Nucleophilic Substitution Reactions of 5-Acetoxymethyl- and 5-*p*-Nitrophenoxymethyluracils (1,2)

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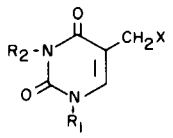
The synthesis of a number of 5-acetoxymethyl- and 5-*p*-nitrophenoxymethyluracils and their nucleophilic substitution reactions with sodium methoxide and sodium borohydride are reported. These reactions all appear to involve intermediates with carbonium ion character, the formation of which are dependent upon structural features of the heterocycle. Most facile reactions occur when the 1-position of the heterocycle can accommodate a negative charge to assist in the formation of highly reactive 5-methenyluracil (VII) intermediates. Where ionization is precluded, as with 1-methyl derivatives (IVb, VIb) displacements are retarded but may be assisted by addition of a nucleophile to the 6-position of the heterocycle. Analogous 1,3-dialkylpyrimidines may react with nucleophiles at the 4-carbonyl group to give anomalous products. Biological connotations of these reactions are discussed.

5-Hydroxymethylpyrimidines and related compounds which represent adducts of an aldehyde to the 5-position of a pyrimidine are of interest because of their biological occurrence and activity (4). Within this class are the 4-amino-5-hydroxymethylpyrimidines such as 5-hydroxymethylcytosine, a constituent of the DNA of T-even bacteriophages of *Escherichia coli* (5) and a number of antimetabolites of thiamine and pyridoxine (4). Also included are certain pyrimidine-2,4-diones which may be exemplified by 5-hydroxymethyluracil (IIa), a constituent of the DNA of certain *Bacillus subtilis* phages (6), 5-bis-(2-chloroethyl)-aminoethyluracil (7), a cancerostatic agent, 5-thymidylyltetrahydrofolic acid, a possible intermediate in the biosynthesis of thymidylate (8), and pseudouridine (9), a minor component of all tRNA species sequenced to date.

We have been particularly interested in the chemistry of pyrimidine-2,4-diones structurally related to I; such compounds appear to demonstrate unusual susceptibility towards acid and base-catalyzed nucleophilic displacements at the 5-methylene group which we believe may be relevant to their function in certain biological systems. One such reaction is the unprecedented alkylation of poorly nucleophilic aromatic amines by 5-hydroxymethyluracil (IIa), which occurs readily in aqueous sodium hydroxide (10). Ethers of IIa are also formed under unusually mild conditions (11) and are hydrolyzed with ease at neutral *p*H. Further amplification of the high reactivity of IIa is provided by the observation that it C-alkylates phenol in the *ortho*- and *para*-positions (12). A similar peculiarity is observed with pseudouridine (9), an unusual *C*-glycoside in



I

which the anomeric carbon of D-ribose is attached to the 5-position of uracil. Although from a chemical standpoint the glycosidic linkage of pseudouridine would *a priori* be expected to be quite stable, in the presence of acid or base the naturally occurring β-anomer readily gives rise to an equilibrium mixture of the α- and β-anomeric species (9c).

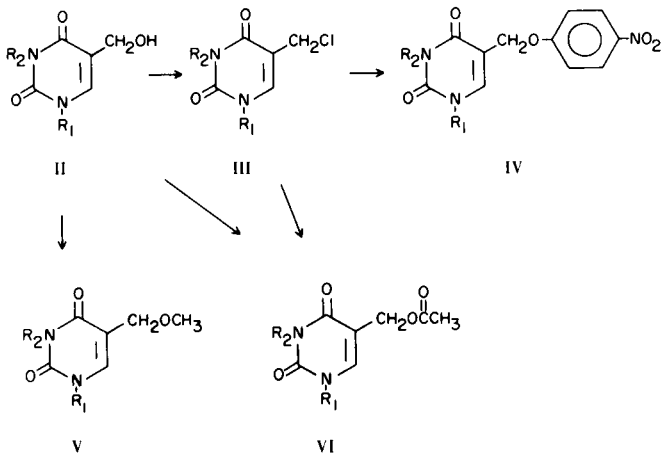
In an effort to obtain insight into the mechanistic features and possible biological implications of these unusual reactions, we have undertaken studies of nucleophilic substitution reactions of relevant models related to I. In this report we describe the reactions of 5-acetoxymethyluracil, 5-*p*-nitrophenoxymethyluracil and their *N*-methylated derivatives with sodium methoxide and sodium borohydride; pertinent structural features of the heterocycle necessary for activation of the 5-methylene group of I towards nucleophilic attack are described.

