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Chemical Differentiation of Histamine H_1- and H_2-Receptor Agonists[1]

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Received March 25, 1975

Histamine exists predominantly as the N^{τ}–H tautomer of the monocation (IIa) at a physiological pH of 7.4 and structure–activity studies indicate that this tautomer is likely to be the pharmacologically active species for both H_1 and H_2 receptors. Effective H_2-receptor agonists appear to require a prototropic tautomeric system whereas H_1-receptor agonists do not need to be tautomeric. This identifies a chemical difference in the receptor requirements which provides the basis for obtaining selective histamine H_1-receptor agonists. Thus 2-(2-aminoethyl)thiazole and 2-(2-aminoethyl)pyridine are nontautomeric and are highly selective agonists for histamine H_1 receptors (H_1:H_2 ca. 90:1 and 30:1, respectively). In conjunction with the selective H_2-receptor agonist, 4-methylhistamine, they are of great value for studying the pharmacology of histamine receptors.

The pharmacological actions of histamine are mediated by at least two distinct classes of receptor. One receptor type, designated H_1 by Ash and Schild,[2] mediates histamine-induced contraction of smooth muscle of the small intestine and bronchi. A second receptor type, designated H_2 and characterized by Black and coworkers,[3] mediates the action of histamine in vivo in stimulating gastric acid secretion and in vitro in inhibiting contractions of the rat uterus and in increasing the rate of beating of the guinea-pig atrium. The receptors are defined pharmacologically from the antagonists which selectively block the responses of these tissues to histamine stimulation.[2,3] The differentiation of these two receptor types is also reflected in the relative agonist activities of certain histamine analogs.[2-4] Thus from an analysis of agonist activities in three H_2-receptor systems and two H_1-receptor systems it was shown that 4-methylhistamine (1, Table I) is highly selective in stimulating H_2 receptors and that 2-methylhistamine (2) is a relatively selective H_1-receptor stimulant[3] (for histamine nomenclature[5] see Scheme I, I). In the work presented herein, H_2-receptor agonist activity is determined by stimulation of rat gastric acid secretion, and H_1-receptor agonist activity is measured on the isolated guinea-pig ileum. In these assays, the relative activities (H_1:H_2) are in the ratios of approximately 1:200 for 4-methylhistamine and approximately 8:1 for 2-methylhistamine. Activity in the H_1- and H_2-receptor systems is highly sensitive to apparently minor structural changes in the agonist molecule. This suggests

Scheme I. Ionic and Tautomeric Equilibria between Histamine Species

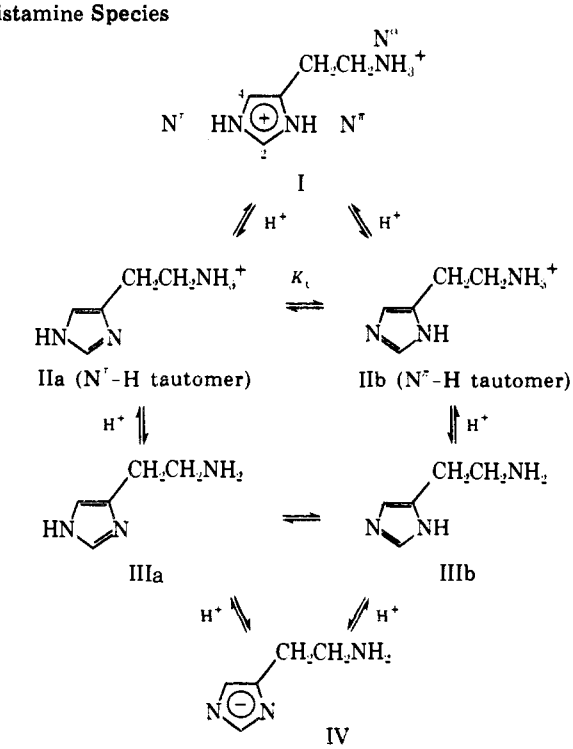


Table I. H_1- and H_2-Receptor Agonist Activities of Some Methylated Histamine Derivatives and of Some Heterocyclic Analogs of Histamine

