Mesylate **26** (0.50 g, 8.75×10^{-4} mole) and NaN_3 (0.550 g, 7.7×10^{-3} mole) in 50 ml of DMF were heated with stirring to 100° overnight. The reaction mixt was poured into salt–ice H_2O and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and evapd under reduced pressure affording 0.360 g of white crystals. Recrystn twice from MeOH afforded 0.290 g (62%) of **27**, mp 128–130°. *Anal.* ($C_{30}H_{51}N_3O_4$) H, N, C: calcd, 69.59; found, 70.48.

6β-Azido-5α-cholestane-3β,5α-diol 3-Acetate (28).—Cathylate **27** (2.80 g, 5.4×10^{-3} mole) was dissolved in 100 ml of hot EtOH. Aq NaOH (10 ml 10%) was added, and the reaction mixt was refluxed for 2 hr cooled, neutralized with aq HCl (10%), and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 2.40 g of white residue. The reaction product was acetylated under the usual condns of Ac_2O in pyridine yielding 2.45 g of **28**. Recrystn twice from MeOH afforded 2.00 g (76%) of **28**: mp 154–155°; lit.[6] mp 154°.

6β-Amino-5α-cholestane-3β,5α-diol 3-Acetate (29) from 6β-Azido-5α-cholestane-3β,5α-diol 3-Acetate (28).—The method of Ponsold[6] was used to prep **29**: mp 190–191°; lit. mp 190–191°.

3,6-Dioximino-5α-cholestan-5α-ol (31).—5α-Cholestane-3,6-dion-5α-ol[2] (4.5 g, 1.1×10^{-2} mole, **30**) and 10.0 g (0.144 mole) of $HONH_2 \cdot HCl$ were suspended in 50 ml of abs EtOH and 20 ml of dry pyridine. The reaction mixt was refluxed for 7 hr, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and evapd under reduced pressure affording 4.7 g of a white residue. Recrystn twice from MeOH afforded 3.6 g (74%) of **31**, mp 188–190° dec. *Anal.* ($C_{27}H_{46}N_2O_3$) C, H, N.

3β-6β-Diamino-5α-cholestan-5α-ol (32).—Compd **31** (1.00 g, 2.2×10^{-3} mole) in 75 ml of Et_2O was added dropwise with stirring to LAH (1.00 g, 2.9×10^{-2} mole) in 50 ml of Et_2O at 0°. The reaction mixt was stirred at room temp for 24 hr. Excess LAH was decompd by dropwise addn of 1 ml of NaOH (10%) and 4 ml of H_2O. The filtrate was collected and the ppt was extd twice with THF. The combined filtrate was distd under reduced pressure affording 0.50 g (47%) of white product which crystd with difficulty from MeOH–H_2O, affording the diamine **32**, mp 145–148°. *Anal.* ($C_{27}H_{50}N_2O$) C, H, N.

3β,6β-Diacetamido-5α-cholestan-5α-ol (33).—Ac_2O (5.0 ml) in 5.0 ml of dry pyridine was added to the diamine **32** (0.01 g, 1.5×10^{-3} mole) dissolved in 2.0 ml of dry pyridine. The reaction mixt was allowed to stand at room temp overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 0.56 g of cryst residue. Recrystn from Et_2O afforded 0.31 g (42%) of diamide **33**, mp 119–120°. *Anal.* ($C_{31}H_{54}N_2O_3$) C, H; N: calcd, 6.69; found, 6.10.

Biological studies *in vitro* were carried out according to methods previously reported by Dempsey and coworkers.[16,17,23,28]

Acknowledgment.—We are grateful to the National Institutes of Health for support of this work through Grants HE-12740, HE-8364, and HE-6314 from the National Heart Institute. This investigation was supported (in part) by National Institutes of Health Research Grant No. FR-328 from the Division of Research Facilities and Resources.

(28) M. E. Dempsey, *Methods Enzymol.*, **15**, 501 (1969).

Potential Folic Acid Antagonists. 5. Synthesis and Biologic Evaluation of N^{10}-Deazapteroic Acid and N^{10}-Deazafolic Acid and Their 9,10-Dehydro Derivatives[1,2]

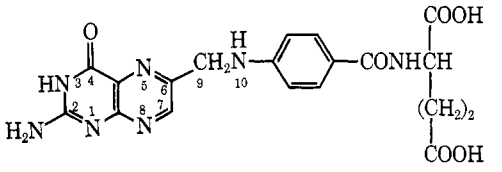
ROBERT F. STRUCK,* Y. FULMER SHEALY, AND JOHN A. MONTGOMERY

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received December 21, 1970

9,10-Dehydro-N^{10}-deazapteroic acid (V) was synthesized from 2-acetamido-6-formylpteridin-4(3*H*)-one (II) and the ylide from *p*-carbomethoxybenzyltriphenylphosphonium bromide by means of a Wittig reaction. Reduction of the acetylated methyl ester of V (IV) gave, after hydrolysis, N^{10}-deazapteroic acid (VI). Coupling of the acetylated derivatives of V and VI with diethyl glutamate by use of dicyclohexylcarbodiimide gave 9,10-dehydro-N^{10}-deazafolic acid (IX) and N^{10}-deazafolic acid (XII), respectively, after removal of acetyl and ester blocking groups. N^{10}-Deazafolic acid (XII), after reduction to the dihydro form, was shown to serve as a substrate for dihydrofolate reductase, whereas the unreduced acid XII was mildly inhibitory of the enzyme but did not serve as a substrate. The antagonistic effect of the analog XII was weak, as demonstrated by facile reversal of growth inhibition of *Streptococcus faecalis* by folic acid. No significant *in vitro* activity against human epidermoid cells or *in vivo* activity against leukemia L1210 was observed for the folic and pteroic acid analogs. Evaluation of the pteroic acid analogs in *Plasmodium berghei* revealed no antimalarial activity.

