baugh, C. M. *J. Med. Chem.* **1974**, *17*, 223.
(16) Nair, M. G.; Chen, S. Y.; Baugh, C. M.; Strumpf, D.; Gaumont, Y.; Kisliuk, R. L. In "Chemistry and Biology of Pteridines"; Kisliuk, R. L.; Brown, G. M., Eds.; Elsevier/North Holland: Amsterdam, 1979; p 255.

Scheme II



acetoxymethyl vinyl ketones[10,17] were investigated. Although these reactions were successful with the use of thiophenols, no reactions took place between the first two vinyl ketones and methyl *p*-hydroxybenzoate under various conditions. However, the reaction proceeded to a small extent with acetoxymethyl vinyl ketone, giving the addition product **9b** in ~25% yield. Attempts to convert this product to the chloromethyl ketone **9** via the corresponding hydroxymethyl ketone were unsuccessful due to the occurrence of the retro reaction when **9b** was treated with either acidic or basic reagents.

Reaction of either bromomethyl ketone 8 or chloromethyl ketone **9** with sodium azide in aqueous acetone or methanol gave methyl *p*-hydroxybenzoate by way of the retro Michael reaction.[18] Therefore, an alternate route to the construction of the side-chain **4** was explored in accordance with the procedures which have been previously developed for the preparation of the sulfur analogue.[10] The carbonyl group of 8 was protected as the oxime by reacting it with hydroxylamine hydrochloride and sodium methoxide in methanol. The oxime **10** thus obtained was reacted with potassium phthalimide in acetonitrile using crown-18 ether as a catalyst to obtain an intermediate **11**, which was potentially capable of yielding **4** (Scheme I).

Hydrazinolysis of **11** in the usual manner[11-14] gave crystalline **4** in good yield. Reaction of this amino compound **4** with 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine[15] (**12**) in methanol, using 1 equiv of *N*-methylmorpholine as a proton acceptor, gave **13**, which on treatment with 1 N HCl in trifluoroacetic acid yielded **14** (Scheme II). The next step involved the reduction of the nitro group of the pyrimidine ring. This was accomplished in 40% yield by treatment of **14** with sodium dithionite in aqueous DMF according to procedures previously reported from this laboratory.[11-14] The low yield of the reduction product was later established to be due to the cleavage of the side chain of **15** by a retro Michael reaction. In order to gain some insight into this cleavage reaction, the stability of intermediate **14** was examined under conditions which were employed for its dithionite reduction, such as pH and temperature. From these experiments, it was established that compound **14** was indeed a very stable compound which could tolerate a wide pH range in solution and was also thermally stable at this pH range. Next, we investigated the stability of some 4-phenoxy-2-butanone derivatives which were found to be unstable under these conditions, and methyl *p*-hydroxybenzoate was formed quantitatively. These results clearly suggested that the unusual stability of **14** must have its origin in the neighboring 5-nitro group of the pyrimidine ring, and this neighboring group participated in stabilizing the system from the retro Michael reaction (structure **21**). The possibility of such a neighboring group participation was invoked by us previously as we tried to explain the resistance of the nitro group of such systems to various reducing agents.[14] In the absence of such a stabilizing effect, such as when the nitro group is reduced to the amino, the butanone system becomes susceptible to the retro Michael reaction.

Compound **15** was dissolved in purified, dimethylamine-free DMF and subjected to thermal cyclization and aerobic oxidation.[15,11] The formation of a pteridine was

(17) Hennion, G. F.; Kupiecki, F. P. *J. Org. Chem.* **1953**, *18*, 1601.
(18) Chen, S. Y.; Nair, M. G. *J. Org. Chem.* **1978**, *43*, 4143.

Table I. Inhibition of Microbial Growth by Various 11-Oxahomofolates

compd[a]	concn determined by	ng/mL required for 50% inhibition			
		S. faecium ATCC8043	*S. faecium* MTX resistant	*L. casei* ATCC7469	*L. casei* MTX resistant
11-oxahomofolate	weight	14	260	320	1000
11-oxadihydrohomofolate	ϵ 9600 at 270 nm, pH 7.4	14	520	240	700
dl-11-oxatetrahydrohomofolate	ϵ 10 250 at 301 nm, pH 7.4	0.7	50	10	19
l-11-oxatetrahydrohomofolate	ϵ 9600 at 270 nm, pH 7.4	9	>200	56	64
methotrexate	weight	0.05	3500	0.07	$>5 \times 10^5$

[a] *l*-11-Oxatetrahydrohomofolate did not support the growth of *Lactobacillus casei* at 200 ng/mL; *l* refers to the natural configuration at carbon-6.

apparent by examination of the UV spectrum of the reaction mixture after 2 h at 120 °C. On treating **15** with dry Me_2SO, identical results were obtained. The NMR and UV spectra of this pteridine were in agreement with structure **16**, formed via the cyclization and oxidation of the retro Michael reaction product **22**.