Compd no.	Het-CH_2CH_2X Het	X	Agonist act. rel to histamine (=100) H_1 receptor[a]	H_2 receptor[b]
1		NH_2	0.23[c]	39
2		NH_2	16.5[d]	2.0
3		$N(CH_3)_2$	44[e,f]	19
4		$N^+(CH_3)_3$	0.09[g]	Weak[h]
5		NH_2	12.7[i,j]	13.7
6		NH_2	5.6[k,l]	~0.2[m]
7		NH_2	<0.001[n]	~0.4[o]
8		NH_2	26[p,q]	~0.3
9		NH_2	0.01	~0.1
10		NH_2	0.12[r,s]	~0.5[t]

[a]Tested for contraction of isolated guinea-pig ileum in the presence of atropine (see Experimental Section). [b]Tested for stimulation of gastric acid secretion in the anesthetized rat (see Experimental Section). [c]95% confidence limits (0.20-0.27). [d]95% confidence limits (15.1-18.1). [e]95% confidence limits (38-51). [f]Lit. data 18%,[29] 80%.[30] [g]Lit.[31] data 1%. [h]Non-dose-related stimulation of gastric acid secretion in the range 8-64 μmol/kg. [i]95% confidence limits (10.9-13.5). [j]Lit.[29] data 73%. [k]95% confidence limits (5.0-6.3). [l]Lit. data 3%,[2] 9%.[13] [m]Lit.[2] data 0.7%. [n]Lit.[2] data 0.01%; reported inactive in ref 11. [o]Lit.[2] data 0.7%. [p]95% confidence limits (19.7-32.7). [q]Lit.[13] data 30%. [r]95% confidence limits (0.10-0.14). [s]Lit. data 0.06%,[2] 1%,[29] [t]Lit.[2] data 4.2%.

that there may be differences in the topography of the two receptors and it raises the interesting question of whether the chemical involvement of histamine differs at the two types of receptor. Thus we need to identify which chemical properties of histamine differentiate its action at H_1 and H_2 receptors and consideration of this problem is the subject of the present publication.

In order to consider relationships between chemical and biological properties it is important to know which is the biologically active form of histamine in the two receptor systems. In aqueous solution, histamine exists as an equilibrium mixture of different ionic and tautomeric species (Scheme I), viz. the dication I, two tautomers of the monocation II, two tautomers of the neutral form III, and the anion IV. The populations of the different ionic species in the physiological pH range, calculated from reported pK_a values,[6] are given in Table II. At a pH of 7.4, the pH usually taken to be that of the extracellular fluids, the main form is the monocation (mole fraction, n = 0.97). The pH at the site of action is unknown, but a pH as low as 5 could

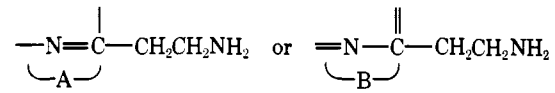
Table II. Population (Expressed as Mole Fraction, n) of Histamine Species in Aqueous Solution[a] at pH 7.4 and 5.4, Respectively

Species	n At pH 7.4	At pH 5.4
Dication I	0.025	0.715
Monocation II	0.966	0.285
Neutral III	0.01	0.0001
Anion IV	2.5×10^{-6}	2.5×10^{-8}

[a]Derived from pK_a values at 37° (ref 6): pK_{a_1} = 5.80; pK_{a_2} = 9.40; pK_{a_3} = 14.

