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.

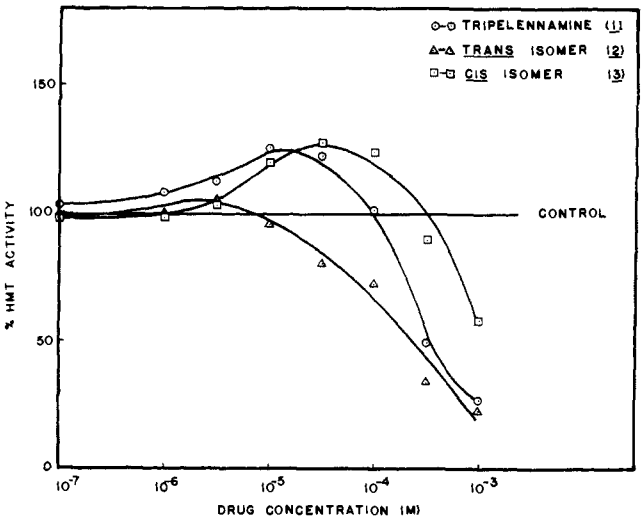
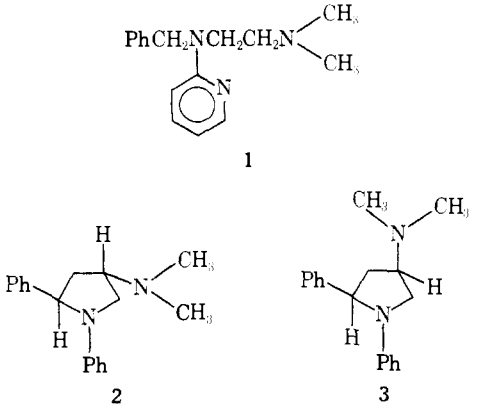


Figure 1. Effect of tripelennamine and *trans*- and *cis*-1,5-diphenyl-3-dimethylaminopyrrolidine on the activity of histamine *N*-methyltransferase (HMT). HMT activity was measured in the presence of increasing concentrations of drugs, while the concentrations of SAM (0.2 m*M*) and histamine (0.2 m*M*) were kept constant. Each point is the mean of two determinations.



1

2 3

diastereomeric histamine antagonists 2 and 3 have been used to study the stereochemical factors associated with inhibition or potentiation of HMT by antihistaminic drugs and the results are reported in this communication.

Results and Discussion

In Figure 1 the effects of increasing concentrations of tripelennamine (1), the trans isomer 2, and the cis isomer 3 on the activity of HMT are shown. These experiments were conducted utilizing a fixed concentration of histamine (0.2 m*M*) with drug concentrations ranging from 0.1 μM to 1 m*M*. At lower concentrations of tripelennamine (1) and the cis isomer 3 enhanced enzyme activity was observed, which is consistent with the effects of other antihistamines as previously reported by Taylor and Snyder.[3] In contrast, the trans isomer 2 under the experimental conditions used showed very little if any potentiation of HMT activity, but rather produced a potent inhibition of enzyme activity. Although a configurational stereoselectivity for the inhibition and potentiation of HMT by enantiomeric histamine antagonists was previously reported, it was found that either of the isomeric forms of a given antihistamine could both inhibit and activate the enzyme.[3] In the present study the diastereoisomers 2 and 3 exhibited qualitative as well as quantitative differences in their effects on HMT. Both tripelennamine and the cis isomer 3 also produced inhibition of HMT activity, but at higher drug concentrations than required for 2. The failure of 2 to enhance HMT activity significantly is similar to the results previously reported for the antimalarial agent quinacrine.[3]