The ring-closure reaction of **15** was also investigated with the use of a mixture of pyridine, HCl, and EtOH as described previously for similar compounds.[11-14] Although the occurrence of this reaction was apparent, it was accompanied by the complete loss of the side chain. In a separate attempt when **15** was treated with 0.1 N NaOH, it was cyclized smoothly to a dihydropteridine in 2 h with the retention of the side chain. Oxidation of this dihydropteridine to a pteridine was carried out by the use of a mixture of DMF and Me_2SO under aerobic conditions. Hydrolysis of this oxidation product according to Mautner's procedure[19] gave the crude pteroic acid analogue, which was purified by ion-exchange chromatography. The NMR and UV spectrum of this compound exhibited all the spectral characteristics expected of a compound having the required structure **19**.

The only step that remained for the completion of the synthesis of 11-oxahomofolic acid (**2**) was the introduction of the glutamate moiety to the pteroiate analogue **19**. The solid-phase method, which has been used successfully for other analogues reported from this laboratory, was found to be unsatisfactory with this compound. The carboxyl group of **19** was activated by the isobutyl chloroformate method to the mixed anhydride **20**, which on reaction with diethyl L-glutamate gave the diethyl ester of **2**. Without isolating the diethyl ester, the reaction product was hydrolyzed with 0.33 N NaOH in acetonitrile,[19] and the mixture was chromatographed on a DEAE-cellulose column. Two clean products were eluted from the column. The less polar fraction was identified to be **19** and the more polar fraction, which was obtained in 55% yield, was proven to be the desired product **2**. The light yellow compound that was obtained by this method showed all the spectral characteristics in complete harmony with structure **2**.

An aqueous solution of the potassium salt of **2**, when treated with excess sodium dithionite at room temperature according to a previous procedure,[10] gave the 7,8-dihydro derivative which was used for both antibacterial and enzyme studies reported in this paper.

Interaction of 11-Oxahomofolates with DHFR and TS. Biological Evaluation. The antifolate activities of four compounds in this series were examined in terms of their ability to inhibit the growth of both the MTX-sensitive and -resistant strains of *Streptococcus faecium* (ATCC 8043) and *Lactobacillus casei* (ATCC 7469). 7,8-Dihydro-11-oxahomofolate was prepared by the dithionite reduction of **2** as described previously.[10] *d,l*-L-Tetrahydro-11-oxahomofolic acid was prepared from the parent compound by catalytic reduction according to the procedure of Blakley.[20] *l*,L-Tetrahydro-11-oxahomofolate was prepared by the enzymatic reduction of the 7,8-dihydro derivative using *L. casei* dihydrofolate reductase.[21] The antifolate activities of these analogues against methotrexate-sensitive and -resistant strains of the above-mentioned organisms are summarized in Table I. The I_{50} values of all the four compounds ranged from 0.7 to 320 ng/mL for the sensitive strains of both *S. faecium* and *L. casei*. The parent compound, 11-oxahomofolic acid, showed moderate activity against MTX-resistant *S. faecium* but showed weak inhibitory potency against MTX-resistant *L. casei*. The corresponding dihydro derivative, i.e., 7,8-dihydro-11-oxahomofolate, although showing good inhibitory potency against both MTX-sensitive *S. faecium* and *L. casei*, was a weaker inhibitor of the growth of the corresponding MTX-resistant strains (I_{50} = 520 and 700 ng/mL).

The catalytic reduction product of **2**, consisting of a mixture of the diastereomers of 5,6,7,8-tetrahydro-11-oxahomofolate, showed powerful inhibition of all strains of the above-mentioned organisms. The antifolate activity of this mixture against methotrexate-resistant *L. casei* is very striking. The I_{50} value of this growth inhibition is 19 ng/mL, compared to $>5 \times 10^5$ ng/mL for MTX. Even more noteworthy is the ability of the "natural diastereomer" of 5,6,7,8-tetrahydro-11-oxahomofolate to inhibit the growth of MTX-resistant *L. casei* by 50% at 64 ng/mL. Examination of Table I also reveals that whether the tetrahydro derivatives are derived from catalytic reduction of the parent compound or by enzymatic reduction of the dihydro derivative, they are equally potent in inhibiting both MTX-sensitive and -resistant strains of *L. casei* (I_{50} = 10 and 19 ng/mL for the former and 57 and 64 ng/mL for the latter).