be considered, as happens in the vicinity of some membranes.[7] At a pH of 5.4, for example, the main form would be the dication (mole fraction, n = 0.72). Thus both the monocation and the dication should be considered as possible active forms in histamine-receptor interactions. The populations of the neutral form and of the anion are relatively much lower in the pH range 5.4-7.4 so that it would seem much less likely for them to be active species in histamine-receptor interactions. We can gain indications for the active species by considering heterocyclic analogs of histamine. Thus we find that the 1,2,4-triazole analog 5, a compound first synthesized by Ainsworth and Jones,[8] has approximately 13-14% of the activity of histamine as an agonist at both H_1 and H_2 receptors (Table I). The ring pK_a of this compound is, however, very low (1.4)[9] and therefore the population of the dication, even at a pH of 5.4, is very small indeed ($n = 1 \times 10^{-4}$) suggesting that it is most unlikely for the dication to be the biologically active species. The dominant ionic species in triazolylethylamine is overwhelmingly the monocation ($n > 0.99$) and this is most likely to be the active form. It seems reasonable to deduce that, correspondingly, the monocation is also the active form of histamine. All agonists that we know to be reasonably active at either H_1 or H_2 receptors are strongly basic compounds, typically primary, secondary, or tertiary amines which exist mainly in cationic forms at physiological pH. We find that the tertiary dimethylamine 3 is active both at H_1 and H_2 receptors but the quaternary trimethylammonium derivative 4 is an exceedingly weak stimulant in both receptor systems (Table I). Therefore, a proton on the N^α-ammonium group appears to be of importance for both H_1- and H_2-receptor agonism.

Histamine monocation is a mixture of two tautomers (Scheme I). pK_a studies[10] suggest that in aqueous solution the N^τ-H tautomer (IIa) is the more prevalent but that a substantial fraction is present in the N^π-H form (IIb); the measured tautomeric equilibrium constant K_t = 4.2 indicates that the relative concentration of N^τ-H:N^π-H ≈ 4:1. The N^τ-H tautomer IIa has previously been associated with the action of histamine on guinea-pig ileum. This followed from the original finding by Walter et al.[11] that 2-pyridylethylamine (6) was active whereas 4-pyridylethylamine (7) was not active on guinea-pig ileum (Table I). Niemann and Hays[12] showed later that 3-pyridylethylamine was also inactive on ileum and concluded that the characteristic pharmacological properties of histamine are associated with structures that have their origin in the tautomer IIa. These workers also asserted that chelation should occur between the nitrogen atoms in the cation. Subsequently, Lee and Jones[13] showed that 2-thiazolylethylamine (8) was a very active stimulant on ileum and proposed a minimal structural fragment V for histamine-like activity



V

where A or B is part of an aromatic nucleus. We have synthesized and retested several of these compounds (Table I) and we can now restate these earlier postulates in the form that H_1-receptor agonist activity is associated with the N^τ–H tautomer of histamine monocation IIa and that there appears to be a minimum structural requirement for an ammonium ethyl group ortho to a heterocyclic nitrogen atom possessing a lone electron pair. 5-Thiazolylethylamine (**9**), which we have found to be an extremely weak H_1-receptor agonist (Table I), and 3-pyridylethylamine are analogs of the N^π–H tautomer of histamine IIb in which the N–H group is replaced by S or C=C, respectively; the very low level of activity of these compounds suggests that the N^π–H tautomer is not an active form of histamine.