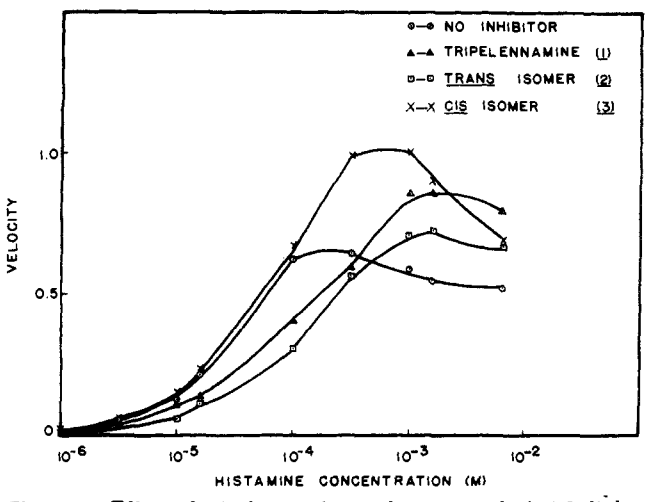


Figure 2. Effect of tripelennamine and *trans*- and *cis*-1,5-diphenyl-3-dimethylaminopyrrolidine on the activity of histamine *N*-methyltransferase (HMT) at various concentrations of histamine. HMT activity was measured in the presence of increasing concentrations of histamine, while the concentration of the drug was maintained at 0.1 m*M*. SAM concentration was also maintained at 0.2 m*M*. Each point is the mean of three determinations. Vel = nmol/mg of protein/min.

Table I. Inhibition Constants for Tripelennamine and *trans*- and *cis*-1,5-Diphenyl-3-dimethylaminopyrrolidine[a]

Compd	Inhibition constants, μM,[b] $K_{is} \pm$ S.E.M.
Tripelennamine (1)	87.7 ± 8.9
trans-1,5-Diphenyl-3-dimethylaminopyrrolidine (2)	55.8 ± 5.9
cis-1,5-Diphenyl-3-dimethylaminopyrrolidine (3)	164 ± 23

[a] HMT was purified and assayed as described in the Experimental Section. [b] Conditions used to determine inhibition constants: SAM concentration, 200 μM; histamine concentrations variable, 5–40 μM; inhibitor concentrations variable, 0.1–1.0 m*M*; phosphate buffer, pH 7.4; incubation time 15 min, 37°. Each of the inhibitors showed competitive kinetics and data fitted to eq 1.

As shown in Figure 2 similar results were obtained when the effects of compounds 1, 2, and 3 on HMT activity were examined at different histamine concentrations. Drug concentrations of 0.1 m*M*, which had produced marked potentiation of HMT activity in the initial experiments, were examined for their effects on enzyme activity at histamine concentrations ranging from 1 μM to 8 m*M*. As previously shown,[3] tripelennamine (1) produced inhibition of enzyme activity at lower histamine concentrations and potentiation at higher concentrations of histamine. Interestingly, the cis isomer 3 under the conditions used showed no inhibition of activity but produced significantly greater potentiation of HMT activity than tripelennamine (1). In contrast, the trans isomer 2 under the same conditions showed potent inhibition of HMT activity and showed significantly less potentiating effect than tripelennamine (1) or the cis isomer 3.

Using reciprocal velocity *vs.* reciprocal substrate plots, the kinetic patterns for inhibition of HMT by tripelennamine (1), trans isomer 2 and cis isomer 3 were determined. In each case a linear competitive pattern of inhibition was observed when histamine was the variable substrate. In Table I is shown the inhibition constants which were calculated for compounds 1, 2, and 3. As indicated from the initial experiments the most potent inhibitor is

the trans isomer **2** and the weakest inhibitor is the cis isomer **3**. The order of activity as inhibitors of HMT appears to correlate with the ability of these drugs to block the action of histamine on the histamine H_1 receptor.[5,6] In contrast, the ability to potentiate the effects of HMT by these drugs appears to be completely opposite to their inhibitory effects on HMT and their antagonistic effects on the histamine receptor.

Since the double-reciprocal plots described above showed linear competitive kinetics (no increase in V_{max}), it can be assumed that the "activation" of HMT by these drugs results only from diminishing the inhibition produced at higher concentrations of histamine. The potentiation of HMT by these compounds, therefore, is not a true "activation" of the enzyme, but rather reversal of substrate inhibition. This substrate inhibition may be the result of allosteric mechanisms; however, neither the present work nor that of Taylor and Snyder provides definitive evidence for allosteric mechanisms.[3]

In summary, it can be concluded that a relationship exists between the HMT inhibitory effects of compounds **2** and **3** and their effects as histamine antagonists on the H_1 receptor. The trans isomer **2** is a better HMT inhibitor and a better histamine antagonist than the cis isomer **3**. However, the cis isomer **3** is more active in potentiating HMT activity, whereas the trans isomer **2** shows little potentiating ability. This may indicate a difference in the conformational requirements associated with the inhibition and potentiation of HMT by antihistamines.

Experimental Section

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/ml and stored at −20°F. SAM iodide (Sigma) was stored as a 0.01 *M* stock solution.