From these results, it is apparent that the tetrahydro derivative possessing the unnatural configuration at C^6 is three to five times more active than the corresponding derivative possessing the natural configuration at this position. Yet, the excellent antifolate activity exhibited by the natural isomer against MTX-resistant *L. casei* is in direct contrast to the behavior of the natural isomer of

(19) Kim, Y. H.; Gaumont, Y.; Kisliuk, R. L.; Mautner, H. G. *J. Med. Chem.* **1975**, *18*, 776.

(20) Blakley, R. L. *Biochem. J.* **1957**, *65*, 331.

(21) Chaykovsky, M.; Rosowsky, A.; Papathanosopoulos, N.; Chen, K. N.; Modest, E. J.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1974**, *17*, 1212.

tetrahydrohomofolate, which has been shown to be a pseudocofactor in the thymidylate synthetase reaction in both *S. faecium* and *L. casei*.[8,9]

In an attempt to gain some insight into the mechanism by which these compounds exhibit their antifolate activities, they were investigated in vitro as substrates or inhibitors of *L. casei* dihydrofolate reductase[21] (DHFR, EC 1.5.1.3) and thymidylate synthetase[22] (TS, EC 2.1.1.4.5). At pH 7.4, 7,8-dihydro-11-oxahomofolate at 5.0×10^{-5} M was reduced by *L. casei* dihydrofolate reductase at 50% the rate of dihydrofolate under the same conditions. None of the 11-oxahomofolate derivatives were inhibitors of *L. casei* dihydrofolate reductase or thymidylate synthetase at concentrations lower than 4×10^{-5} M.

Folate analogues that are altered in the C^9–N^{10} bridge region are designed and evaluated in our laboratories to achieve the following main purpose. We are interested in developing synthetic substrates of dihydrofolate reductase,[23] which in their enzymatically reduced tetrahydro form are capable of interfering with tetrahydrofolate utilization. Since the enzyme dihydrofolate reductase is elevated in many MTX-resistant tumor cells,[1,24,25] we hope to exploit this elevation of the enzyme for the selective generation of a potential antifol in these cell lines to achieve selective toxicity in the chemotherapy of MTX-resistant tumors. Results with 7,8-dihydro-11-oxahomofolate (which is a substrate for the enzyme DHFR) and the antifolate l-L-tetrahydro-11-oxahomofolate (which is the enzymatic reduction product) suggest that such an approach is experimentally feasible. One limiting factor to the accomplishment of the proposed concept of in vivo lethal synthesis is the transport of the potential substrate to the tumor cells. Since there is no direct correlation between the transport receptors of MTX-sensitive and -resistant bacteria and those of mammalian cell lines, we are currently in the process of evaluating the biological activities and transport characteristics of these oxahomofolate derivatives in HeLa cells.

Experimental Section

Melting points were determined on a Fisher Model 355 digital melting point analyzer and NMR spectra were run in $CDCl_3$ or CF_3COOH on a 90 MHz Perkin-Elmer R-32 spectrometer with Me_4Si as internal lock signal. Field strength of the various proton resonances are expressed in δ (parts per million) and coupling constants in hertz. Peak multiplicity is depicted as usual: s, singlet; d, doublet; t, triplet; q, quartet; br, broadened singlet or unresolved multiplet, the center of which is given. UV spectra were determined on a Beckman Model 25 spectrophotometer. Ion-exchange chromatography was carried out on DEAE-cellulose in the chloride form with 1.2 × 22 cm packing, unless otherwise specified. A linear NaCl gradient of 1 L each of 0–0.5 M NaCl in 0.005 M phosphate buffer, pH 7, was used to elute the column. Elemental analyses were either by Galbraith Laboratories, Inc., Knoxville, TN, or Integral Microanalytical Lab, Raleigh, N.C. Where analyses are indicated only by symbols of elements, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values. Yields represent the amount of pure compound isolated.