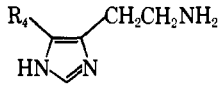
It is obvious from the activity of 2-pyridyl- and 2-thiazolylethylamine that the imidazole tautomeric system is not essential for H_1-receptor agonist activity and these results imply that, for histamine, imidazole tautomerism is probably not functionally involved in H_1-receptor stimulation. These nontautomeric heterocyclic ethylamines have a very low level of activity in stimulating gastric secretion in the rat (Table I), i.e., they are very weak H_2-receptor agonists. In fact, 2-pyridylethylamine (**6**) and its *N*-methyl derivative, betahistine, are known to be either inactive or very weak stimulants of gastric acid secretion in several mammalian species, including man.[14] It has been noted previously by Jones[4] that although compounds **6** and **8** are active on ileum they do not stimulate acid secretion in the dog. 2-Thiazolylethylamine (**8**) and 2-pyridylethylamine (**6**) may be described therefore as highly selective H_1-receptor agonists (H_1:H_2 ca. 90:1 and 30:1, respectively). In our studies which have included many analogs of histamine (unpublished results), we find that all H_2-receptor agonists with a reasonable level of activity in stimulating the secretion of gastric acid are compounds which are capable of undergoing 1,3-prototropic tautomerism. This clear chemical differentiation between H_1- and H_2-receptor agonists has not been reported previously. Care must be taken when assuming that the agonist activity of an analog is the consequence of a direct interaction with histamine receptors. The response could be caused by other actions of the compound, for example, by release of endogenous histamine. Analysis of dose–response curves often helps to establish the mode of action of the agonist. Analysis is difficult, however, with weakly active compounds. Thus 3-pyrazolylethylamine (betazole, **10**) which possesses a 1,2-prototropic tautomeric system but not a 1,3-tautomeric system is only weakly active as an agonist on rat gastric secretion (Table I). Betazole has also been used in place of histamine as a stimulant of gastric secretion in man.[15] Such use does not necessarily reflect a high degree of receptor selectivity (H_2:H_1), however, since betazole has been shown to release histamine from gastric tissues.[16] Conversely, lack of activity may be due to other factors such as difficulties of access, protein binding, or to metabolic effects rather than failure to activate receptors. However, there is no evidence to suggest that these factors account for the observed activity differences in the present investigation.

The importance of tautomerism for H_2-receptor agonist activity gains support from a comparison of the activities of 4-substituted histamine derivatives (Table III). 4-Methylhistamine is about half as active as histamine; replacement of methyl by electron-withdrawing substituents reduces activity. Thus 4-chlorohistamine (**11**) has about one-tenth and 4-nitrohistamine (**12**) has less than one-hundredth of the activity of histamine. Electron-withdrawing substituents in the 4 position of the imidazole ring change the relative tautomer concentrations and they alter the electron densities at the nitrogen atoms and affect proton acidities; the effect is more pronounced at the nearer nitrogen atom so that the relative stabilities of the tautomers change in comparison with histamine.[17] Thus the population of the N^τ–H tautomer of 4-methylhistamine is similar to that of histamine but is reduced by one order of magnitude for 4-chlorohistamine and by two orders of magnitude for 4-nitrohistamine (Table III). These reductions in populations of the N^τ–H tautomer approximately parallel the changes in H_2-receptor agonist activities. One is led to the speculative deduction that either the N^τ–H tautomer is the biologically active form of histamine at the H_2 receptor or that the free-energy difference between the two tautomers must be small for effective biological activity.

Thus the active form of histamine for both receptors is likely to be the N^τ–H tautomer IIa of the monocation which is also the most prevalent species in water at around neutrality. However, different chemical properties of histamine may be associated with interactions at the two receptor types (Chart I). At the H_1 receptor, imidazole tautomerism is not a functional requirement, but the ammoniumethyl group should be ortho to a heterocyclic nitrogen atom. The ring may also need to be able to freely rotate or at least to achieve coplanarity with the side chain.[18] At the H_2 receptor, the tautomeric property of the imidazole ring of histamine appears to be of importance and histamine might be involved as a proton-transfer agent. Pictorially, one can envisage the imidazole ring catalyzing the transfer of a proton from site A to site B and perhaps a catalytic mechanism of some kind may be involved in the events leading to an effective H_2-receptor response.

The requirements for agonist activity at H_1 and H_2 receptors differ sufficiently to permit the identification of highly selective agonists. In particular 2-pyridylethylamine (**6**) and 2-thiazolylethylamine (**8**), which lack a tautomeric system, are selective H_1-receptor agonists and 4-methylhistamine (**1**) [and to some extent 4-chlorohistamine (**11**)] is a

Table III. Tautomer Concentration Ratios K_t. Corresponding Mole Fractions $n_{N^\tau\text{-H}}$ of N^τ–H Tautomer at pH 7.4, and H_1- and H_2-Receptor Agonist Activities of 4-Substituted Histamine Derivatives

