Inhibition of *Lactobacillus casei* Thymidylate Synthetase by 5-Substituted 2'-Deoxyuridylates.[1] Preliminary Quantitative Structure-Activity Relationship

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The binding of a series of 5-substituted 2'-deoxyuridylates to *Lactobacillus casei* thymidylate synthetase has been determined by their inhibition of thymidylate formation and of dehalogenation of 5-bromo-2'-deoxyuridylate. As inhibitors of thymidylate formation, the apparent dissociation constants of this series span over three orders of magnitude, and quantitative structure-activity relationships (QSAR) could be established. The most important parameters are the electronic σ^- constant and the polar $\mathcal{F}$ constant which indicates that electron withdrawal from the uracil heterocycle increases affinity for the enzyme. The effect cannot be solely ascribed to changes in acidity of the 3-NH and must involve other electronic perturbations of the uracil moiety. The steric parameter, MR, has a negative coefficient indicating that in the series tested the bulkier 5-substituents are detrimental to binding. Hydrophobic effects do not appear to be important in the differential binding of the 5-substituted analogues examined. The same series of analogues shows similar affinities for the enzyme in the absence of the cofactor, as determined by their inhibition of dehalogenation of 5-bromo-2'-deoxyuridylate in the absence of the cofactor, 5,10-methylenetetrahydrofolate. Although QSAR could not be established using this assay, it is clear that the binding regions of 5-substituted 2'-deoxyuridylates are dramatically different in the enzyme and enzyme-cofactor complex.

Thymidylate synthetase catalyzes the conversion of 2'-deoxyuridylate (dUMP[1]) to thymidylate (dTMP), with concomitant transfer and reduction of the one carbon unit of 5,10-methylenetetrahydrofolate (CH_2-H_4folate).[2] Because this enzyme represents the sole de novo pathway for dTMP synthesis, it has been a popular target for design of inhibitors with potential chemotherapeutic applications. Thus far, the most useful inhibitors of thymidylate synthetase have been 5-substituted 2'-deoxyuridylates.[2-4] The nature of the 5-substituents has dramatic effects on the affinity for this enzyme, and such analogues seemed ideal candidates for construction of quantitative structure-activity relationships.[5] In this report, we describe equations which correlate the inhibition of *Lactobacillus casei* thymidylate synthetase by nine 5-substituted 2'-deoxyuridylates. The results permit assessment of the features of the 5-substituent which are important for binding and provide a basis for rational development of more potent inhibitors of this enzyme.

Experimental Section

Thymidylate synthetase obtained from an amethopterin-resistant strain of *L. casei*[6] was the purified preparation previously described.[7] 5-Hydroxymethyl- and 5-trifluoromethyl-2'-deoxyuridine were obtained from P-L Biochemicals, Inc., and converted to the corresponding nucleotides with thymidine kinase;[8] after DEAE-cellulose chromatography,[9] the nucleotides were desalted and further purified by paper chromatography using *n*-BuOH-AcOH-H_2O (7:1:2). FdUMP was obtained from Terra-Marine Bioresearch and other 5-halogenated nucleotides were from sources previously described.[7] dUMP and dTMP were obtained from P-L Biochemicals and *dl*-L-H_4folate was prepared by the method of Hatefi et al.[10]

Assays of thymidylate synthetase catalyzed TMP formation were performed spectrophotometrically at 30 °C under conditions previously described.[11] Initial velocity experiments were performed as follows: in 1.1 mL of NMM buffer (54.5 mM *N*-methylmorpholine, pH 7.4, 27.3 mM $MgCl_2$, 1.1 mM EDTA, and 81.8 mM 2-mercaptoethanol) was contained 0.13 mM *dl*-L-H_4-folate, 5.6 mM formaldehyde, specified amounts of dUMP (4-35 μM), and the inhibitor. Reactions were initiated by addition of limiting amounts of enzyme (ca. 0.01 mmol) in 0.1 mL to give a total volume of 1.2 mL. Controls omitted dUMP.