Results and Discussion.

Synthesis.

The studies described in this report required the synthesis of a number of new pyrimidines (Scheme I) to be used as reactants and authentic samples to establish the identity of the products obtained. A modification of the

SCHEME I



series a, $R_1 = H$, $R_2 = H$
b, $R_1 = CH_3$, $R_2 = H$
c, $R_1 = H$, $R_2 = CH_3$
d, $R_1 = CH_3$, $R_2 = CH_3$

base-catalyzed hydroxymethylation of uracil (I) was utilized to prepare IIc in high yield. Compound IIb, which cannot be prepared by base-catalyzed hydroxymethylations (13), could be obtained by treatment of 1-methyluracil with acidic formalin solution. However, the product was difficult to recover and most preparations of IIb utilized the method previously described (14).

1-Methyl-5-chloromethyluracil (IIIb) was obtained by treatment of IIb with concentrated hydrochloric acid as described for IIIa (15). The high reactivity of IIIb, coupled with its insolubility and poor chromatographic properties, made purification and analysis difficult. Structure assignment was based upon lack of OH stretch in the ir spectrum, positive *p*-nitrobenzylpyridine test for active halogen (16) and direct conversion to Vb upon treatment with methanol. The greatly enhanced solubilities of IIc and IId permitted their facile conversion to the 5-chloromethylpyrimidines, IIIc and IIId by treatment with a slight excess of thionyl chloride in an inert solvent.

The 5-methoxymethylpyrimidines (Va-d) were prepared by heating the corresponding 5-hydroxymethyluracils (IIa-d) in 1% methanolic-hydrochloric acid. The acetate esters VIc and VId were obtained in good yield after treatment of IIc and IId with acetic anhydride-acetic acid. Compound VIb was prepared by the methods previously reported for the synthesis of VIa (11,17). Interestingly, whereas VIa and VIc were quantitatively converted to the starting hydroxymethylpyrimidines (IIa and IIc) upon attempted recrystallization from water, VIb and VId were not hydrolyzed after similar treatment.

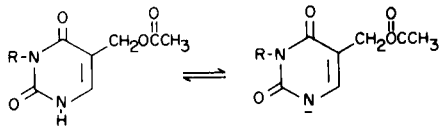
Sodium *p*-nitrophenoxide rapidly reacted with the chloromethyluracils IIIa-c in an acetone suspension to afford the corresponding 5-*p*-nitrophenoxymethyluracils IVa-c in good yield. Under identical reaction conditions, alkylation of *p*-nitrophenoxide with IIId was unsuccessful, and after as long as 12 hours tlc showed only starting materials; similar results were obtained with DMF as solvent. However, when DMF was used as solvent and sodium iodide added as a catalyst, IVd was obtained in good yield after 6 hours at ambient temperature. In contrast, treatment of phenol with IIa has been reported to give *C*-alkylated products (12) and no less than five products were detected by tlc when phenol was reacted with IIIa under the conditions described above. That *C*-alkylated products were not obtained with sodium *p*-nitrophenoxide was primarily demonstrated by ir and nmr. In addition, as described elsewhere in this report, treatment of the *p*-nitrophenoxymethyluracils IVa-d with base results in the rapid liberation of *p*-nitrophenolate, a property not consistent with a *C*-alkylated product.

Nucleophilic Substitution Reactions.