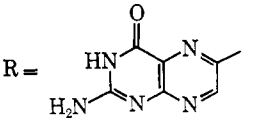
Chemistry.—Analogs of folic acid (I) are of interest for the determination of the structural features necessary for binding and inhibiting the enzymes involved in folic acid metabolism.[3] Replacement of the N in position 10 of folic acid (I) with CH_2 would alter the nucleophilicity of this portion of the molecule without significantly changing its steric properties. In addition, lacking the N^5,N^{10}-grouping, the compound would be incapable of being formylated and, therefore, functioning as a 1-C transfer intermediate.[4]

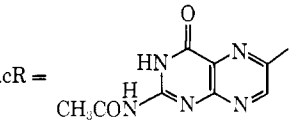


I

AcRCHO
II

AcRCH=CHC$_6$H$_5$
III





A potentially facile synthesis of N^{10}-deaza analogs of pteroic acid involved reaction of 2-acetamido-2-for-

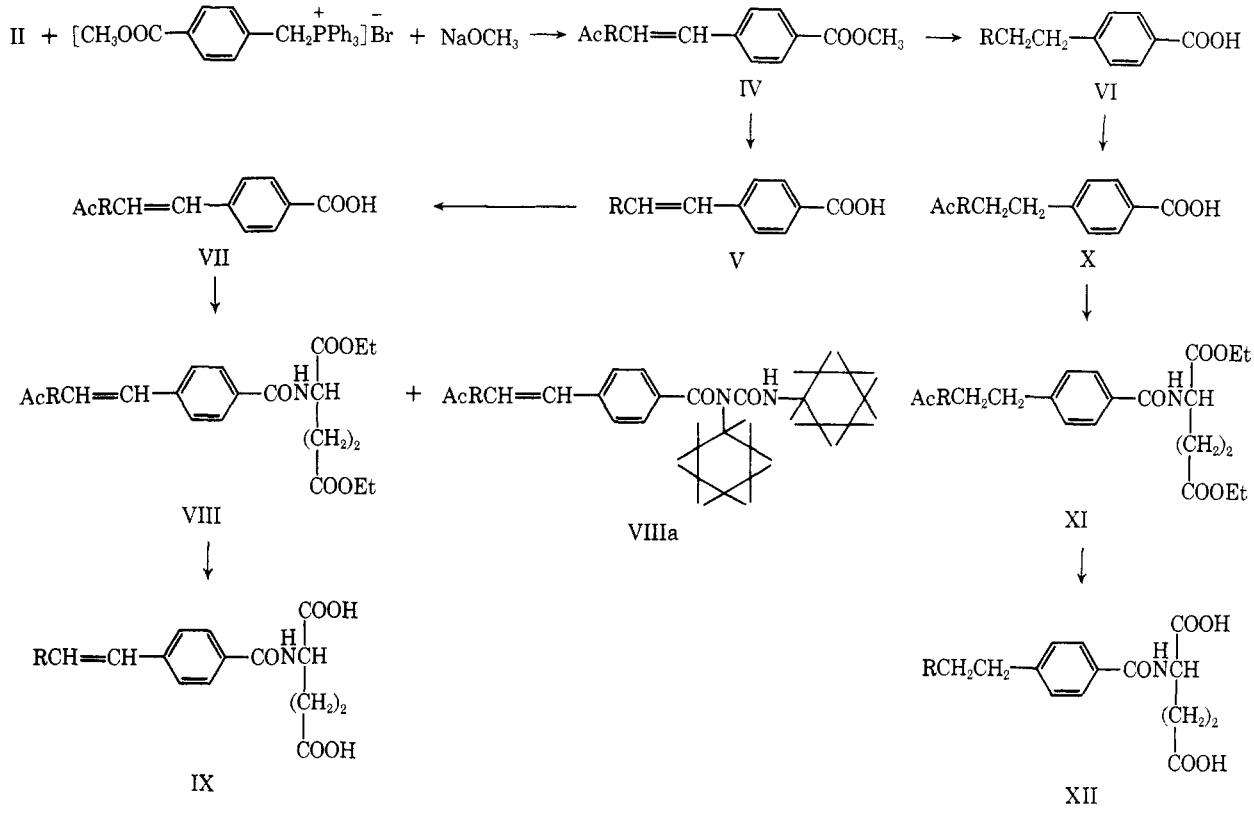
(1) This investigation was supported by Contract NIH-71-2021 with Chemotherapy, National Cancer Institute, National Institutes of Health.

(2) Part 4: R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **35**, 1676 (1970).

(3) J. A. Montgomery, T. P. Johnston, and Y. F. Shealy, in "Medicinal Chemistry," 3rd ed, A. Burger, Ed., Wiley, New York, N. Y., 1970, p 680.

(4) E. C. Roberts and Y. F. Shealy, *J. Med. Chem.*, **14**, 125 (1971).