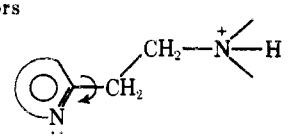


Compd no.	R_4	K_t[a]	$n_{N^\tau\text{-H}}$[b] × 100	Agonist act. rel to histamine (=100) H_1 receptor[c]	H_2 receptor[d]
Histamine	H	2.4	69	100	100
1	CH_3	4.1	70	0.23[e]	39
11	Cl	0.13	12	1.7[f]	12
12	NO_2	0.009	0.9	0.17[f]	0.6

[a] $K_{t,R}$ = [IIa]/[IIb] = antilog $[3.4(\sigma_{m,CH_2CH_2NH_3^+} - \sigma_{m,R})]$;[17] σ_m values taken as +0.11 ($CH_2CH_2NH_3^+$),[17] −0.07 (CH_3), +0.37 (Cl), +0.71 (NO_2). [b] At pH 7.4 using pK_a values from ref 17, corrected to 37°: viz. 6.5 (R = CH_3); 3.1 (R = Cl); <0 (R = NO_2). [c,d] See footnotes *a* and *b*, Table I. [e] See footnote *c*, Table I. [f] Limited data; tested in one nonatropinized ileum preparation by comparing single doses of the compound at two dose levels with single doses of histamine at two dose levels in 2 + 2 assay.

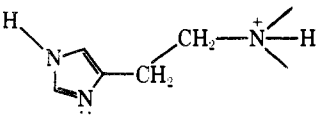
Chart I. Functional Requirements of Agonists

(a) at H_1 receptors



indicating (i) side-chain cation and NH;
(ii) ring rotation and basic nitrogen atom (with lone pair of electrons) in ortho position

(b) at H_2 receptors



indicating (i) side-chain cation and NH;
(ii) N'-H tautomer of ring which may function as a proton transfer agent, e.g.

selective H_2-receptor agonist. These selective agonists are of great value for studying the pharmacology of histamine receptors.

Experimental Section

Chemistry. Melting points were determined on an "Electrothermal" electrically heated apparatus using a thermometer corrected for stem exposure. Microanalyses for elements indicated were within 0.4% of the theoretical values.

Compound **10** (dihydrochloride, betazole hydrochloride) was obtained from Eli Lilly and Co. The syntheses of compounds **1** (dihydrochloride, mp 239–242°, MeOH–Et_2O), **2** (dihydrochloride, mp 222–224°, EtOH–Et_2O), and **3** (dihydrochloride, mp 188–191°, *n*-PrOH) have been described[19a] and will be discussed in a future publication.[19b] Compounds **6** [dihydrochloride, mp 184–185°, MeOH–H_2O (lit.[11] mp 195–196°)] and **7** [dihydrochloride, mp 220–222°, MeOH (lit.[11] mp 222°)] were obtained from commercially available amines (Koch-Light and Co., England; Midland Tar Distillers, England). 4-Nitrohistamine [**12**, hydrochloride mp 274–276°, MeOH (lit.[20] mp 272°). Anal. ($C_5H_8N_2O_2$·HCl) C, H, N, Cl] was synthesized in these laboratories by Dr. G. Crank from histamine dihydrochloride by direct nitration. 4-Chlorohistamine (**11**, dihydrochloride mp 234–236°, EtOH–Et_2O) was synthesized in these laboratories by Dr. G. Crank from N^{α}-acetyl-4-nitrohistamine [mp 241–243°. Anal. ($C_7H_{10}N_4O_3$) C, H, N] by hydrogenation (Pd/C) followed by diazotization with $NaNO_2$–Cu_2Cl_2–HCl. Compounds **5** [dihydrochloride, mp 211–213°, MeOH–Et_2O (lit.[8] mp 215°)] and **8** [dihydrochloride, mp 163–165°, MeOH–*i*-PrOH (lit.[21] mp 154–158°)] were prepared by literature methods.[8,21]

2-[4(5)-Imidazolylethyl]trimethylammonium Chloride (4). 4(5)-(2-Chloroethyl)imidazole hydrochloride[22] (2.0 g, 0.012 mol) was converted to the base with NaOMe (0.65 g, 0.012 mol) in EtOH and allowed to react with 33% Me_3N–EtOH (20 ml) in a pressure vessel at steam bath temperature for 18 hr. Concentration, followed by two recrystallizations from *i*-PrOH–hexane afforded **4** (0.9 g, 40%), mp 205–206°. Anal. ($C_8H_{16}ClN_3$) H, N; C: calcd, 50.65; found, 49.96. Cl: calcd, 18.69; found, 19.44. The compound has been reported previously[23] as the hydrochloride salt.