Preparation of 3-[*p*-(Carbomethoxy)phenoxy]propionic Acid (7). To 400 mL of distilled water in an Erlenmeyer flask was added 15.2 g (0.1 mol) of methyl *p*-hydroxybenzoate, and the mixture was slowly heated to ~80 °C under stirring with the dropwise addition of 100 mL of 1 N NaOH. The clear solution thus obtained was treated with 8 mL of β-propiolactone, and the mixture was allowed to stir at this temperature for 0.5 h. The solution was then cooled to room temperature and acidified with 6 N HCl to pH 3. The precipitated material was extracted with three 100-mL portions of ether, and the ether extract was washed twice with distilled water and extracted several times with 50-mL portions of saturated sodium bicarbonate until all the effervescence ceased on further addition of $NaHCO_3$. The bicarbonate extracts were combined and acidified to pH 3 with 6 N HCl. The white precipitate thus obtained was washed with water and dried: yield 7.4 g (33%); mp 146–147 °C. Anal. ($C_{11}H_{12}O_5$) C, H, O.

Conversion of 7 to 1-Bromo-4-[*p*-(carbomethoxy)phenoxy]-2-butanone (8). A suspension of 11.2 g (50 mmol) of **7** in 100 mL of benzene was treated with 50 mL of thionyl chloride and refluxed for 2 h. The solution was evaporated to dryness under reduced pressure and dried overnight under vacuum. This acid chloride was dissolved in 50 mL of methylene chloride and added slowly through a dropping funnel to an ice-cold ethereal solution of 150 mmol of diazomethane, and the reaction was allowed to proceed at room temperature for 1 h. After this period, dry HBr was bubbled through the reaction mixture for 20 min and the solvents were immediately removed by evaporation under reduced pressure. The crude product, which was obtained in 80% yield, was recrystallized from a mixture of benzene and hexane: mp 101–102 °C; MS *m/e* 300 and 302, with equal intensity; TLC single spot on plate in several solvent systems; NMR δ 3.15, 4.35 (t, t, 4 H, ethylene), 4.0 (s, 3 H, carbomethoxy), 4.09 (s, 2 H, bromomethyl), 7.0, 8.1 (d, d, 4 H, aromatic). This compound did not give a satisfactory elemental analysis.

Since compound **8** failed to give a satisfactory elemental analysis, the corresponding chloromethyl ketone was made by substituting HCl for HBr in the above Arndt–Eistert procedure. The crude chloromethyl ketone **9** thus obtained was recrystallized from a mixture of CH_2Cl_2 and hexane as white clusters of needles: yield 76%; mp 100 °C. Anal. ($C_{12}H_{13}ClO_4$) C, H, Cl.

Preparation of 1-Acetoxy-4-[*p*-(carbomethoxy)phenoxy]-2-butanone (9b). In a round-bottom flask a solution of 3 g of acetoxymethyl vinyl ketone (23.4 mmol) in 100 mL of benzene was refluxed with 4 g of methyl *p*-hydroxybenzoate (22.7 mmol) and 0.1 mL of triethylamine for 1 h. The solvent was removed by evaporation, and the residue was dissolved in CH_2Cl_2 and extracted with two 20-mL portions of 0.1 N NaOH. The methylene chloride layer was washed, dried with Na_2SO_4, and evaporated to a gum, which on standing gave yellow crystals. The colorless crystalline product, **9b**, was obtained in 27% yield (1.8 g) after recrystallization of the crude product from a mixture of chloroform and pentane: mp 82–83 °C; the compound showed NMR resonances δ 7.95, 6.85 (d, d, 4 H, aromatic), 4.7 (s, 2 H, acetoxymethyl), 4.3, 2.9 (t, t, 4 H, ethyl), 3.85 and 2.15 (s, s, 6 H, methoxy, acetyl) in complete agreement with the desired structure. Anal. ($C_{14}H_{16}O_6$) C, H, O.

Treatment of **9b** with either acid or base in an attempt to convert it to the corresponding hydroxymethyl compound gave methyl *p*-hydroxybenzoate.