Assays of dehalogenation of BrdUMP were performed at 30 °C by monitoring the decrease in absorbance at 290 nm ($\Delta\epsilon$ 4880) concomitant with conversion to dUMP.[7] Initial velocity experiments were performed as follows: in 1.1 mL of NMM buffer was contained specified amounts of BrdUMP (10-80 μM). Reactions were initiated by addition of a limiting amount of enzyme (ca. 0.2 nmol) in 0.1 mL to give a total volume of 1.2 mL. Controls omitted enzyme. Initial velocities were determined before 5% of the reaction had occurred to avoid errors resulting from inhibitory effects of dUMP.

Results

Table I shows K_i values for a number of 5-substituted 2'-deoxyuridylates as assessed by their ability to inhibit thymidylate synthetase catalyzed dTMP formation and dehalogenation of 5-BrdUMP. As described below, an assay of these compounds as inhibitors of dTMP formation is complicated by the fact that, with the exception of dTMP, CldUMP, and $HOCH_2$dUMP, the compounds are in themselves capable of undergoing reactions in the presence of thymidylate synthetase and CH_2-H_4folate. To obtain the apparent K_i values reported, enzyme was added to solutions containing *all* components of the reaction mixture, and initial velocities were determined as fast as possible. In this manner, measurements are made under conditions where the effects of slower, competing reactions would be minimized. Figure 1 shows representative double reciprocal plots which clearly indicate competitive inhibition kinetics.

Two of the compounds examined, BrdUMP and IdUMP, are substrates for thymidylate synthetase and in the presence of CH_2-H_4folate are converted to dTMP.[7] Clearly, if under the conditions used these reactions were rapid compared to the normal enzymic reaction, the validity of the determined K_i values would be questionable. To ascertain whether this was the case, the initial velocities of dTMP formation from BrdUMP, IdUMP, and dUMP were compared under conditions identical with those used in determination of apparent K_i values. These experiments used concentrations of the halogenated nucleotides (7.5 μM) which were well above their apparent K_i (K_m) values. The rates of formation of dTMP from BrdUMP and IdUMP were 3 and 2.5%, respectively, of those from dUMP; thus, competing reactions of these halogenated derivatives are sufficiently slow that they are not significant over the time of measurement and the reported numbers reflect true K_i values.

Table I. Inhibition of Thymidylate Synthetase by 5-Substituted 2'-Deoxyuridylates[a]

Abbreviation used	5-Substituent	TMP formation, K_i (μM) ± CV (%)[b]	BrdUMP dehalogenation, K_i, μM
dTMP	CH_3	15.5 ± 10	8.69
$HOCH_2$dUMP	CH_2OH	8.3 ± 3.7	3.9
dUMP	H	5.2 ± 35 (K_m)	2.26
IdUMP	I	1.6 ± 20	1.44 (K_m)
BrdUMP	Br	1.4 ± 18	5.7 (K_m)
CldUMP	Cl	0.19 ± 20	9.39
CF_3dUMP	CF_3	0.039 ± 4.0	
FdUMP	F	0.014 ± 25	14.9
CHOdUMP	CHO[c]	0.017 ± 42	

[a] All inhibitors were competitive with respect to dUMP or BrdUMP as determined by double reciprocal plots. K_i values were calculated using K_m values determined in the same experiment; reported K_i values are the mean of three determinations for dTMP formation and two for BrdUMP dehalogenation. [b] Coefficient of variation in percentage form is calculated as 100 × SD/mean K_i (K_m). The K_m values for dUMP and BrdUMP are the means of ten and six determinations, respectively. [c] Value obtained from ref 11.