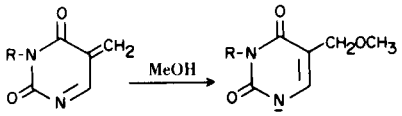
The reaction products obtained upon treatment of the 5-acetoxymethyluracils (VI) and 5-*p*-nitrophenoxymethyluracils (IV) with sodium methoxide and sodium borohydride are given in Table I. When 5-acetoxymethyluracil (VIa) was treated with two equivalents of sodium methoxide-methanol (run 1), 5-methoxymethyluracil (Va) was rapidly ($t_{1/2} < 10$ seconds) formed in 73% yield but 5-hydroxymethyluracil (IIa), the product expected from a normal $B_{AC}2$ hydrolysis, could not be detected. The chromatographic and spectral properties of the remaining products indicated that they were polymeric substances, a supposition which received further support from the observations that the amount formed was proportional to the concentration of VIa and the mass spectrum which gave peaks up to about m/e = 400. Similar treatment of 3-methyl-5-acetoxymethyluracil (VIc) (run 3) rapidly ($t_{1/2} < 10$ seconds) gave the methyl ether Vc in quantitative yield; again, the expected hydroxymethylpyrimidine IIc could not be detected. In contrast, the 1-methylpyrimidine VIb reacted with sodium methoxide slowly ($t_{1/2} > 23$ minutes) and afforded only the 5-hydroxymethylpyrimidine IIb (run 2).

These data provide evidence that the base-catalyzed methanolysis of VIa and VIc proceed exclusively by oxygen-alkyl scission of the corresponding pyrimidine 1-anions to give the highly reactive VII; the suggested intermediate (VII) is closely related to those found in the base-catalyzed hydrolysis of 4(5)-hydroxymethylimidazole acetate (18) and nucleophilic substitutions of indoles of the gramine type (19). With the 1-methylpyrimidine VIb, where ionization of the 1-position is precluded, the normal $B_{AC}2$ mechanism appears to be operative.

p-Nitrophenyl ethers are normally quite resistant to nucleophilic displacement reactions in basic media (20);

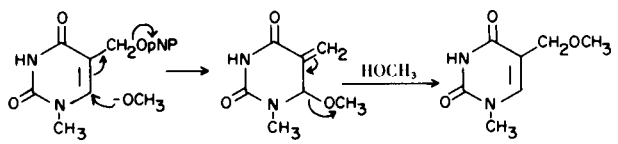


VIa, VIc VIa⁻, VIc⁻



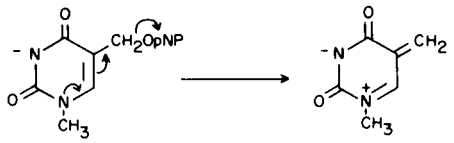
VII Va⁻, Vb⁻

however, in the presence of sodium methoxide-methanol transetherification of IVa and IVc (runs 14,16) occurred so rapidly that the reaction mixtures could not be quenched for analysis prior to completion. The facility of these reactions strongly suggests that, as with VIa and VIc, assistance is provided by the 1-anion to form the highly reactive VII. With the 1-methylpyrimidine IVb (run 15) the reaction is considerably slower ($t_{1/2} \cong 90$ minutes), but unlike VIb the methyl ether Vb is the sole product formed. This is not surprising since under the anhydrous conditions employed IIb could only arise from methoxide attack on the phenyl ring of IVb. A mechanism involving S_N2 displacement of *p*-nitrophenoxide from IVb is discounted on the basis of the known chemistry of related *p*-nitrophenyl ethers (20), kinetics of the hydrolysis of IVb which implicate sp^2 hybridization of the 5-methylene group in the rate determining step (21), and reactions of IVb with sodium borohydride which are discussed later. The assistance needed to displace *p*-nitrophenoxide from IVb may be provided by addition of methoxide to the 6-position of the heterocycle to give the intermediate VIII; there is much precedent for the addition of nucleophiles to the 6-position of the uracil heterocycle (13,22) and the intermediacy of VIII is not eliminated by kinetic data (21).



IVb VIII Vb

An alternative pathway which cannot be discounted at this time involves the spontaneous elimination of *p*-nitrophenoxide from the 3-anion of IVb to give the intermediates IX.