Conversion of 8 to 1-Bromo-4-[*p*-(carbomethoxy)phenoxy]-2-butanone Oxime (10). To a solution of 1.512 g (28 mmol) of sodium methoxide in 250 mL of methanol in a round-bottom flask was added 1.96 g (35 mmol) of hydroxylamine hydrochloride. A solution of 8.43 g (28 mmol) of compound **8** in 80 mL of THF was added to the methanolic solution of hydroxylamine, and the reaction was allowed to proceed under stirring at 25 °C for 18 h. After this period, the solvents were removed under vacuum and the viscous residue was chromatographed on a column made of silica gel, CC_4. A 1:1 mixture of benzene–chloroform eluted the product. Evaporation of the benzene–chloroform fraction gave a white solid, which was shown to be a mixture of the expected anti and syn isomers (NMR) of the oxime: yield 6.45 g (73%); mp 96 °C; NMR δ 2.95 (t, 2 H, ethylene), 3.89 (s, 3 H, carbomethoxy), 4.1 (s, 2 H, bromomethyl), 4.3 (t, 2 H, ethylene), 6.9 and 7.95 (d, d, 4 H, aromatic); MS *m/e* 315, 317 (M^+, equal intensity).

Preparation of 1-Phthalimido-4-[*p*-(carbomethoxy)phenoxy]-2-butanone Oxime (11). To a solution of 7.086 g of crown-18 ether (300 mg/mmol of **10**) in 500 mL of acetonitrile was added 8.739 g (47.24 mmol) of powdered potassium

(22) Wahba, A. J.; Friedkin, M. *J. Biol. Chem.* **1962**, *237*, 3794.
(23) Nair, M. G.; Kisliuk, R. L. "Abstracts of Papers", ACS/CSJ Chemical Congress, Honolulu, Hawaii, 1979; MEDI-053.
(24) Friedkin, M.; Crawford, E. J.; Humphreys, S. R.; Goldin, A. *Cancer Res.* **1962**, *22*, 600.
(25) Hillcoat, B. L.; Swett, V.; Bertino, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 1632.

phthalimide, and the mixture was allowed to stir for 5 min when most of the potassium phthalimide went into solution. This was followed by the addition of 7.46 g (23.62 mmol) of **10** and stirring was continued for a period of 4 h at 25 °C. The reaction mixture was evaporated to dryness under vacuum, and the residue was dissolved in 30 mL of chloroform and chromatographed over a silica gel CC_4 column. Elution with chloroform and evaporation of the solvent gave compound 11 as a white crystalline compound in 84% yield: mp 230 °C; NMR (Me_2SO–$CDCl_3$) δ 2.85 (t, 2 H, ethylene), 3.85 (s, 3 H, carbomethoxy), 4.3 (t, 2 H, ethylene), 4.5 (s, 2 H, methylene), 6.85, 7.9 (d, d, 4 H, aromatic), 7.85 (C, 4 H, phthalimide). Anal. ($C_{20}H_{18}N_2O_6$) C, H, N, O.

Hydrazinolysis of 11. Preparation of 1-Amino-4-[*p*-(carbomethoxy)phenoxy]-2-butanone Oxime (4). A solution of 1.91 g (5 mmol) of **11** was made in a three-neck round-bottom flask by refluxing with 200 mL of absolute ethanol in an atmosphere of nitrogen. The solution was gradually cooled to 40 °C, a solution of 165 mg (5 mmol) of 95% hydrazine in 20 mL of alcohol was added, and the reaction was stirred under nitrogen for 72 h at 25 °C. Examination of the reaction mixture by TLC revealed that ~95% of the starting material had reacted during this period. The mixture was refluxed in nitrogen for an additional 30 min to complete the reaction, cooled, and 5 mL of 1 N HCl was added. The solution thus obtained was evaporated to ~10 mL, diluted to 100 mL, and acidified to pH 4.0 with 1 N HCl. The precipitated phthaloylhydrazide was removed by filtration and the hydrazinolysis product which was present in the filtrate was precipitated by the addition of 30% NH_4OH so that the pH was raised to 8. The white crystalline material thus obtained in 45% yield was washed several times with water and dried: mp 121–123 °C; NMR (CF_3COOH) δ 2.95 (t, 2 H, ethylene), 3.85 (s, 3 H, carbomethoxy), 4.1 (s, 2 H, methylene), 4.25 (t, 2 H, ethylene), 6.85 and 7.9 (d, d, 4 H, aromatic).

Preparation of 1-[*N*-(2-Amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]-4-[*p*-(carbomethoxy)phenoxy]-2-butanone Oxime (13). The hydrazinolysis product (**4**) was condensed with 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine (**12**) by refluxing a mixture of 1 mmol each of these compounds with 75 mL of methanol in the presence of 1 equiv of *N*-methylmorpholine for 4 h. After this period, the reaction mixture was concentrated to 25 mL by boiling off methanol in a stream of N_2. On cooling in an ice bath, compound **13** separated from the reaction mixture as light yellow crystals. The product was washed several times with water and finally with methanol: yield 227 mg (56%); mp 152 °C; UV (0.1 N NaOH) λ_{max} 347 and 255 nm. Anal. ($C_{16}H_{18}N_6O_7 \cdot 0.25H_2O$) C, H, N, O.