SCHEME I



mylpteridin-4(3*H*)-one (II)[5] with an appropriate P ylide in a normal Wittig reaction. In order to investigate the method, benzyltriphenylphosphonium chloride was converted to the corresponding ylide with NaOEt in EtOH and treated with II. Isolation of the expected product III in good yield indicated that the route should be applicable to the synthesis of para-substituted types. The method was applied using *p*-carbomethoxybenzyltriphenylphosphonium bromide in place of the unsubstituted phosphonium salt. Because of the precipitation of the ylide upon addition of the phosphonium salt to NaOEt solution, the reaction yielded the desired product contaminated with the ylide. When the ylide was generated in DMF with solid NaOMe, the ylide remained in solution and gave essentially a quantitative yield of the olefin IV upon treatment with II (Scheme I). Alkaline hydrolysis of IV gave 9, 10-dehydro-N^{10}-deazapteroic acid (*p*-[2-(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]benzoic acid, V). Conversion of V to 9,10-dehydro-N^{10}-deazafolic acid (*N*-{*p*-[2-(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]benzoyl}glutamic acid, IX) was accomplished by acetylation of V to improve solubility and subsequent treatment of VII with diethyl glutamate (DEG) and dicyclohexylcarbodiimide (DCI) in pyridine–DMF to give VIII contaminated with the byproduct VIIIa. Hydrolysis of VIII in dil base after purification gave IX. Catalytic hydrogenation of IV gave, after reoxidation of the pyrazine ring with H_2O_2, N^{10}-deazapteroic acid (*p*-[2-(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)ethyl]benzoic acid, VI). N^{10}-Deazafolic acid (*N*-{*p*-[2-(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)ethyl] benzoyl}glutamic acid, XII) was synthesized from VI *via* its Ac derivative X in a fashion (X–XII) similar to that used for the corresponding 9,10-dehydro analog.

Biologic Evaluation.—The antifolic acid activity of the pteroic and folic acid analogs (V, VI, IX, XII) was determined using *Streptococcus faecalis*.[6] The results are shown in Figure 1. Results for tetrahydrohomofolic acid, a potent growth inhibitor of *S. faecalis*,[7] and for methotrexate (amethopterin), a "pseudoirreversible" inhibitor of dihydrofolate reductase,[8] are included for comparison purposes. Although only limited antifolic acid activity was shown by both pteroic acid analogs (V and VI) and 9,10-dehydro-N^{10}-deazafolic acid (IX), N^{10}-deazafolic acid (XII) showed growth inhibition of essentially the same order as tetrahydrohomofolic acid and approximately 0.01 that of methotrexate.

In order to determine whether N^{10}-deazafolic acid (XII) was acting as a reversible or irreversible inhibitor of growth of *S. faecalis*, the effect of XII was evaluated in the presence of increasing concentration of folic acid. Figure 2 shows that growth inhibition is reversed by folic acid. The facile reversal by folic acid shows that the antagonistic effect of XII is weak. The action of XII is paralleled by 10-methylfolic acid, which is an effective antagonist of folic acid;[9] inhibition

(5) M. Sletzinger, D. Reinhold, J. Grier, M. Beachem, and M. Tishler, *J. Amer. Chem. Soc.*, **77**, 6365 (1955).

(6) We wish to thank Dr. R. F. Pittillo and associates of the Microbiology Division, Chemotherapy Research Department, Southern Research Institute, for the *S. faecalis* assays performed according to the procedure of E. E. Snell, in "Vitamin Methods, Microbiological Methods in Vitamin Research," P. György, Ed., Academic Press, N. Y., 1950, p 327.

(7) L. Goodman, J. DeGraw, R. L. Kisliuk, M. Friedkin, E. J. Pastore, E. J. Crawford, L. T. Plante, A. Al-Nahas, J. F. Morningstar, Jr., G. Kwok, L. Wilson, E. F. Donovan, and J. Ratzan, *J. Amer. Chem. Soc.*, **86**, 308 (1964).

(8) W. C. Werkheiser, *J. Biol. Chem.*, **236**, 888 (1961).

(9) D. B. Cosulich and J. M. Smith, *J. Amer. Chem. Soc.*, **70**, 1922 (1948).

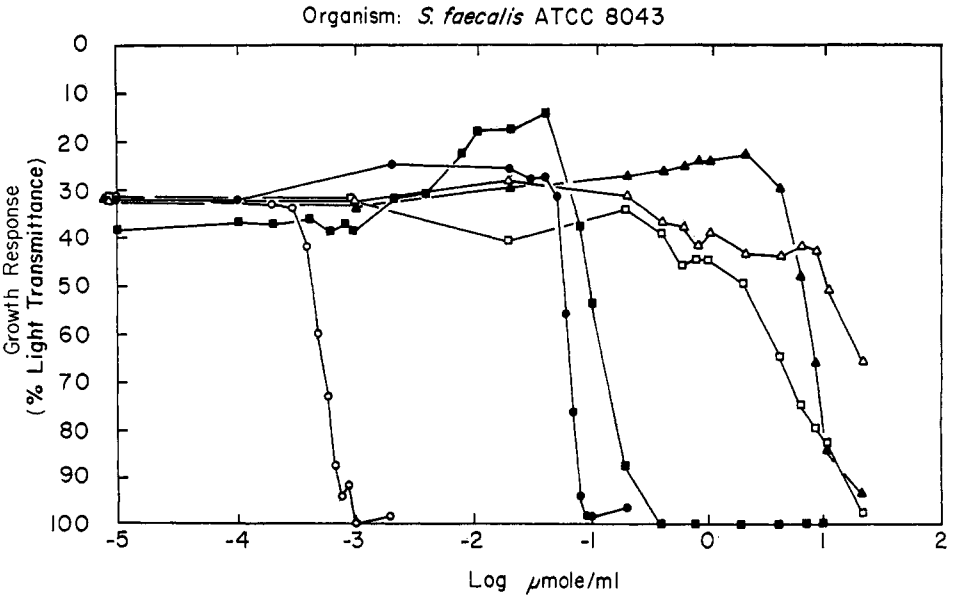


Figure 1.—Analogs *vs.* folic acid at 0.0004 μmole/ml in Flynn's broth: △, V; □, VI; ▲, IX; ●, XII; ○, methotrexate; ■, tetrahydrohomofolate.