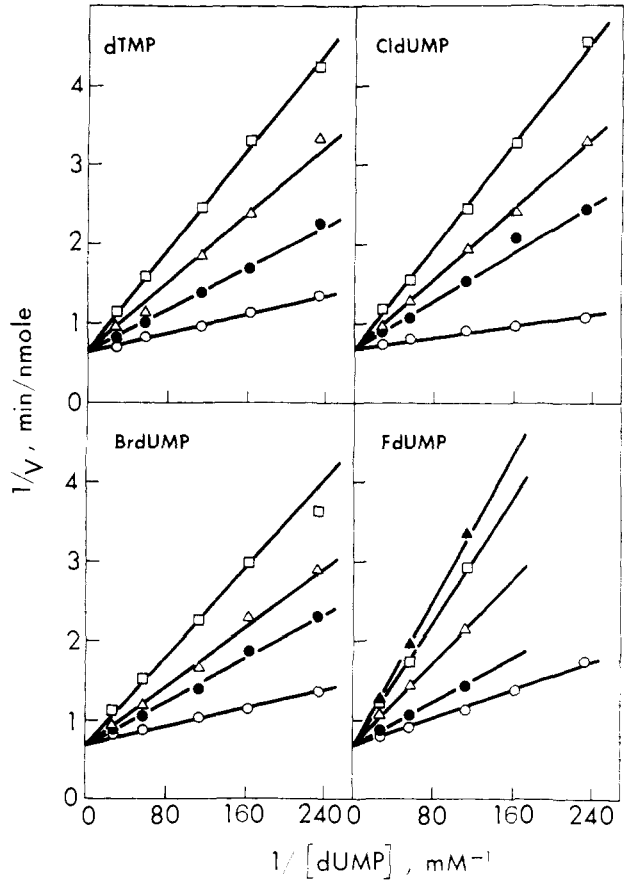

Figure 1. Double reciprocal plots for inhibition of *L. casei* thymidylate synthetase. The procedure is as described in the Experimental Section and inhibitor concentrations are as follows: for dTMP, none (○), 18.7 μM (●), 37.5 μM (△), 74.9 μM (□); for CldUMP, none (○), 0.40 μM (●), 0.80 μM (△), 1.61 μM (□); for BrdUMP, none (○), 1.66 μM (●), 3.32 μM (△), 6.64 μM (□); for FdUMP, none (○), 13.0 nM (●), 26.0 nM (△), 46.8 nM (□), 52.1 nM (▲).

Three compounds examined (FdUMP, CF_3dUMP, and CHOdUMP) specifically interact with the enzyme in the presence of cofactor and then undergo covalent bond changes which, in effect, inactivate the enzyme.[11-16] The general scheme depicting these reactions is

$$E\cdot CH_2\text{-}H_4folate + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\cdot CH_2\text{-}H_4folate\cdot I \xrightarrow{k_2} \text{inactive enzyme}$$

where E·CH_2-H_4folate·I represents the initially formed reversible complex which undergoes unimolecular changes leading to inactivation. The assumption made here is that k_{-1} is comparable to or greater than k_2 so that apparent K_i values reflect the dissociation constants (k_{-1}/k_1) of the initially formed E·CH_2-H_4folate·I complexes. The fact that apparent competitive inhibition kinetics are observed does not distinguish the alternate possibility (i.e., $k_2 > k_{-1}$) since, in either case, the velocity at extrapolated infinite substrate concentration would be V_{max}; however, if $k_2 > k_{-1}$ the apparent K_i value obtained does not reflect the dissociation constant of the inhibitor from the initially formed ternary complex. Present information does not permit us to distinguish between these two possibilities for FdUMP and CF_3dUMP, and only indirect evidence may be cited in support of the aforementioned assumption. The most studied of these inhibitors is FdUMP.[13-18] This analogue reacts with the E·CH_2-H_4folate complex to form covalent bonds with both the enzyme and cofactor, and in the context of the previous discussion this form would represent the "inactive enzyme". Nevertheless, covalent bond formation is slowly reversible and the apparent dissociation constant of FdUMP from this complex is ca. 10^{-12} M,[15] some 10^4-fold lower than the apparent K_i reported here. However, when certain cofactor analogues are used which do not permit covalent bond formation, the dissociation constant of FdUMP from the reversible ternary complex can be as low as 10^{-8} M.[17,19] The agreement of this value with the K_i reported here is evidence, but not proof, that under the conditions used here, the kinetically determined K_i of FdUMP reflects the dissociation constant of the initially formed reversible complex.

The aforementioned complications do not apply to K_i values listed for inhibition of dehalogenation of BrdUMP since, with the exception of CF_3dUMP, the inhibitors examined do not react with thymidylate synthetase in the absence of CH_2-H_4folate. It was not possible to obtain a K_i value for CF_3dUMP since this compound undergoes reaction with thymidylate synthetase[12] more rapidly than dehalogenation of BrdUMP. The values listed for BrdUMP and IdUMP are K_m values for their dehalogenation and are assumed to represent their dissociation constants.