5-(2-Aminoethyl)thiazole Dihydrochloride (9). 5-Formylthiazole (6.44 g, 0.058 mol, prepared from chloromalondialdehyde[24] and thioformamide[25] as described[26] and purified by steam distillation) and nitromethane (3.12 g, 0.058 mol) were mixed, *n*-butylamine (8 drops) was added, and the mixture was set aside for 4 days at room temperature. A dark solid separated, which was collected, chromatographed on a column of SiO_2 ($CHCl_3$), and recrystallized from EtOH to give 5-(2-nitrovinyl)thiazole as needles (4.3 g, 49%), mp 175–177.5°. Anal. ($C_5H_4N_2O_2S$) C, H, N.

5-(2-Nitrovinyl)thiazole (2.40 g) was reduced with excess $LiAlH_4$ (2.4 g) in Et_2O (100 ml)–benzene (325 ml) under N_2 and the crude product obtained was purified using ion-exchange resin Zeo-Carb 225 (H^+) with elution by 2 *N* HCl. Recrystallization from EtOH–C_6H_6 gave **9** (0.50 g, 17%), mp 147–148° (hot-stage microscope). Anal. ($C_5H_8N_2S$·2HCl) C, H, N, Cl.

Pharmacology. H_2-Receptor Agonist Activity Assay on Rat Gastric Acid Secretion. The preparation used was a modification of the rat stomach perfusion technique of Ghosh and Schild.[27] The stomach of the starved atropinized (1 mg/kg im), urethane anesthetized rat was perfused via cannulae placed in the esophagus and in the pyloric antrum. The perfusate was collected via a funnel placed in the nonsecretory rumen. The pyloric cannula had a solid piece of perspex at one end designed to fill dead-space in the stomach cavity. Perfusion was carried out with 5.4% w/v glucose solution warmed to 37° and the perfusate was passed through a micro flow type glass electrode system. Changes in the hydrogen ion activity were recorded continuously on a potentiometric pen recorder. Histamine and the other compounds were administered by rapid iv injection through a cannula in a jugular vein. The atropinization and the sectioning of the vagus nerves carried out during the operation reduced basal secretion to a minimum. The body temperature of the rat was maintained at 37° throughout the experiment.

Analytical dilution assays were carried out on compounds 1–3, 5, and 11 using 3 + 3 doses in a Latin square design (doses 1, 2, and 4 μmol/kg iv of histamine normally being used). Three animals were used for each assay, each animal receiving 12 doses or two lines of the block design. The results of all assays were treated by an analysis of variance. Compounds 1–3 and 5 were tested for inhibition of stimulant activity by the H_2-receptor antagonist metiamide.[28] Antagonism was quantitatively similar to the antagonism of histamine by metiamide.

The other compounds in Tables I and III have a low order of activity and it was not possible to carry out parallel line assays. For these compounds estimates of potency were obtained by comparing responses to low doses of histamine with very high doses of the compound. A maximum of three doses of the compound was used in each preparation and a minimum of three preparations was used for each approximate potency estimate.

H_1-Receptor Agonist Activity Assay on Guinea-Pig Ileum. Guinea pigs of either sex weighing between 400 and 700 g were used. Immediately after the animal was killed, the terminal ileum was removed, washed, and mounted in a 15-ml organ bath containing magnesium-free Tyrode solution. The bath solution was gassed with a mixture of 95% O_2–5% CO_2 and the temperature was maintained at 30°. The tissue was loaded with 0.5 g and isometric contractions were detected by a force transducer and displayed on a potentiometric recorder.