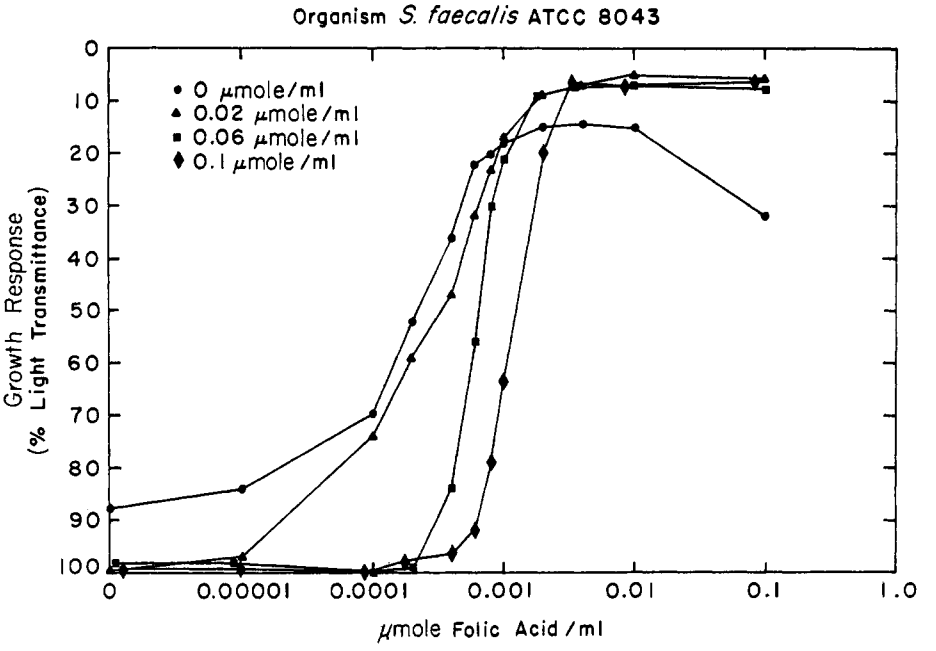


Figure 2.—XII *vs.* folic acid at varying concentrations in Flynn's broth.

of growth of *S. faecalis* by 10-methylfolic acid is similarly easily reversed by folic acid.[10]

A direct measurement of the degree of inhibition of dihydrofolate reductase by IX and XII was performed using the enzyme from pigeon liver.[11] Methotrexate gave 50% inhibition at 0.014 μM. Folic acid was 50% inhibitory at 20 μM. Again, IX showed only limited inhibition (50% at 85 μM), whereas N^{10}-deazafolic acid (XII), although much less effective than methotrexate, was 3-fold more inhibitory (50% at 6.1 μM) than folic acid (Table I). N^{10}-Deazafolic acid (XII) and its dihydro derivative[12] were tested for substrate activity along with folic acid and dihydrofolic acid. The unreduced acid XII and folic acid were inactive as substrates,[13] whereas the dihydro derivative of XII was approximately 20% as effective as dihydrofolic acid. The substrate activity of dihydro-N^{10}-deazafolic acid suggests that the analog XII is being bound to dihydrofolate reductase at the site of folic acid binding and not at the enzyme's hydrophobic site.[14] Binding at the

(10) T. H. Jukes, A. L. Franklin, and E. L. R. Stokstad, *Ann. N. Y. Acad. Sci.*, **52**, 1336 (1950).

(11) We wish to thank Miss Suzanne Straight of the Biochemistry Research Department, Southern Research Institute, for the dihydrofolate reductase experiments performed as reported by B. R. Baker, B.-T. Ho, and T. Neilson, *J. Heterocycl. Chem.*, **1**, 79 (1964).

(12) Prepd as described by R. L. Blakley, *Nature (London)*, **188**, 231 (1960).

(13) Since the spectrophotometric method (uv) used in the enzymatic assay would not detect low levels of reduction of TPNH, it appears that folic acid itself is not serving as a substrate. A more sensitive detection method (*e.g.*, spectrophotofluorometric) would have shown folic acid to be a substrate.

(14) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967, p 192.

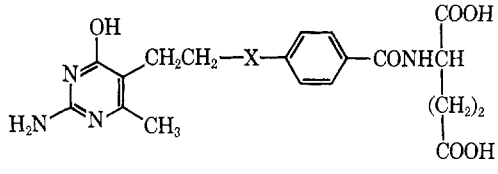
TABLE I[a]
INHIBITION OF DIHYDROFOLATE REDUCTASE BY FOLATE ANALOGS

Compd	Concn for 50% inhibition, M
Methotrexate	0.014
N^{10}-Deazafolic acid (XII)	6.1
Folid acid	20
9,10-Dehydro-N^{10}-deazafolic acid (IX)	85
N^{10}-Deazapteroic acid (VI)	90
9,10-Dehydro-N^{10}-deazapteroic acid (VII)	110
Pteroic acid	120

[a] See ref 11.

hydrophobic site was considered to be a possibility due to the nonpolar nature of the 6-phenethyl moiety.[14] The substrate activity of the dihydro derivative of XII adds another point of similarity with 10-methylfolic acid.[15]

After discovery of the hydrophobic binding region of dihydrofolate reductase, Baker[14] concluded that the basic amino group (N^{10}) of the *p*-aminobenzoyl-L-glutamate moiety of folic acid contributes little to binding to the enzyme since benzoyl-L-glutamic acid was about as good an inhibitor as the corresponding *p*-amino compound. The activity of N^{10}-deazafolic acid (XII) supports this conclusion. Earlier, Baker, *et al.*,[16] had suggested that the N^{10}-amino group was necessary for good binding. This suggestion was supported by an 80-fold increase in the concn of XIII (8000 μM) necessary for 50% inhibition in comparison with XIV (100 μM), the increase being attributed to decreased basicity of N^{10} by the C_9 carbonyl function. Since N^{10}-deazafolic acid is comparable in binding strength to folic acid, it appears now that the reason for the decreased activity of XIII was probably a steric factor; in addition,