Deprotection of 13. Preparation of 1-[*N*-(2-Amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]-4-[*p*-(carbomethoxy)phenoxy]-2-butanone (14). A solution of 1.37 g (3.38 mmol) of the oxime **13** was made in 25 mL of trifluoroacetic acid and kept in a water bath which was maintained at 55 °C. To this solution was added 25 mL of 1 N HCl dropwise during a period of 15 min while the temperature of the water bath was raised to 60 °C. The reaction mixture was evaporated to a small volume under reduced pressure at this temperature, and on addition of crushed ice, a light yellow precipitate was obtained. This precipitate was filtered and washed several times with water and dried: yield 1.1 g (83.5%); mp >300 °C; NMR (TFA) δ 3.0 (t, 2 H, ethylene), 3.8 (s, 3 H, carbomethoxy), 4.3 (t, 2 H, ethylene), 4.6 (br, 2 H, methylene), 6.8 and 7.9 (d, d, 4 H, aromatic); UV (0.1 N NaOH) λ_{max} 295 nm with a shoulder at 330 nm. Anal. ($C_{16}H_{17}N_5O_7$) C, H, N.

Dithionite Reduction of 14. Preparation of 15. Several modifications of this reduction have been carried out, each of which gave varying yields of the reduction product. The procedure that follows is typical of an experiment which gave the best result. To 25 mL of DMF in a 250-mL Erlenmeyer flask was added 1.2 g (3 mmol) of **14** and heated on a hot plate under stirring to obtain a clear solution. This solution was cooled to 50 °C and 5.0 g of solid sodium dithionite was added. This suspension was stirred vigorously while distilled water (40 mL) was added very slowly during a period of 15 min. The temperature of the reaction mixture was maintained at 50 °C during the entire 15 min. The reaction mixture was cooled and diluted to 500 mL. The precipitated reduction product was filtered, washed several times with water, and dried: yield 450 mg (42%); NMR examination revealed that this product retained the phenoxybutanone side chain; UV (0.1 N NaOH) λ_{max} 325 (s), 270 (s), 250 nm. This reduction product is unstable on exposure to air and had to be used immediately for the next step.

The filtrate of this reaction mixture was evaporated to dryness under reduced pressure and dried overnight under vacuum over P_2O_5. To the solid thus obtained, 10 mL of ethyl acetate was added, triturated with a spatula, and filtered. The filtrate on TLC examination revealed the presence of a single product which cochromatographed with authentic methyl *p*-hydroxybenzoate. The NMR spectrum of this compound was identical with that of methyl *p*-hydroxybenzoate.

Attempts to Convert 15 to Methyl 11-Oxahomopteroate (18). (A) Initial attempts to convert **15** to **18** involved procedures similar to the one-step cyclization–oxidation technique we have previously developed for converting dithionite reduction products of analogous compounds to pteridines.[13,14] In a representative experiment, 361 mg (1 mmol) of the dithionite reduction product was added to a 1:1 solution of ethanol and pyridine (whose pH was adjusted to 5.0 by the addition of concentrated HCl) in an atmosphere of nitrogen and refluxed for 1 h. The reaction mixture was cooled and evaporated to dryness under reduced pressure. The product was triturated with ether and filtered. The dark brown solid left on the filter showed a UV spectrum consistent with a pteridine structure. However, an NMR spectrum of this material showed no resonances which are attributable to either the ethylene bridge or a carbomethoxy group but exhibited complex signals in the vinyl and aromatic region. Evaporation of the ether filtrate gave a white solid which was identified as pure methyl *p*-hydroxybenzoate by comparison with an authentic sample.

(B) A small amount of the dithionite reduction product (50 mg) was suspended in 50 mL of dry DMF and heated slowly to 100–120 °C during a period of 1 h. The reaction mixture was monitored for the appearance of an absorption maxima at 365 nm in 0.1 N NaOH, which is a diagnostic procedure for the formation of a pteridine nucleus. During a period of 2 h at this temperature, a stable 365-nm λ_{max} was observed. The solvent was evaporated off under reduced pressure and the residue that was left was identified as a mixture of the pteridine and methyl *p*-hydroxybenzoate.