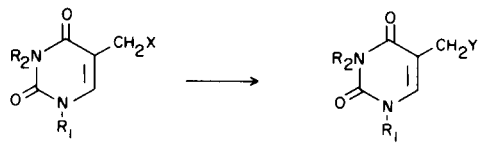
IVb⁻ IX

In the absence of sodium methoxide, none of the aforementioned reactions of IV and VI occurred under the conditions employed. The possibility was considered that the initial products of the sodium methoxide mediated methanolysis of IVa,c and VIa,c were the hydroxymethyluracils IIa,c which might rapidly give rise to the methyl ethers Va and Vc. This was shown not to be the case by demonstrating that all of the 5-hydroxymethyluracils (IIa-d) remained unchanged under identical reaction conditions for as long as three days (run 25).

The numerous resonance hybrids that can be drawn for VII and VIII indicate that, if such intermediates exist, the exocyclic methylene group would possess substantial carbonium ion character. Bell and Brown (23) have described the use of sodium borohydride as a convenient carbonium ion trapping agent in solvolytic media and we observed (runs 8 and 12) that when VIa and VIc were treated under the suggested conditions (2 *M* sodium borohydride in 80% aqueous diglyme), quantitative yields of thymine (Ia, X = H) and 3-methylthymine (Ic, X = H) were obtained. In order to assimilate conditions used in the previously described solvolytic experiments, the concentration range of sodium borohydride used was lowered to 0.02-0.10 *M* and anhydrous methanol was used as the solvent. With 0.10 *M* sodium borohydride all of the pyrimidines possessing a free 1-NH (VIa, run 7; VIc, run 11; IVa, run 19; IVc, run 23) were quantitatively converted to thymine or 3-methyl thymine. When the concentration of sodium borohydride was lowered to 0.02 *M* (runs 6, 10, 18 and 22), competition for VII occurred between methanol and sodium borohydride resulting in the formation of both the corresponding thymines (Ia,c, X = H) and methyl ethers (Va,c); the fact that the product distribution was essentially constant regardless of the leaving group or 3-substituent is consistent with the suggestion that a similar intermediate (VII) is formed in all cases where the pyrimidine is unsubstituted at the 1-position. It will be noted that although these reactions were very rapid ($t_{1/2} < 15$ seconds), sodium borohydride is relatively unstable in methanol (24) and the actual concentration of the reducing agent in the reaction mixtures is probably lower than the amount introduced.

In contrast to the facile reductions of VIa and VIc by sodium borohydride, the 1-methylpyrimidine ester (VIb) underwent a slow transesterification ($t_{1/2} \cong 23$ minutes) to give IIb and methyl acetate (run 9). The analogous *p*-nitrophenyl ether (IVb) was unchanged after reaction with the

TABLE I (a)