XIII, X = CONH
XIV, X = $NHCH_2$

the C_9 carbonyl group may have electronically interfered with binding by this portion of the molecule. The binding characteristics of XIII were not discussed in Baker's more recent work.[14]

Comparison of XII with N^{10}-deazapteroic acid (VI, 50% inhibitory at 90 μM) suggests a strong contribution to binding by the glutamate moiety.[14,15] We similarly observed a 6-fold increase in the inhibition concn of pteroic acid (50% at 120 μM) compared to folic acid (50% at 20 μM); using the enzyme from Ehrlich ascites carcinoma cells, Bertino, *et al.*,[17] observed a 70-fold increase for pteroic acid (50% at 400 μM) compared to folic acid (50% at 5.5 μM). The nearly identical inhibition concns observed by us for pteroic acid, N^{10}-deazapteroic acid (VI), and 9,10-dehydro-N^{10}-deazapteroic acid (VII, 50% at 110 μM) suggest that the three may be binding at the same site on the enzyme. As suggested by Baker,[14] rotamers of these pteroic acids bound at the pteridine site, with extension of the para-substituted benzoic acid moiety into the hydrophobic region, could account for the observed similarity in binding strength.

The large difference observed between 9,10-dehydro-N^{10}-deazafolic acid (IX) on one hand and XII and folic acid on the other (Table I) suggests either that these 3 are binding at the normal folic acid loci on the enzyme but that the trans-substituted olefinic moiety of IX renders it sterically less compatible for binding at this site or that IX is binding as a rotamer[14] which extends into the hydrophobic region of the enzyme. The latter explanation is supported by the close agreement between the 50% inhibition concn of IX (85 μM) and those for pteroic acid (120 μM), VI (90 μM), and VII (110 μM). Examination of Dreiding models of folic acid (IX) and XII reveals that, although folic acid and XII are able to duplicate closely all conformations of IX, IX is unable to assume certain conformations possible for XII and folic acid. Because of the difference in binding observed for the saturated and the olefinic types, it appears likely that conformations of folic acid and XII, which cannot be assumed by IX, are those responsible for strong binding. Certain conformations of a model of the cis isomer of IX, on the other hand, closely matched the conformations of folic acid and XII that could not be assumed by the trans isomer. Synthesis of *cis*-IX and determination of its binding strength could provide additional information on the preferred conformation for optimal binding to the enzyme.

The folic and pteroic acid analogs were tested for cytotoxicity against human epidermoid cells in culture. Slight activity was observed for III (ED_{50}[18] = 64 μg/ml) and IV (ED_{50}[18] = 21 μg/ml).

The folic acid analogs IX and XII and the model compound III were evaluated against leukemia L1210. Although some toxicity was observed, none of the compounds showed significant antileukemic activity. N^{10}-Deazafolic acid (XII) was toxic at a dose of 80 mg/kg when administered by the intraperitoneal route on days 1–9, but nontoxic under identical conditions at a dose of 60 mg/kg.

The 2 pteroic acid analogs V and VI were tested for antimalarial activity against *Plasmodium berghei* in mice at doses of 10, 20, 40, 80, 160, and 320 mg/kg. No toxicity or increase in survival time was observed for either compound.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were determined with a Kofler Heizbank apparatus. Nmr data were determined in F_3CCO_2H, unless stated otherwise, with a Varian A-60A spectrometer, and chemical shifts in parts per million and line positions in hertz/60 are given downfield from internal Me_4Si. Uv data were determined with a Cary Model 14 recording spectrophotometer. Ir data were obtained with a Perkin-Elmer 521 or 621 spectrophotometer in KBr disks.

(15) S. F. Zakrzewski, *J. Biol. Chem.*, **235**, 1780 (1960).
(16) B. R. Baker, D. C. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, **7**, 24 (1964).
(17) J. R. Bertino, J. P. Perkins, and D. G. Johns, *Biochemistry*, **4**, 839 (1965).
(18) Cancer Chemotherapy National Service Center, *Cancer Chemother. Rep.*, **25**, 57 (1962).

N-(**3,4-Dihydro-4-oxo-6-styryl-2-pteridinyl)acetamide (III).**—2-Acetamido-6-formylpteridin-4(3*H*)-one[4] (500 mg, 2.15 mmoles) was added under N_2 to a yellow mixt of the ylide prepared by addn of benzyltriphenylphosphonium chloride (950 mg, 2.45 mmoles) to Na (50 mg, 2.15 mg-atoms) in 5 ml of EtOH. The mixt was stirred 4 days at room temp under N_2. H_2O (10 ml) was added, and the mixt was filtered. The residue was washed with Me_2CO and PhMe, giving a yellow solid. Recrystn from DMF gave yellow needles: mp >260°; yield 400 mg (60%). An anal. sample was obtained by recrystg again from DMF: ir (cm^{-1}), 3430, 3280, 3120, 1700, 1675, 1610, 1550, 1420, 1350, 1215, 970, 760, 720, 680; uv pH 13 [λ_{max} in mμ ($\epsilon \times 10^{-3}$)]. 315 (25.8), 398 (16.2); nmr, 2.6 (CH_3C); complex multiplet centered at 7.6 (phenyl and vinyl), 8.15, 8.42 (vinyl, J = 16 Hz, trans), 9.5 (pyrazine). *Anal.* ($C_{16}H_{13}N_5O_2$) C, H, N.