Cumulative dose–response curves to histamine (normal concentration range 0.11–2.7 μM) and the analogs were obtained in the presence of 1 μM atropine to establish the approximate agonist potency. From the cumulative curves, doses which produced approximately 25 and 75% of the maximum response were chosen and a 2 + 2 assay of the analog against histamine was carried out using a 2-min time cycle. The results were put through the IBM 370 computer on the Ranova 2 program for factorial analysis and the potency calculation with limits. Compounds in Tables I and III which have activities quoted with limits gave maximal responses similar to those of histamine and parallel line assays could be carried out.

Acknowledgments. The authors acknowledge the contribution of Dr. G. Crank for synthesizing 4-chloro- and 4-nitrohistamine. We also thank J. M. Loynes and P. D. Miles (synthetic chemistry), R. C. Blakemore (pharmacology), and M. J. Graham (microanalysis) for their contributions to the experimental work.

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Methotrexate Analogs. 6. Replacement of Glutamic Acid by Various Amino Acid Esters and Amines

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A series of methotrexate (MTX) analogs was prepared in which the glutamic acid moiety is replaced by various amino acid esters and amines. The synthetic method consisted of the reaction of 4-amino-4-deoxy-N^{10}-methylpteroic acid with various reagents to form intermediate mixed anhydrides, which then reacted with amino acid esters or amines to give the MTX analogs. These compounds were tested for antibacterial activity against *Streptococcus faecium* and for antitumor activity against L1210 leukemia in mice. Several compounds showed significant antibacterial activity; the MTX homocysteinethiolactone and MTX aspartate analogs showed marginal in vivo antitumor activity.

For the past several years, work has been conducted in this laboratory aimed at the structural modification of the antitumor agent methotrexate (4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid, amethopterin, MTX)[1] in order to prepare analogs with modified transport properties and improved biological activity. The initial work involved a total synthesis scheme whereby analogs were prepared in which the carboxyl groups of the glutamic acid moiety of MTX were replaced by less polar groups such as CH_2OH and CH_3 and analogs in which glutamic acid was completely replaced by adamantylamine.[2,3] Lipophilic alkyl esters of MTX and 3′,5′-dichloromethotrexate have also been prepared and investigated as "latent" forms of these drugs.[3,4] Other MTX analogs prepared in this laboratory include N^8-oxides,[3] 7-methyl derivatives,[5] and 7,8-dihydro-8-methylmethotrexate.[6]

During the course of this work, an efficient synthesis of 4-amino-4-deoxy-N^{10}-methylpteroic acid (1) was developed,[2,3] which allowed this compound to be prepared in large quantities for use as an intermediate for the preparation of other side-chain modified MTX analogs. We were interested in preparing additional MTX analogs for biological evaluation in which glutamic acid is replaced by various amino acid esters and amines. Many folate analogs and aminopterin analogs in which the glutamate moiety is replaced by other amino acids have been reported,[7,8] but relatively few MTX analogs of this type have been prepared and evaluated for biological activity. It has been shown that the MTX aspartate analog[9] and D-glutamate analog[10] exhibit antitumor activity against L1210 leukemia in mice.

Starting with the pteroic acid analog **1**, the compounds listed in Table I were prepared and evaluated for biological activity. The synthetic method employed consisted of the reaction of **1** with various reagents to form intermediate mixed anhydrides,[11] which then reacted with amines or amino acid esters to give the products, in which the side-chain R was varied to include straight-chain, branched-chain, and cyclic structural features.

Chemistry. The pteroic acid analog **1** is not appreciably soluble in most organic solvents. However, as its partial hydrochloride hydrate ($C_{15}H_{15}N_7O_2$·0.5HCl·1.5H_2O)[3] it is sufficiently soluble in DMF (1–2 g/100 ml) to permit this solvent to be used as a reaction medium. It was found that **1** reacted rapidly with an excess of isobutyl or isopropyl chloroformate, in DMF at room temperature and in the presence of Et_3N, to form mixed anhydrides (Scheme I).