(C) The above experiment was repeated in Me_2SO at room temperature, and the results were similar to those of the previous two experiments. Treatment of the reduction product with trifluoroacetic acid also induced the facile and quantitative retro Michael reaction.

Preparation of 11-Oxahomopteroic Acid (19). A suspension of 722 mg (2 mmol) of the dithionite reduction product (**15**) in 100 mL of 0.1 N sodium hydroxide was stirred at room temperature for 2 h. During this period, the shoulder at 325 nm of the UV spectrum of **15** changed to a sharp well-defined peak at 320 nm and the one at 275 became more well defined at 275. The ratio of the absorbance at 275 to the one at 320 nm was approximately 2.5, which is indicative of the formation of a 7,8-dihydropteridine. The reaction mixture was acidified to pH 4.5 with glacial acetic acid and filtered. The precipitate of the dihydropteroate was washed with water and dried under vacuum for 18 h.

The dihydro derivative was dissolved in 100 mL of DMF at room temperature under stirring. To this solution, 5 mL of Me_2SO was added dropwise during a period of 8 h under aerobic conditions, and the mixture was allowed to stir for 48 h. The bright yellow solution thus obtained contained a pteridine ring system as evidence by the UV spectrum. Evaporation of DMF under vacuum at 65 °C gave a viscous yellow residue, which after cooling was stirred with a 0.33 N NaOH solution (15 mL) in acetonitrile for 6 h. The acetonitrile was evaporated off under vacuum, and the contents of the flask were diluted to 100 mL, the pH was adjusted to 7.4 with 0.1 N HCl, and the solution was chromatographed over DEAE-cellulose using a 0 to 0.5 N linear NaCl gradient at pH 7.0. The major effluent from the column which corresponded to the desired product (vide infra) was pooled, concentrated to a small volume, and acidified to pH 4.0 with glacial acetic acid. The precipitated 11-oxahomopteroic acid was collected by filtration, washed, and dried: yield 220 mg (34%); NMR (TFA) δ 3.25, 4.23 (t, t, 4 H, ethylene bridge), 6.65, 7.75 (d, d, 4 H,

aromatic), 8.68 (s, 1 H, pteridine ring); UV (0.1 N NaOH) λ_{max} 362 nm (ϵ 6502), 253 (36 800). Anal. ($C_{15}H_{13}N_5O_4$) C, H, N.

Preparation of 11-Oxahomofolic Acid (2). In an oven-dried round-bottom flask, a solution of 327 mg (1 mmol) of 11-oxahomopteroic acid in 50 mL of Me_2SO was treated with 35 mL of dry tetrahydrofuran. To this solution, 0.14 mL (1.25 mmol) of *N*-methylmorpholine was added and stirred closed for 15 min. To this reaction mixture, 0.131 mL (1 mmol) of freshly distilled isobutyl chloroformate was added and let stir for 20 min.

During this period, a solution of 480 mg (2 mmol) of diethyl-L-glutamic acid hydrochloride was made in 30 mL of Me_2SO containing 0.226 mL (2 mmol) of *N*-methylmorpholine. This solution was added to the flask containing the mixed anhydride and the coupling was allowed to proceed for 18 h. The reaction mixture was made 0.1 N with respect to sodium hydroxide by the proper addition of 1 N NaOH and hydrolyzed for 4 h. After dilution of the reaction mixture to ~500 mL with distilled water, the pH was adjusted to 7.3 and applied on a DEAE-cellulose column. The column was washed thoroughly with distilled water and eluted with a linear NaCl gradient as described before. Three products were eluted from the column. The least polar material was identified to be the monoethyl ester of 11-oxahomofolate (NMR) and the most polar compound was identified to be the desired final product. The compound which was eluted from the column possessing intermediate polarity was identified to be the starting pteroic acid analogue by comparison with an authentic sample. Rehydrolysis of the least polar material by 0.33 N NaOH in acetonitrile converted it to **2**. The pteroic acid analogue which was recovered from this reaction could be recycled to get additional amounts of **2**: NMR (TFA) δ 2–3 (C, 4 H, glutamic acid), 3.62, 4.6 (t, t, 4 H, ethylene bridge), 7.05, 7.83 (d, d, 4 H, aromatic), 8.95 (s, 1 H, pteridine ring); UV (0.1 N NaOH) λ_{max} 253 nm (ϵ 37 027), 362 (6976). Anal. ($C_{20}H_{20}N_6O_7$) C, H, N, O.