Methyl *p*-[2-(2-Acetamido-3,4-dihydro-4-oxo-6-pteridinyl)-vinyl]benzoate (IV).—α-Bromo-*p*-toluic acid (20 g, K & K Laboratories) was added batchwise to CH_2N_2 in Et_2O (from 40 g of Diazald) in 30 min at 0° with stirring. After stirring 1 hr at room temp, the soln was evapd and yielded crystalline methyl α-bromo-*p*-toluate. The ester was refluxed 4 hr in 200 ml of PhMe containing an equal wt of PPh_3. At the end of the reflux period, the mixt was cooled to room temp and filtered to collect cryst ***p*-carbomethoxybenzyltriphenylphosphonium bromide**: yield 34 g; mp 258°. *Anal.* ($C_{27}H_{24}BrO_2P$) C, H, Br, P. The phosphonium bromide (21.1 g, 0.043 mole) in 150 ml of DMF (molecular sieve dried) was treated with 2.33 g of NaOMe (0.043 mole). After stirring the orange soln for 15 min at room temp, 2-acetamido-6-formylpteridin-4(3*H*)-one[4] (10 g, 0.043 mole) in 800 ml of dry DMF was added, and the resulting soln was stirred 3 days at room temp under N_2. Removal of the solvent *in vacuo* gave a solid residue (34 g), which was stirred vigorously with PhMe (250 ml) for 1 hr and filtered. The PhMe-insol material was crystd from hot pyridine and gave yellow needles: yield 12.4 g (79%); mp >260°; ir (cm^{-1}), 3420, 3290, 3160, 2950, 1710, 1685, 1620, 1555, 1480, 1460, 1360, 1280, 1260, 1240, 1220, 1110, 1020, 1000, 880, 820, 790, 760, 750, 730, 700, 620. *Anal.* ($C_{18}H_{15}N_5O_4 \cdot 0.25C_5H_5N$) C, H, N.

***p*-[2-(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]benzoic Acid (V).**—IV (330 mg) in 50 ml of 1 *N* KOH was stirred 20 hr at room temp and filtered. The filtrate was acidified to pH 3 with concd HCl, and the resulting yellow ppt was collected by centrifugation and washed 3 times with dil HCl (pH 3). The solid was suspended in 100 ml of H_2O and treated dropwise with stirring with 1 *N* KOH until homogeneous. After filtration, the filtrate was acidified to pH 3. The ppt was collected by filtration, washed 3 times with dil HCl (pH 3), and dried *in vacuo:* yield 140 mg; mp >260°; ir (cm^{-1}), 3400, 1680, 1640, 1600, 1565, 1520, 1480, 1370, 1260, 1170, 1110, 940, 870, 800 770; uv pH 13 [λ_{max} in mμ ($\epsilon \times 10^{-3}$)], 253 (19.1), 310 (14.8), 386 (14.7); nmr, 6.8, 7.0, 7.3, 7.5, 8.1, 8.2 (phenyl and vinyl), 8.6 (pyrazine). *Anal.* ($C_{15}H_{11}N_5O_3 \cdot H_2O$) C, N. Calcd for H: 4.00. Found: 3.51.

***p*-[2-(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)ethyl]benzoic Acid (VI).**—IV (3 g) in 165 ml of hot DMF was hydrogenated for 24 hr at 70° at an initial pressure of 3.5 kg/cm² in the presence of 5 g of 5% Pd/C. After cooling to room temp, the mixt was filtered, and the filtrate was evapd to dryness *in vacuo*. The solid residue in 50 ml of H_2O was treated with concd, aq KOH until basic (pH 10–11). A small amount of insol material was removed by filtration, and the filtrate was treated with 10 ml of 30% H_2O_2 and stirred 2 hr at room temp. Acidification to pH 4 with concd HCl and filtration gave a solid. This (1.3 g) was suspended in 200 ml of H_2O; 1 *N* KOH was added dropwise with stirring until homogeneous. After filtration and acidification of the filtrate (pH 3), the ppt was collected by filtration, washed 3 times with dil HCl (pH 3), and dried *in vacuo*: yield 700 mg; mp >260°; ir (cm^{-1}), 3300, 3120, 1700, 1680, 1650, 1610, 1575, 1535, 1480, 1410, 1370, 1240, 1180, 1115, 1020, 820, 780; uv pH 13 [λ_{max} in mμ ($\epsilon \times 10^{-3}$)], 253 (23.2), 364 (5.9); nmr, 3.5 (CH_2CH_2), multiplet centered at 7.75 (phenyl), 8.7 (pyrazine). *Anal.* ($C_{15}H_{13}N_5O_3 \cdot 1.5H_2O$) Calcd: C, 53.25; H, 4.77; N, 20.70. Found: C, 52.83; H, 4.32; N, 20.94.

***p*-[2-(2-Acetamido-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]benzoic Acid (VII).**—V (7 g) in 150 ml of Ac_2O was refluxed 16 hr with stirring. Filtration of the hot mixt gave a yellow solid, which was suspended in 150 ml of H_2O and treated with 1 *N* KOH with stirring until homogeneous. After filtration, acidification of the filtrate to pH 6 with concd HCl and filtration gave a yellow solid, which was washed 3 times with H_2O and dried: yield 3.1 g; mp >260°; nmr, 2.55 (CH_3CO), 7.65, 7.75, 7.9, 8.04, 8.3 (multiplet, Ph and vinyl), 9.2 (pyrazine). More of VII was obtained by evapn of the Ac_2O filtrate and treatment of the resulting residue as described.

