Antimicrobial Activity of 8-Deazafolic Acid

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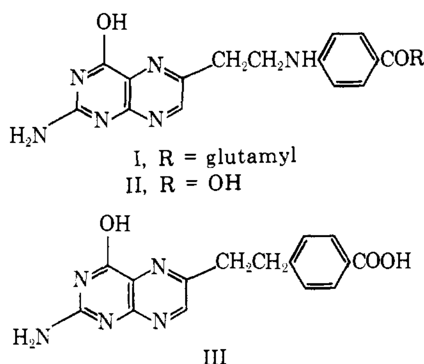
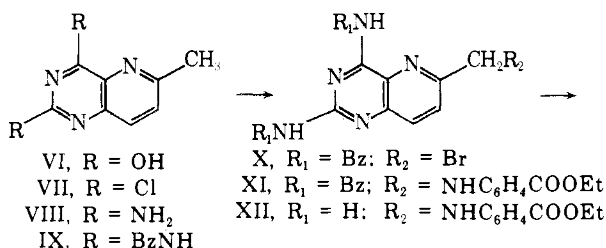
The synthesis and antimicrobial and enzyme inhibitory activity of homofolic acid (I) and its tetrahydro derivative were reported several years ago.[1,2] Homopteroic acid (II), an intermediate in the synthesis of I, was later found to possess antimalarial activity[3] in the tetrahydro form and some analogs of II were prepared in an attempt to develop more potent antimalarial agents.[4,5] The latter investigations were unsuccessful; however, the potent antimicrobial action of 10-deazapteroic acid[5,6] (III) was noted. The antifolate activity of these relatively nontoxic 2-amino-4-hydroxypteridine compounds is distinctly different from 2,4-diaminopteridines such as methotrexate. The diamino analogs function as strong, irreversible inhibitors of cellular dihydrofolic reductase,[7] while the aminohydroxy compounds apparently act by interference with folic uptake by the cells,[8] the powerful inhibition of bacterial thymidylate synthetase by tetrahydrohomofolate[1] not withstanding. As part of a continuing interest in the potential antitumor and antimicrobial activities of folate analogs, we wish to report the preparation and antimicrobial activity of 8-deazapteroic (IV) and 8-deazafolic acid (V) and reduced derivatives of V.



The synthesis of IV was first reported by Oakes, *et al.*[9] Starting from 2,4-dihydroxy-6-methyl-1,3,5-triazanaphthalene (VI)[10] we have reprepared IV *via* the literature method as outlined below. Prolonged hydrolysis of the diamino ester XII in 1 N NaOH-2-HOC_2H_4OMe gave the aminohydroxy acid IV. Treatment of IV with boiling $(CF_3CO)_2O$ afforded the N^{10}-CF_3CO intermediate XIII, which was directly acetylated to yield the blocked 8-deazapteroate (XIV). Coupling of XIV with diethyl glutamate *via* the mixed anhydride method[2] and subsequent alkaline hydrolysis (0.1 N NaOH) afforded 8-deazafolic acid (V). The yield of V was dramatically increased from 17 to 60% when a solid-phase resin technique[11] was employed for coupling of XIV and glutamic acid.

In Table I the microbiological activities of the 8-deaza compounds against folate dependent organisms are presented. 8-Deazafolic acid (V) was as equipotent a growth inhibitor of *Streptococcus faecium* as methotrexate; however, partial reduction to the dihydro-V increased the potency about 1.5-fold. Complete reduction to the 5,6,7,8-tetrahydro-V considerably reduced the activity by a factor of 8. It is noteworthy that all three forms of V were significantly inhibitory against the methotrexate resistant strains of *S. faecium*. Against *Lactobacillus casei*, unreduced V was most active; however, none of the forms were comparable to methotrexate. The compounds V and dihydro-V showed moderate activity against a resistant strain of *L. casei*, but tetrahydro-V was quite inactive. 8-Deazapteroic acid (IV) also showed surprisingly good inhibition of the nonresistant strains of *S. faecium* and *L. casei*. In view of the close structural similarity between tetrahy-