Stability Studies of Compounds 4, 8, and 9b. In a typical experiment, 50 mg of each of **4**, **8**, or **9b** were dissolved in 5 mL of trifluoroacetic acid and heated to 60 °C. To this solution, during a period of 20 min, 5 mL of 1.0 N HCl was added and the reaction mixture was evaporated to dryness. After drying under vacuum for a few hours, the residue was dissolved in 5 mL of ethyl acetate and tested for the presence of the respective starting materials and methyl *p*-hydroxybenzoate by TLC. Under these conditions, only the presence of methyl *p*-hydroxybenzoate was detected in the ethyl acetate layer.

To check the stability of these compounds under conditions which were employed for the dithionite reduction of **14**, each compound was subjected to these reduction conditions. After the addition of water, each reaction mixture was evaporated to dryness, dried under vacuum, and checked for the formation of methyl *p*-hydroxybenzoate. In each experiment, a clean single product was obtained, which was identified as methyl *p*-hydroxybenzoate.

Methods Used for Biological Testing. The preparation of 7,8-dihydro-11-oxahomofolic acid was carried out as described previously for the corresponding 11-thio analogue.[10] Catalytic reduction of 11-oxahomofolic acid gave a mixture of the diastereomers of the tetrahydro derivative, *d,l*-L-tetrahydro-11-oxahomofolic acid. The preparation of tetrahydro-11-oxahomofolic acid possessing the "natural" configuration at C^6 was carried out by substituting 7,8-dihydro-1-oxahomofolic acid for the natural substrate in the assay medium previously described for the assay of *L. casei*, DHFR,[21] and isolating the tetrahydro derivative thus formed after chromatography.

Dihydrofolate reductase,[21] thymidylate synthetase,[22] and microbiological assays[26] were carried out as described.

Acknowledgment. This investigation was supported by grants from the American Cancer Society (CH-53A) and National Institutes of Health (CA-10914 and CA-27101).

(26) Kisliuk, R. L.; Strumpf, D.; Gaumont, Y.; Leary, R. P.; Plante, L. *J. Med. Chem.* **1977**, *20*, 1531.

Heterocyclic Analogues of the Antihypertensive β-Adrenergic Blocking Agent (*S*)-2-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3-cyanopyridine

John J. Baldwin,* Edward L. Engelhardt, Ralph Hirschmann, Gerald S. Ponticello,*

Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486

Joseph G. Atkinson, Burton K. Wasson,

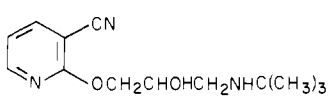
Merck Frosst Laboratories, Montreal, Canada

Charles S. Sweet, and Alexander Scriabine

Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486. *Received July 19, 1979*

The syntheses of a series of isoelectronic analogues of (*S*)-2-[3-(*tert*-butylamino)-2-hydroxypropoxy]-3-cyanopyridine (**1**) are described; included in this group are examples of thiazole, isothiazole, thiadiazole, pyrazine, and the structurally related naphthyridines. All of the compounds are similar to **1** in that they contain a cyano group ortho to the aminohydroxypropoxy side chain and meta to the nitrogen heteroatom. In addition, several related examples, having additional nuclear substituents and/or groups other than CN in the position adjacent to the aminohydroxypropoxy group, were prepared, and β-adrenoceptor antagonist activity and vasodilating potency were determined. Three compounds, thiazole **2** and isothiazoles **3** and **27**, effectively lowered mean arterial pressure in the SH rat at 5 mg/kg. Compounds **2**, **3**, and **27** increased iliac blood flow and exhibited β-adrenergic blocking properties in the dog.

An interest in the synthesis of compounds capable of lowering blood pressure by a vasodilating mode of action without inducing a concomitant increase in heart rate led to the discovery of (*S*)-2-[3-(*tert*-butylamino)-2-hydroxypropoxy]-3-cyanopyridine (**1**).[1a] This compound lowered blood pressure acutely in the spontaneously hypertensive (SH) rat and was a potent nonselective β-adrenergic blocking agent in the anesthetized dog. In this latter species, a non-β-agonist type of vasodilator action was also indicated.



1

(1) (a) Baldwin, J. J.; Lumma, W. C.; Lundell, G. F.; Ponticello, G. S.; Raab, A. W.; Engelhardt, E. L.; Hirschmann, R.; Sweet, C. S.; Scriabine, A. *J. Med. Chem.* **1979**, *22*, 1284. (b) Unpublished results.

0022-2623/80/1823-0065$01.00/0