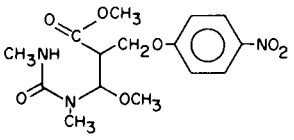
Run	Compound	R_1	R_2	Nucleophile (M conc.)	Method of isolation (b)	Product -Y (% yield)
1	VIa	H	H	NaOMe (0.02)	A,B,C	OCH_3 (73)
2	VIa	CH_3	H	NaOMe (0.02)	A	OH (100)
3	VIc	H	CH_3	NaOMe (0.02)	A,C	OCH_3 (98)
4	VId	CH_3	CH_3	NaOMe (0.02)	D	OH (77); OCH_3 (23)
5	VId	CH_3	CH_3	NaOMe (2.0)	D	OH (81); OCH_3 (19)
6	VIa	H	H	$NaBH_4$ (0.02)	B	H (67); OCH_3 (33)
7	VIa	H	H	$NaBH_4$ (0.10)	B,C	H (97)
8	VIa	H	H	$NaBH_4$ (2.0)	B	H (100)
9	VIb	CH_3	H	$NaBH_4$ (0.02)	A	OH (100)
10	VIc	H	CH_3	$NaBH_4$ (0.02)	B,C	H (57); OCH_3 (43)
11	VIc	H	CH_3	$NaBH_4$ (0.10)	B,C	H (98)
12	VIc	H	CH_3	$NaBH_4$ (2.0)	B	H (100)
13	VId	CH_3	CH_3	$NaBH_4$ (0.02)	D	OH (100)
14	IVa	H	H	NaOME (0.02)	A,B	OCH_3 (98)
15	IVb	CH_3	H	NaOMe (0.03)	A	OCH_3 (99)
16	IVc	H	CH_3	NaOMe (0.02)	A,C	OCH_3 (98)
17	IVd	CH_3	CH_3	NaOMe (0.02)	D	OCH_3 (97)
18	IVa	H	H	$NaBH_4$ (0.02)	B	H (64); OCH_3 (36)
19	IVa	H	H	$NaBH_4$ (0.10)	B,C	H (98)
20	IVb	CH_3	H	$NaBH_4$ (0.02)	A	– (c)
21	IVb	CH_3	H	$NaBH_4$ (0.02) NaOMe (0.10)	C	H (100)
22	IVc	H	CH_3	$NaBH_4$ (0.02)	C	H (60); OCH_3 (40)
23	IVc	H	CH_3	$NaBH_4$ (0.10)	A,C	H (95)
24	IVd	CH_3	CH_3	$NaBH_4$ (0.02)	D	– (d)
25	IIa-d			NaOMe (0.02)	C	– (e)
26	IIa-d			NaOMe (0.02)	C	– (e)
27	Va-d			NaOMe (0.02)	C	– (e)
28	Va-d			$NaBH_4$ (0.02)	C	– (e)
29	Ia-d (X = H)			NaOMe (0.02)	C	– (e)
30	Ia-d (X = H)			$NaBH_4$ (0.02)	C	– (e)

(a) See experimental section for details. (b) Reactions were also performed on a 1 mmole scale and products isolated by conventional techniques for identification; in these cases yields were 10-15% lower than determined spectrophotometrically due to losses encountered in isolation. (c) No reaction was observed after 0.5 hour. (d) Rapid loss of uv absorption. (e) Only starting material could be detected by tlc after 72 hours and no changes in uv spectra were observed throughout this period.

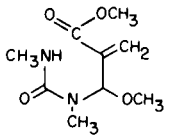
same amount of sodium borohydride for 30 minutes (run 20). However when 0.02 *M* sodium methoxide is added, IVb is reduced by sodium borohydride to give 1-methylthymine (run 21) as the sole product. It will be recalled that in the absence of reducing agent, this concentration of sodium methoxide is sufficient to form significant amounts of the methyl ether Vb (run 15); furthermore, Vb was not an intermediate in the reduction as shown by its inertness toward sodium borohydride (run 28). These data provide additional evidence against S_N2 type displacement reactions since, firstly, under these conditions significant amounts of the methyl ether Vb would be expected to form, and secondly, it is difficult to visualize why this type of reaction would be dependent upon sodium methoxide concentration. These data are in best accord with the proposed intermediates VIII and IX, which require methoxide for formation, and would rapidly be trapped by sodium borohydride.

As with VIb, treatment of VId with sodium borohydride (run 13) resulted in a rather slow transesterification ($t_{1/2} \cong$ 154 minutes) to give IId and methyl acetate. Unexpectedly, when IVd was treated with sodium borohydride (run 24) the pyrimidine chromophore was rapidly ($t_{1/2} < 15$ seconds) destroyed. If an intermediate such as VIII were formed, it would be anticipated to react with borohydride or methanol to give either 1,3-dimethylthymine (Id, X = H) or the methyl ether Vd; both of these possible products were shown to be stable towards sodium borohydride under the conditions employed (runs 28 and 30) and could therefore not be intermediates in the destruction of IVd. These results suggested that nucleophilic substitution reactions of the dimethylpyrimidines IVd and VId might proceed by an entirely different mechanism than previously suggested for the 1-substituted derivatives (IVb, VIb).