Diethyl L-*N*-{*p*-[2-(2-Acetamido-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]benzoyl}glutamate (VIII).—VII (3 g, 8.55 mmoles) was added to a soln of diethyl L-glutamate·HCl (2.05 g, 8.55 mmoles) in 550 ml of pyridine (dried over molecular sieves) and 200 ml of dry DMF, and the mixt was heated until homogeneous. The red soln was allowed to cool slightly, treated with dicyclohexylcarbodiimide (2.22 g, 8.55 mmoles), and stirred 3 days at room temp. The soln was concd *in vacuo* to 75 ml and filtered to remove dicyclohexylurea (1.6 g). Evapn of the filtrate to dryness, trituration with EtOH, and filtration gave a yellow solid (3.8 g), which was crystd from $CHCl_3$–EtOH–MeCOEt (12:2:1) (100 ml); yield 2.3 g. An anal. sample was obtd by recryst twice from pyridine: mp > 260°; nmr, 2 triplets centered at 1.3 and 1.4 (OCH_2CH_3), 2.5 (CH_3CO), 2.6 (multiplet, CH_2CH_2CO), 4.4 (multiplet, OCH_2CH_3), 5.0 (NHCH), 7.4 and 7.65, 8.05 and 8.3 (2 H, vinyl, J = 16 Hz, trans), 7.9, 7.95 (Ph), 9.2 (pyrazine). *Anal.* ($C_{26}H_{28}N_6O_7$) C, H, N.

***N,N'*-Dicyclohexyl-*N*-{*p*-[2-(2-acetamido-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]benzoyl}urea (VIIIA).**—The filtrate resulting from crystn of VIII from $CHCl_3$–EtOH–MeCOEt (12:2:1) was passed through a silica gel column (4.5 × 20 cm) developed in the same solvent. After removal of the frontal band (50 ml), 200 ml of eluate contg a yellow component was collected. Evapn gave a yellow solid: yield 300 mg; mp >260°; nmr in DMSO-d_6, 1.3 (poorly resolved complex multiplet, cyclohexyl CH_2), 2.2 (CH_3CO), 3.05 (NH), 4.1 (multiplet, >NCH), 7.7 (multiplet, Ph and vinyl), 9.05 (pyrazine). *Anal.* ($C_{30}H_{35}N_7O_5$) C, H, N.

L-*N*-{*p*-[2-(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)vinyl]-benzoyl}glutamic Acid (IX).—VIII (170 mg) in 100 ml of 0.1 *N* KOH was stirred 5 hr at room temp. After filtration, concd HCl was added dropwise with stirring to pH 3. The orange ppt was collected by centrifugation, washed twice with dil HCl (pH 3), and collected after each washing by centrifugation. The solid was dried *in vacuo* at 78° for 5 hr: yield 150 mg: mp >260°; nmr 2.7 (complex multiplet, CH_2CH_2), 5.1 (NHCH), 7.35, 7.6 (doublet, 1 vinyl H, J = 16 Hz, trans), complex multiplet centered at 7.9 (Ph and 1 vinyl H), 9.05 (pyrazine). *Anal.* ($C_{20}H_{18}N_6O_6 \cdot 0.5H_2O$) C, H, N.

***p*-[2-(2-Acetamido-3,4-dihydro-4-oxo-6-pteridinyl)ethyl]benzoic Acid (X).**—VI (1.65 g) in 25 ml of Ac_2O was refluxed 4 hr with stirring, and the resulting dark soln was evapd to dryness *in vacuo*. Trituration of the residue with ether and filtration gave a brown solid (2 g), which was added to 50 ml of H_2O. The mixt was stirred and treated with 1 *N* KOH until essentially homogeneous. After filtration, stepwise acid treatment to pH 7, 6, and 3 with a filtration at each pH gave 3 samples of solid. Tlc showed that the material collected at pH 3 was superior in purity to those collected at higher pH. The yield of product collected at pH 3 was 380 mg: nmr in DMSO-d_6, 2.2 (CH_3CO), 3.2 (CH_2CH_2), 7.3, 7.4, 7.8, 7.9 (Ph), 8.75 (pyrazine).

Diethyl L-*N*-{*p*-[2-(2-Acetamido-3,4-dihydro-4-oxo-6-pteridinyl)ethyl]benzoyl}glutamate (XI).—X (340 mg, 1 mmole) dissolved in 45 ml of dry pyridine (molecular sieve dried) was treated with 240 mg of diethyl L-glutamate·HCl (1 mmole) and 260 mg of DIC (1 mmole), and the soln was stirred 2 days at room temp. The soln was concd to 10 ml *in vacuo* and filtered to remove dicyclohexylurea (220 mg). Evapn of the filtrate to dryness gave a glassy residue which was crystd from 2 ml of EtOH: yield 170 mg; mp 194–195°. An anal. sample was obtained by recrystn from EtOH: nmr in DMSO-d_6, 2 triplets centered at 1.2 and 1.25 (OCH_2CH_3), complex multiplet centered at 2.2 (CH_3CO and CH_2CH_2CO), 3.2 (pyrazine CH_2CH_2), 4.05 (multiplet, OCH_2CH_3), 4.5 (NHCH), 7.35, 7.45, 7.8, 7.9 (phenyl), 8.55, 8.7 (CONHCH), 8.8 (pyrazine). *Anal.* ($C_{26}H_{30}N_6O_7$) C, H, N.

L-*N*-{*p*-[2-(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)ethyl]-benzoyl}glutamic Acid (XII).—XI (50 mg) in 50 ml of 0.1 *N* KOH was stirred 5 hr at room temp. The yellow soln was concd by lyophilization to 10 ml. After filtration, concd HCl was added to pH 4. The solid was collected by filtration and resuspended in 20 ml of H_2O; 1 *N* KOH was added with stirring until the solid dissolved (pH 8.5). The soln was filtered and the filtrate acidified with dil HCl to pH 3.3. The ppt was collected by centrifugation. The solid was washed 3 times with 10-ml portions of dil HCl (pH 4), collection being accomplished by centrifugation

after each washing: yield 20 mg; mp >260°; uv pH 13 [λ_{max} in mμ ($\epsilon \times 10^{-3}$)], 253 (31.3), 363 (7.5); nmr, 2.6 (multiplet, CH_2-CH_2CO), 3.4 (pyrazine CH_2CH_2), 5.1 (NHCH), 7.35, 7.45, 7.8, 7.9 (phenyl), 8.7 (pyrazine). *Anal.* ($C_{20}H_{20}N_6O_6$) C, H, N.