Tripelennamine was a gift from Dr. A. J. Plummer and compounds **2** and **3** were prepared by previously described procedures.[6]

Histamine *N*-Methyltransferase (HMT) Purification. HMT was purified from guinea pig brain (Pel-Freez Biologicals) according to the methods previously described by Brown, *et al.*[2] The enzyme was purified through the dialysis step resulting in a preparation which contained 15.2 mg of protein per milliliter. This represented a ninefold purification from the crude supernatant.

Measurement of Enzyme Activity. Kinetic experiments were carried out in 15-ml screw cap culture tubes and the basic incubation mixture contained the following components (in micromoles) added in this sequence: water, so that final volume was 0.25 ml; histamine (variable); inhibitor (variable); *S*-adenosyl-L-methionine (variable); 0.05 μCi of *S*-adenosyl-L-methionine-^{14}C; phosphate buffer, pH 7.40 (10); and the enzyme preparation (0.05 ml). The reaction was started by addition of enzyme and incubated for 15 min at 37°. The reaction was stopped by addition of 0.25 ml of 0.5 *M* borate buffer, pH 10.0, and the aqueous mixture extracted with 10 ml of toluene–isoamyl alcohol (1:1). After centrifugation, an aliquot (5 ml) of the organic phase was transferred to a scintillation vial, a dioxane-based phosphor solution (10 ml) added, and the radioactivity measured in a Beckman LS-150 scintillation spectrophotometer.

Data Processing. Reciprocal velocities were plotted graphically against reciprocals of the substrate concentrations. In all cases, for the substrate concentration ranges used, reasonably linear relationships were obtained.

Data fitting linear competitive inhibition patterns were fitted to eq 1 using a Hewlett-Packard 2100A digital computer and a Fortran IV program.[7,8]

$$v = VA/(K[1 + I/K_{is}] + A) \qquad (1)$$

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Synthesis of Some Trinucleoside Monophosphates of Biological Interest

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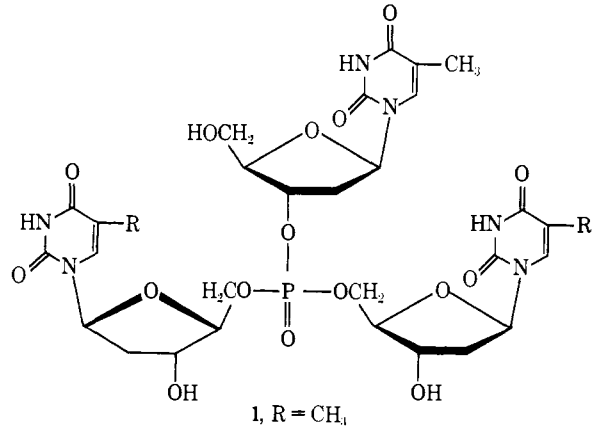
Synthesis and studies of some trinucleoside monophosphates were undertaken in the hope of finding a way of introducing nucleotide analogs into mammalian cells. Phosphotriesters of intermediary stability seemed suitable to achieve this goal, in spite of negative results reported for the diethyl FdUrd-5′-P.[1,†] Trinucleoside monophosphates resemble the nucleosides in that they are both neutral and possess free hydroxyl groups, but they also contain an internucleotide linkage. These compounds *per se* might exhibit interesting biological properties. So far, tri(uridine 5′-) phosphate and di(uridine 5′-) thymidine 3′-phosphate were the only compounds of this class obtained by a deliberate synthesis.[2] Here we describe the synthesis of di(thymidine 5′-) thymidine 3′-phosphate (**1**, $dThd_3$-P), bis(5-bromodeoxyuridine 5′-) thymidine 3′-phosphate [**2**, $(BrdUrd)_2$dThd-P], and bis(5-fluorodeoxyuridine 5′-) thymidine 3′-phosphate [**3**, $(FdUrd)_2$dThd-P].



1, R = CH_3
2, R = Br
3, R = F

Of the several synthetic approaches which we explored, the most successful one was the esterification of 5′-*O*-tritylthymidine 3′-phosphate (TrdThd-P) with an excess of unprotected nucleosides using triisopropylbenzenesulfonyl chloride as the condensing agent. After acidic removal of the trityl groups, the triesters **1**, **2**, and **3** were obtained by passing the mixtures through Dowex-1 resin, followed by silica gel chromatography. The generally poor

†Abbreviations: FUra, 5-fluorouracil; FdUrd, 5-fluoro-2′-deoxyuridine; BrdUrd, 5-bromo-2-deoxyuridine; dThd, 2′-deoxythymidine; FdUrd-5-P, the corresponding nucleotide.