A number of reports have appeared which demonstrate that the 4-carbonyl group of uracil derivatives is susceptible to nucleophilic attack (25). These reactions are most facile when both the 1- and 3-positions are substituted and often result in cleavage of the N_3-C_4 amide bond with concomitant loss of uv absorption. Furthermore, in cases investigated, addition across the 5,6-double bond appears to be necessary for activation of the 4-carbonyl towards nucleophilic attack. Notable examples are the facile base catalyzed N_3-C_4 cleavages of 5,6-dihydrouracils (25a), 1,3-dimethyluracil (25b) and 1(β-D-arabinofuranosyl)-5-fluorouracil (25c). It was considered possible that a similar pathway might be involved in the reaction of IVd with sodium methoxide and sodium borohydride. In the latter case, hydride attack at the 4-carbonyl position would result in destruction of the heterocycle. Precedence for a reaction of this type has been established by Witkop *et al.* (26) who have shown that analogous borohydride reductions occur with 5,6-dihydrouracils and thymines. A similar reaction with methoxide would provide an acyclic ester (X or XI) which could ultimately cyclize to a pyrimidine product.



X



XI

A test for this pathway would simply involve substitution of hydroxide for methoxide in the reaction of IVd. If, as proposed above, attack were to occur at the 4-carbonyl group an acylic carboxylate would result which could not revert to the pyrimidine precursor; if a displacement reaction at the 5-methylene group is involved, formation of the 5-hydroxymethylpyrimidine (IId) would be expected. When IVd was treated with 0.1 *N* sodium hydroxide, *p*-nitrophenolate was rapidly liberated, followed by a slower loss of the uv chromophore. The reaction is complicated by the presence of numerous short-lived intermediates, as evidenced by uv spectral scans, but the following points support the above proposal. Using 6-deuterated IVd, liberation of *p*-nitrophenolate shows a kinetic secondary deuterium isotope effect of $k_H/k_D = 0.91$ (21). This demonstrates that C-6 undergoes a change of hybridization from sp^2 to sp^3, as would occur upon addition of hydroxide, in the rate-determining step or in a pre-equilibrium step. Furthermore, since IId is stable toward hydroxide under similar conditions, it cannot be an initially formed product or intermediate which subsequently undergoes degradation. Although the exact reaction course of the reactions of IVd is not known at this time, it is clear that it does not proceed by the same pathway as do the mono- and unsubstituted pyrimidines (series a-c), and must involve reaction of a nucleophilic species at C-6 and the 4-carbonyl prior to the introduction of a nucleophile at the 5-methylene group.

The results described above demonstrate that thyminyl derivatives related to I are quite susceptible toward nucleophilic displacement reactions. Although all apparently involve carbonium ion type intermediates, the facility and mechanism of these reactions are dependent upon structural features of the heterocycle. Most facile reactions occur when the 1-position of the pyrimidine can accommodate an anion to give stabilized intermediates of type VII. Where ionization is precluded, the reactions are retarded, but may be assisted by addition of a nucleophile to the 6-position of the heterocycle. Direct enzymic counterparts to these reactions involving general-base or nucleophilic catalysis may be indicated in biological transformations of some of the aforementioned naturally occurring derivatives of I. The relevance of these studies to specific enzymic processes shall be described in a future

report (21). The high susceptibility of these compounds toward nucleophilic displacements further suggests that they might be useful biological alkylating agents. However, the tendency of IVa,c and VIa,c to hydrolyze ($t_{1/2} \cong 2$ minutes at *p*H 7) (21) under typical assay conditions would necessitate modification of the leaving group to balance the reactivity and permit evaluation; such studies are currently in progress.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are corrected. Infrared spectra (potassium bromide) were obtained on a Perkin-Elmer model 337 spectrophotometer. Ultraviolet (uv) spectra were obtained on a Cary model 15 recording spectrophotometer. Thin layer chromatography (tlc) was performed on silica gel GF_{254} or Kodak polygram silica gel sheets. Ascending paper chromatography was run with Whatman No. 3 paper strips with ethyl acetate:formic acid:water (7:2:1) for development. Spots were detected visually under short-wave uv light. Sodium borohydride was recrystallized from diglyme (24) shortly before use. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

General Procedures for Reactions of IV and VI with Sodium Methoxide and Sodium Borohydride.