Quantitative Structure–Activity Relationships. Multiple regression analysis[5] of the K_i values for inhibition of TMP synthesis yielded eq 1 as the best single-variable equation, eq 2 as the best two-variable result, and eq 3 as the best three-variable relationship. The parenthesized values in these equations are the 95% confidence intervals; n represents the number of data points employed, r is the correlation coefficient, s is the standard deviation, and $F_{1,X}$ is the stepwise F statistic ($F_{1,7;\alpha=0.05} = 5.59$; $F_{1,6;\alpha=0.10} = 3.78$;

Table II. Constants Employed in the Derivation of Eq 1–3 for Inhibition of Thymidylate Synthetase by 5-X-2'-Deoxyuridylates[a]

X	Log $1/K_i$ obsd	Log $1/K_i$ calcd	Δ log $1/K_i$	σ^-	$\mathcal{F}$	MR	π
CH_3	4.80	4.67	0.13	−0.17	−0.04	0.56	0.45
CH_2OH	5.08	4.84	0.24	0.00	0.00	0.72	−0.85
H	5.29	5.73	0.44	0.00	0.00	0.10	0.00
I	5.79	5.56	0.23	0.18	0.40	1.39	1.11
Br	5.85	6.50	0.65	0.23	0.44	0.89	0.86
Cl	6.72	6.81	0.09	0.23	0.41	0.60	0.72
CF_3	7.41	7.51	0.10	0.65	0.38	0.50	1.11
F	7.85	7.33	0.52	0.06	0.43	0.10	0.22
CHO	7.77	7.75	0.02	1.13	0.31	0.69	0.04

[a] For substituent constants σ^-, $\mathcal{F}$, and MR, see ref 5; π values listed were determined using corresponding 5-substituted uracils as described in ref 5.

Table III. Squared Correlation Matrix for Interrelationship of Variables of Table II

	σ^-	$\mathcal{F}$	MR	π
σ^-	1.00	0.20	0.02	0.03
$\mathcal{F}$		1.00	0.08	0.42
MR			1.00	0.13
π				1.00

$F_{1,5;\alpha=0.025} = 10.0$). While the terms in eq 3 are justified by the F test, the number of data points (three per variable) is too low to place complete confidence in the correlation. The constants employed to construct eq 1–3 are provided in Table II as well as π values for the substituents which were determined using corresponding 5-substituted uracils;[5] variables not listed were found insignificant in the correlations. The collinearity among significant variables is given in Table III.

In essence, eq 3 represents the Yukawa–Tsuno modification[20,21] of the Hammett equation plus the term in MR. The Yukawa–Tsuno approach uses either a σ^+ or σ^- term in linear combination with σ or σ^0; the former variables, σ^+ and σ^-, differ from σ only for conjugated positions (e.g., para) and allow different weighting factors to be given to resonance and inductive effects. Although a more direct approach might be to linearly combine σ_I and σ_R^-, σ_R^- values for CHO and CH_2OH substituents are not available, and we have used the Yukama–Tsuno approach by combining a resonance term (σ^-) with a pure inductive term ($\mathcal{F}$). Although there is no a priori reason to believe that $\mathcal{F}$ constants from benzene systems should be applicable to the uracil heterocycle, data are insufficient to permit statements as to why $\mathcal{F}$ is a better parameter than σ in the present case. Qualitatively, σ^- and $\mathcal{F}$ have the same effect although the two vectors are not parallel. As shown in Table II the range and distribution of σ^- are good. The $\mathcal{F}$ values have a fair range, but a better distribution would be desirable; in effect, there are only two values, one centering at 0.0 and the other at 0.4. The parameter MR was first introduced in biological structure–activity work by Pauling and Pressman.[22] Agin et al.[23] also employed the parameter assuming, as did Pauling and Pressman, that it was primarily a measure of London dispersion forces. More recently, this parameter has been found to be extremely important in QSAR of inhibitors of enzymic reactions.[24,25] When MR models dispersion forces, one finds a positive coefficient with MR terms; however, MR is also a measure of volume (units of cc/mol), and we interpret the negative coefficient associated with this parameter in eq 3 to indicate that the MR term reflects a steric effect between the 5-substituent of the ligands and the enzyme. The range and dispersion of MR values