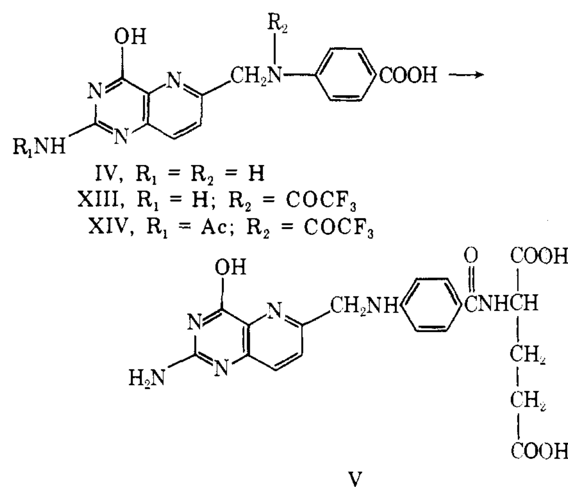


Table I. Bacterial Growth Inhibition of 8-Deazafolates, MIC (ng/ml) for 50% Inhibition[a]

	S. faecium[b] (ATCC 8043)	*S. faecium*[b] methotrexate resistant	*L. casei*[b] (ATCC 7649)	*L. casei*[b] methotrexate resistant	*Pediococcus cerevisiae*[c]
8-Deazapteroic acid (IV)	2.0	>200	2.0	>200	
8-Deazafolic acid (V)	0.15	130	0.6	900	>2000
Dihydro-V[d]	0.09	88	1.2	600	>2000
5,6,7,8-Tetrahydro-V[e]	0.70	460	1.2	>2,000	>2000
Methotrexate	0.15	6000	0.01	38,000	

[a]None of the compounds supported growth in the absence of folate at a level of 2000 ng/ml. MIC denotes minimum inhibitory concentration. [b]Folate, 1 ng/ml. [c]5-Formyltetrahydrofolate, 1 ng/ml. [d]From $Na_2S_2O_4$ reduction of V; uv spectrum was only slightly changed, but inhibition significantly increased. The 5,6-dihydro compound is the probable reduction product. The structure is currently under investigation. [e]From H_2–PtO_2 reduction of V. Uv was similar to tetrahydrofolate.

Table II. Inhibition of Dihydrofolic Reductase and Thymidylate Synthetase by 8-Deazafolates

	Molar concn for 50% inhibition	
	Dihydrofolic reductase[a]	Thymidylate synthetase[a]
V	1.2×10^{-5}	1.4×10^{-5}
Dihydro-V	2.2×10^{-5}	3.0×10^{-5}
5,6,7,8-Tetrahydro-V	5.5×10^{-5}	7.5×10^{-5}

[a]Derived from *L. casei* (ATCC 7649).

dro-V and tetrahydrofolate, particularly around the important N^5-N^{10} region, it is remarkable that tetrahydro-V does not have growth factor activity.

Enzyme inhibitory data shown in Table II indicate that V and its reduced forms were ineffective inhibitors of dihydrofolic reductase and thymidylate synthetase as derived from *L. casei*. The lack of activity against thymidylate synthetase for tetrahydro-V is in sharp contrast to tetrahydrohomofolate.[1] The diminished growth inhibition of *S. faecium* by tetrahydro-V as compared with unreduced V is likewise contrary to the effect noted with the homofolates. Whether this contrast is due to a difference in mechanism of action between the two series remains to be established.

Experimental Section†

8-Deazapteroic Acid (IV). 2,4-Diamino-6-*p*-carbethoxyanilinomethyl-1,3,5-triazanaphthalene (XII) was prepared by the method of Oakes, *et al.*,[9] and was homogeneous by tlc (R_f 0.15, silica gel, EtOAc–Me_2CO, 5:1). 2,4-Dihydroxy-6-methyl-1,3,5-triazanaphthalene (VI), the starting material, was obtained by the one-step synthesis of Irwin and Wibberley.[10] Considerable difficulty was encountered in chlorination of VI to VII with $POCl_3$ in runs larger than 5 g. A variety of other reagents and conditions failed to improve the preparation. A mixture of XII (0.94 g), 50 ml of 1 *N* NaOH, and 20 ml of 2-methoxyethanol was heated 20 hr on a steam bath. The solution was diluted with 170 ml of H_2O, adjusted to pH 8 with HOAc, and filtered. The filtrate was adjusted to pH 5–6 (HOAc) to give a pale yellow crystalline precipitate which was collected, H_2O washed, and dried (0.66 g, 77%). *Anal.* ($C_{15}H_{13}N_5O_3 \cdot 2H_2O$) C (50.9), H, N.