Acknowledgments.—The authors express their appreciation to Dr. W. C. Coburn, Jr., and associates for microanalytical and spectral determinations. Some of the analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Cytotoxicity and antitumor tests were performed by the Chemotherapy Department of Southern Research Institute under Contract PH43-65-594 with Chemotherapy, National Cancer Institute, National Institutes of Health; antitumor testing was carried out under the supervision of Drs. F. M. Schabel, Jr., and W. R. Laster, Jr., and cytotoxicity testing was under the supervision of Dr. L. J. Wilkoff. Antimalarial screening data was supplied to us by the Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

Muscarinic Receptors. Derivatives of 7-Oxabicyclo[2.2.1]heptane[1]

WENDEL L. NELSON,* DAVID R. ALLEN, AND FRANK F. VINCENZI

College of Pharmacy and Department of Pharmacology, University of Washington, Seattle, Washington 98105

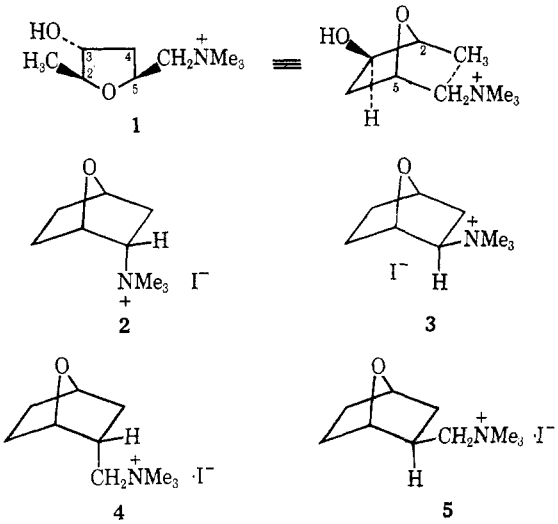
Received September 28, 1970

Syntheses of muscarinic analogs *endo-* and *exo*-2-trimethylammonium-7-oxabicyclo[2.2.1]heptane iodides and *endo-* and *exo*-2-dimethylaminomethyl-7-oxabicyclo[2.2.1]heptane methiodides are described. Muscarinic assays are reported.

Steric and electronic effects in the drug, as well as conformational differences in drug and in the drug–receptor interaction, have been suggested as major reasons why certain analogs of acetylcholine (ACh) are more active than others at various cholinergic sites.[2] Preparation and screening of conformationally rigid or semirigid analogs of ACh has met with some success in applications to muscarinic and AChE sites.[2]

In this study of analogs of various conformers of cholinergic agents, muscarine (**1**) was chosen as a model, and analogs in the 7-oxabicyclo[2.2.1]heptane series were prepared. The C-5 methylene ammonium side chain of muscarine has considerable flexibility in models, and various conformations of it and of muscarone have been suggested to explain the difference in absolute stereochemistry of the most active isomer in each case.[3,4] Calculations (extended Hückel theory)[5–7] are not consistent with earlier drug–receptor proposals.[3]

In this series of compounds the position of the N^+Me_3 is restricted to only certain distances from the ether O, and the agents incorporate few or no additional atoms in the C skeleton, which potentially allows for accumulation of some evidence concerning the conformation of muscarine in this drug–receptor interaction, although separation of optical isomers would be necessary to obtain data concerning the muscarine–muscarone controversy.



1 2 3 4 5

Bicyclic analogs **2**, **3**, **4**, and **5** were prepared as rigid desoxymuscarine analogs. Compounds **2** and **3** differ from desoxymuscarine only by connection of the C-2 methyl and C-5 CH_2 groups through a single C–C bond. Compounds **4** and **5**, being homologs of **2** and **3**, were prepared because the necessary starting materials were intermediates in the preparation of **2** and **3**. These compounds (**4** and **5**) have the disadvantages of conformational freedom of the quaternary head with respect to the O, but could provide information concerning possible steric interaction between the N^+Me_3 cation and portions of the bicyclic skeleton when compared to **2** and **3**.

Initial attempts were made to find a facile route to 7-oxabicyclo[2.2.1]hept-2-ene (**8**) through Diels–Alder reaction of furan and maleic anhydride to form 7-oxabicyclo[2.2.1]hept-5-ene-*exo*-2,3-dicarboxylic anhy-

(1) (a) An account of this work was presented to the 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 1971, Abstract MEDI 32. (b) This work was supported in part by Grant NS-08121 from the National Institute of Neurological Diseases and Stroke, U. S. Public Health Service, Bethesda, Md.

(2) For recent reviews see: (a) M. Friedman, *Drugs Affecting Peripheral Nerv. Syst.*, **1**, 79 (1967); (b) S. Ehrenpreis, J. H. Fleisch, and T. W. Miggag, *Pharmacol. Rev.*, **21**, 131 (1969); (c) P. S. Portoghese, *Annu. Rev. Pharmacol.*, **10**, 51 (1970).

(3) P. G. Waser, *Pharmacol. Rev.*, **13**, 465 (1962).

(4) B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963).

(5) L. B. Kier, *Mol. Pharmacol.*, **3**, 487 (1967).

(6) L. B. Kier, in "Fundamental Concepts in Drug–Receptor Interactions," J. F. Danielli, J. F. Moran, and D. J. Triggle, Ed., Academic Press, New York, N. Y., 1970, pp 15–28.

(7) S. M. Liquori, A. Daimani, and J. L. DeCoen, *J. Mol. Biol.*, **33**, 445 (1968).