$$\log 1/K_i = 2.19\ (\pm 1.86)\ \sigma^- + 5.74\ (\pm 0.85) \quad (1)$$
$$n = 9;\ r = 0.725;\ s = 0.888;\ F_{1,X} = 7.77$$

$$\log 1/K_i = 1.54\ (\pm 1.83)\ \sigma^- + 2.77\ (\pm 3.52)\ \mathcal{F} + 5.19\ (\pm 1.02) \quad (2)$$
$$n = 9;\ r = 0.841;\ s = 0.754;\ F_{1,X} = 3.71$$

$$\log 1/K_i = 1.58\ (\pm 1.17)\ \sigma^- + 3.49\ (\pm 2.33)\ \mathcal{F} - 1.43\ (\pm 1.11)\ \mathrm{MR} + 5.88\ (\pm 0.84) \quad (3)$$
$$n = 9;\ r = 0.953;\ s = 0.461;\ F_{1,X} = 11.1$$

(Table II) are good. The parameter E_s does not correlate as well as MR, but this may in part be due to the fact that E_s values are not available for all groups and some estimated values were used.

Clearly, the QSAR of eq 3 should be considered only as a starting point for further development of inhibitors of thymidylate synthetase. With this qualification, certain conclusions may be reached regarding the effects of 5-substituents on the inhibitory power of deoxyuridylates. The first and second most important variables are σ^- and $\mathcal{F}$, respectively. The positive coefficients associated with these terms indicate that electron withdrawal from the uracil heterocycle increases inhibitory power. The negative coefficient of the third variable of eq 3, MR, suggests that large substituents (high MR values) have detrimental steric effects upon interaction with the enzyme. Lastly, since MR and π are not highly collinear (Table III) and since the use of π in eq 3 results in a much poorer correlation with almost no improvement over eq 2 ($r = 0.871$), hydrophobic effects of the substituents do not appear to be important in binding.

Attempts to obtain quantitative structure–activity relationships for inhibitors of dehalogenation of BrdUMP were unsuccessful. This may be a result of the similarity of K_d values of the inhibitors examined and, hence, the relative uncertainty of absolute values or the fact that two of the seven values obtained are K_m values and may not accurately represent dissociation constants.

Discussion

A number of 5-substituted 2'-deoxyuridylates are potent inhibitors of thymidylate synthetase. Although some undergo further reaction after formation of reversible complexes which contribute to their potency, it is clear that the nature of the 5-substitutent can have dramatic effects on the dissociation constants of the reversible complexes. From the results of a number of studies, it has been suggested that the effects of 5-substituents on binding of such analogues to thymidylate synthetase include steric effects which are detrimental to binding,[3] enhanced acidity of the 3-NH which favors the interaction,[3,26] and mesomeric effects on the heterocycle.[27] To date, the relative importance of these parameters has not been quantitated, perhaps because the data reported have been obtained using different sources of enzyme and differing assay conditions. Here, we have evaluated the inhibitory properties of a series of 5-substituted 2'-deoxyuridylates on two distinct reactions catalyzed by *L. casei* thymidylate synthetase and, for one reaction, have ascertained a preliminary quantitative structure–activity relationship which should be of use in designing further inhibitors.

The K_i values obtained by inhibition of the conversion of dUMP and CH_2-H_4folate to dTMP and H_2folate presumably reflect the dissociation constants of the analogues from the reversible enzyme–CH_2-H_4folate–inhibitor complexes. The large span of K_i values ($>10^3$) amounts to a difference in binding energy of over 4 kcal/mol which is unlikely to result from singular intermolecular interactions between the 5-substituents and their corresponding

binding regions of the enzyme; clearly, the substituents must also effect regions of the heterocycle which are important to binding. Using multiple regression analysis, equations could be derived which relate the K_i values to physical properties of the 5-substituents. One of the equations (eq 3) shows a high correlation coefficient and provides insight into the effects of the 5-substituents on binding to the enzyme–cofactor complex.