2-Acetamido-10-trifluoroacetyl-8-deazapteroic Acid (XIV). A mixture of 495 mg of IV and 20 ml of $(CF_3CO)_2O$ was stirred at reflux for 1.75 hr. The solvent was evaporated *in vacuo* and the residue stirred with ice H_2O for 2 hr. The crystals (XIII) were collected, washed (H_2O), and dried (468 mg): λ^{Nujol} 3.0, 3.2 μ (OH, NH), 5.90 (COOH), 8.3, 8.6 (CF_3). A sample suitable for analysis was not obtained because of hydrolytic instability of the CF_3CO group.

The N^{10}-$COCF_3$ acid (XIII, 460 mg) and 10 ml of Ac_2O were stirred at 105° for 3 hr. After removal of solvent the residue was dissolved in DMF (3 ml) and diluted with 3 ml of H_2O, and the solution was chilled 15 hr to afford tan crystals (376 mg, 53% from IV). *Anal.* ($C_{19}H_{14}F_3N_5O_5$) C, H, N.

8-Deazafolic Acid (V). A solution of XIV (88 mg, 0.19 mmol) in 1.5 ml of DMF was chilled to 0–5°, and Et_3N (0.026 ml, 0.19 mmol) was added, followed by *i*-BuOCOCl (0.025 ml, 0.19 mmol). After 30 min at 0–5° the mixture was treated with diethyl glutamate hydrochloride (47 mg, 0.19 mmol) in 0.5 ml of DMF containing Et_3N (0.026 ml, 0.19 mmol). The mixture was kept at ambient temperature for 3 days and evaporated *in vacuo* and the residue stirred with 10 ml of H_2O for 1 hr. The pale yellow crystals were collected (94 mg) and stirred with 5 ml of saturated $NaHCO_3$ for 1 hr. The blocked ester was then hydrolyzed in 0.1 *N* NaOH (20 ml) at 95–100° for 25 min. The pH was adjusted to 3–4 with 2 *N* HCl to precipitate the acid product, which was collected by centrifugation. The solid was washed with H_2O (3 × 10 ml) and the centrifuge pellet dried *in vacuo* to leave 15 mg (17%) of pale yellow solid.

Alternatively, V was prepared by coupling the mixed anhydride formed with the blocked acid XIV and *i*-BuOCOCl to the α-benzyl-γ-glutamyl Merrifield resin ester as described previously.[11] The product was cleaved from the resin and deprotected by alkaline hydrolysis. This was accomplished by vigorous mechanical shaking for 1 hr at room temperature and 20 min at 50° of the resin suspended in 2 *N* NaOH; an equal volume of *p*-dioxane was added to swell the resin. After chromatography on DEAE-cellulose (0.01 *M* phosphate, pH 7, and 0.3 *M* NaCl) a 60% yield of V was obtained, identical with material prepared by the above procedure; λ (pH 13) 285 nm (ϵ 22,200); λ (pH 1) 252 nm (ϵ 14,950), 305 (10,300); paper chromatography, R_f 0.32 (5% Na_2HPO_4). *Anal.* ($C_{20}H_{20}N_6O_6 \cdot H_2O$) C, H, N.

†Compounds followed by empirical formulas were analyzed for C, H, and N; values found were ±0.4% of theory, except as noted.

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Histamine *N*-Methyltransferase. Inhibition and Potentiation by *trans*- and *cis*-1,5-Diphenyl-3-dimethylaminopyrrolidine

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The inactivation of histamine in most mammalian tissues is dependent upon the enzyme histamine *N*-methyltransferase (HMT) (E.C. 2.1.1.6).[†,1,2] Of recent interest has been the observation that antihistamine drugs could inhibit or potentiate the activity of HMT depending on the concentration of the substrate.[3,4] In addition, there appears to be a partial correlation between the effects on the activity of HMT and the antihistaminic activity of these drugs. Therefore, it has been suggested that there might be structural similarities between the active site of HMT and the histamine receptors.[3]

The possibility of similarities in the binding specificity of HMT and the histamine receptors has been further investigated in this study by comparing the effects of the antihistamine tripelennamine (1) and the semirigid analogs *trans*- and *cis*-1,5-diphenyl-3-dimethylaminopyrrolidine (**2** and **3**) on HMT isolated from guinea pig brain.

Compounds **2** and **3** have previously been shown to be potent antagonists for the histamine H_1 receptor.[5,6] These

†Abbreviations used are: SAM, *S*-adenosyl-L-methionine; HMT, histamine *N*-methyltransferase; K_{is}, inhibition constant for the slope.