From the correlation it is apparent that the major effect of the 5-substituents examined is electronic in nature; that is, electron-withdrawing groups enhance the affinity of such analogues for the enzyme. The manifestations of electron withdrawal which are directly responsible for the different affinities of the inhibitors are unknown and likely a composite of numerous effects. The increased acidity of the 3-NH of the pyrimidines would enhance their ability to act as hydrogen-bond donors; however, since hydrogen bonds in water probably contribute less than 1 kcal/mol in binding energy,[28] differences of the pK_a values of the 3-NH cannot be the only significant factor in binding of these analogues. Electron-withdrawing effects of the 5-substituent will alter the electron distribution of the heterocycle, and this effect is probably the most important factor in the differential affinities of the analogues examined. Unfortunately, this cannot be analyzed in detail at this time. The other important factor in inhibition of TMP formation by the 5-substituted nucleotides examined is the size of the 5-substituent; from eq 3 it appears that larger 5-substituents are more detrimental to binding. The hydrophobicity of the substituents examined does not appear to play an important role in affinity of the analogue for the enzyme–H_4folate complex. It is of interest to note that the factors in binding of 5-substituted 2′-deoxyuridylates to thymidylate synthetase which we are able to ascertain with QSAR are analogous to those qualitatively predicted by empirical observations of a few similar inhibitors.[3]

Surprisingly, inhibition of the thymidylate synthetase catalyzed dehalogenation of BrdUMP by 5-substituted 2′-deoxyuridylates differs dramatically from that previously discussed for dTMP formation. With this assay, K_i values of the inhibitors examined spanned only tenfold and quantitative structure–activity correlations could not be made. Nevertheless, qualitative considerations are of some value in analysis of the interactions of these inhibitors with thymidylate synthetase. The difference in K_i values obtained with the two assays most likely reflects differing affinities of the analogues for the enzyme and enzyme–cofactor complexes. Supporting the contention that K_i values obtained with the dehalogenation reaction reflect dissociation constants from binary enzyme–inhibitor complexes is the fairly close correlation of the K_i values obtained for dUMP and FdUMP with K_d values directly measured by equilibrium dialysis.[15] The 5-CH_3, 5-CH_2OH, and 5-H substituted analogues bind tighter to the enzyme than to the enzyme–cofactor complex, and 5-IdUMP has approximately the same affinity for both. In contrast, other analogues examined which possess electronegative, relatively small 5-substituents appear to have a higher affinity for the enzyme–cofactor complex than the enzyme.

It is interesting that the apparent reversible binding of FdUMP is some 10^3-fold tighter to the enzyme when cofactor is present, and the covalent complex which ultimately results has a K_d some 10^7 lower than that for the binary complex.[15] It is clear from the above that the presence of bound CH_2-H_4folates alters the binding site of analogues of dUMP and has dramatic effects on their binding ability; thus, in considering usage of such analogues in vivo, the intracellular concentration of the cofactor could be an important factor in the potency and disposition of such analogues.

The QSAR of K_i values to the nature of the 5-substituents as defined by eq 3 should not be considered final since the number of data points per variable is not sufficiently great to place complete confidence in the correlation; the equation will almost certainly be modified or expanded as more data become available. Nevertheless, this equation constitutes a useful starting point for future design of inhibitors; also, substituents could be placed in the 6 position which might show similar favorable electronic effects but may be devoid of the detrimental steric effects observed with 5-substituents. Lastly, it would be interesting to ascertain whether enzymes from mammalian sources exhibit the same quantitative structure–activity relationships as the bacterial enzyme used in this study. If not, it should be a simple matter to rationally design inhibitors of this enzyme which would demonstrate species selectivity.

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(1) Abbreviations used are listed in Table I.
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Ergot Alkaloids. Synthesis of 6-Alkyl-8-ergolenes and 6-Methyl-8-aminoergolines as Potential Prolactin Inhibitors

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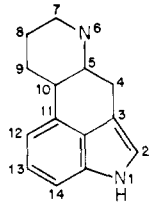
The synthesis of several N-6 derivatives of elymoclavine (**3**) and potential alkylating derivatives of 6-methyl-8-aminoergolines (**12**) is described. These compounds were screened for prolactin-inhibiting ability and 6-propyl-8-hydroxymethyl-8-ergolene (**9**) was found to be as active as the most potent prolactin inhibitors reported to date. The total synthesis of racemic methyl dihydrolysergate I (**23**), having a trans C,D ring fusion, from the tricyclic ketone **18** is also described.

A variety of compounds containing the ergoline (**1**) ring system has been shown to be effective inhibitors of prolactin release.[1-7] Two compounds developed by Semonsky and co-workers,[8,9] VUFB 6605 (**2a**) and VUFB 6683 (Deprenon, **2b**), are currently undergoing clinical trials in Europe. Compound **2c** which was developed by the group of Kornfeld and Clemens at Eli Lilly and Company is also undergoing clinical evaluation as a prolactin inhibitor and an anti-Parkinson agent.[4]



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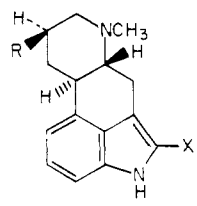

2a, X = H; R = CH_2CN; VUFB 6605
b, X = H; R = $CONH_2$; VUFB 6683
c, X = Cl; R = CH_2CN; lergotrile

Based on the work of several groups, a number of conclusions can be drawn about the relationship between the ergoline structure (**1**) and prolactin inhibitory activity.[1-7] Although Barfknecht and co-workers[10] have reported that an aminotetralin derivative exhibits prolactin inhibition, it seems that in order to produce significant inhibition the intact ergoline skeleton (**1**) is necessary. Reduction of the double bond at the 2,3 position to the corresponding indoline derivative decreases activity. Compounds with an 8,9 double bond are generally more active than the corresponding 9,10 isomers. However, several compounds in which the D ring is completely saturated are quite potent prolactin inhibitors. The ergoline nucleus (**1**) can accommodate substituents at the 1, 6, and 8 positions without a significant decrease in activity, but substitution at positions 7 or 9 is not tolerated.

Previously, elymoclavine (**3**) and several C-17 derivatives were shown to be potent prolactin inhibitors.[2,6] Our present investigation involved preparation of N-6 derivatives of elymoclavine in order to determine (a) if a basic nitrogen at the 6 position was necessary for activity and (b) if dopaminergic activity could be increased by changes in the alkyl substituent. Dopaminergic activity has been demonstrated for several ergoline compounds[11,12] and it appears that these compounds inhibit prolactin release by a dopaminergic mechanism.[13] The potency of dopaminergic agonists has been shown in some cases to depend on the length of the *N*-alkyl substituent.[14] Furthermore, in activation of reserpinized mice, an effect which is used as a criterion for dopaminergic activity, *n*-ethyl- and *n*-propylapomorphine were reported to be twice as potent as apomorphine in reversing reserpine depression.[15] Thus, introduction of the proper N-6 substituent could result in a compound with extremely potent prolactin inhibitory activity.

The second phase of our investigation was concerned with preparation of potential alkylating derivatives of 6-methyl-8-aminoergoline (**12**). Our choice of alkylating derivatives was based on the fact that compounds in the 8-aminoergoline series are generally good prolactin inhibitors.[8,9] The compounds designed contain alkylating groups ($NHCOCH_2Cl$, $NHCOCH_2Br$, NHCOCH=CHCOOH, and maleimide) that are capable of reacting with biological nucleophiles (SH, NH_2, ^-OH, or COO^-). Proper positioning of the alkylating group to a nucleophilic species at or near the active site of an enzyme or protein can result in a rapid neighboring group reaction with covalent bond formation.[16]

If an ergoline could first reversibly bind at the prolactin-inhibiting factor[1] (PIF) receptor, followed by alkylation of an appropriate nucleophile at or near the receptor, then covalent bond formation could result in irreversible prolactin inhibition. An ergoline capable of irreversibly inhibiting prolactin release could be useful in the treatment of prolactin-dependent tumors.

Previously we reported the total synthesis[2] of DL-methyl dihydroisolysergate II (**24**). This paper reports the total synthesis of DL-methyl dihydrolysergate I (**23**), which serves as a precursor for entry into the 8-aminoergoline series.

Chemistry. Preparation of N-6 ergolenes (Table I) utilized elymoclavine (**3**), which is available from submerged cultures of *Claviceps* strain SD-58,[17] as the starting material. Reaction of **3** with cyanogen bromide gave **4** which was converted via a dissolving metal reduction[18] to 8-hydroxymethyl-8-ergolene (**5**). Selective acylation of **5** was carried out using acetic and propionic anhydrides in methanol to give the 6-acetyl and 6-propionyl derivatives