To a vigorously stirred solution of the pyrimidine (usually 0.01 *M*) in 8 ml. of anhydrous methanol was added 0.30 ml. of a stock solution of sodium borohydride in dimethylformamide or a methanolic solution of sodium methoxide (usually giving a 1 *M* excess of the nucleophile). After stirring for 15 minutes at ambient temperature, the solution was neutralized with glacial acetic acid, diluted with methanol to exactly 10.0 ml. and analyzed by one of the following procedures:

Method A.

A 0.100 ml. aliquot of the neutralized reaction mixture was applied to a prewashed Whatman No. 3 paper strip and developed (ascending) with ethyl acetate:formic acid:water (7:2:1). The uv-absorbing bands corresponding to the pyrimidines were cut into small pieces, packed into a micro-column and eluted with 10.0 ml. of 0.01 *N* hydrochloric acid for quantitation by uv. An identical procedure was used to isolate *p*-nitrophenol with the exception that elution was performed with 0.01 *N* sodium hydroxide.

Method B.

A 0.50 ml. aliquot of the neutralized reaction mixture was applied to a Sephadex G-10 column (0.9 x 100 cm) and eluted with 0.05 *M* sodium phosphate (*p*H 7.0) (27). The fractions corresponding to purified products were combined and quantitated by uv.

Method C.

A 0.050 ml. aliquot of the neutralized reaction mixture was applied to a Kodak polygram silica gel sheet which had been previously washed with petroleum ether (b.p. 60-110°): ethanol:iso propanol (8:1:1). The chromatogram was twice developed with the same solvent and the appropriate bands analyzed as described in Method A.

Method D.

A 0.075 ml. aliquot of the neutralized reaction mixture was applied to a silica gel column (1.6 x 38 cm) and eluted with ethyl acetate:methanol (20:1). Fractions were combined and analyzed as previously described.

In each of the above methods summation of the uv absorbance of the purified reaction products corresponded to within 3% of the absorbance of the crude reaction mixture after quenching. With the 5-*p*-nitrophenoxymethyl pyrimidines (IV), the amount of *p*-nitrophenol liberated was determined and in all cases was equivalent to the pyrimidine product(s). Data required for isolation and quantitation of products are given in Table II.

TABLE II

Compound	R_f (a)	$\lambda^{H_2O}_{max}$ (mμ)	ϵ_{max}	*p*H
Ia, X = H	0.61	261	7720	2
Ib, X = H	0.79	273	9190	2
Ic, X = H	0.88	266	7300	2
Id, X = H	0.95	273	7900	2
IIa	0.29	261	8000 (b)	2
IIb	0.52	270	9650	2
IIc	0.60	262	6892	2
IId	0.77	270	8106	2
Va	0.57 (c)	261	8000 (b)	2
Vb	0.78 (d)	270	9400	2
Vc	0.84 (d)	261	7330	2
Vd	0.96 (e)	269	8592	2
p-nitrophenolate	1.00	402	18300	13

(a) Paper chromatography, Method A. (b) Reference 11. (c) Separated from Ia by column chromatography (Sephadex G-10), Method B. (d) Separated from corresponding thymine derivatives by tlc, Method C. (e) Separated from Id by column chromatography (silica gel), Method D.

5-*p*-Nitrophenoxymethyluracil (IVa).

To a vigorously stirred suspension of 0.591 g. (3.0 mmoles) of sodium *p*-nitrophenoxide dihydrate in 7 ml. of reagent grade acetone was added 0.480 g. (3.0 mmoles) of IIIa. After stirring at ambient temperature for 20 minutes, the mixture was filtered, washed with four 20 ml. portions of methanol and dried to give 0.510 g. (64.6%) of crude product, m.p. 224.5-227.5° dec. Reprecipitation from dioxane gave 0.438 g. (55.6%) of white powder, m.p. 214.5-216° dec.; ν max 3150 (NH); 3040 (C_6H_4); 1700-1600 (C=C, C=N, C=O); 1340 (NO_2); 1110 (C-O); 840 cm^{-1} (*p*-C_6H_4); λ max (water) (*p*H 2) 261, 315 mμ. Tlc using ethyl acetate-dioxane (6:4) showed one spot.

Anal. Calcd. for $C_{11}H_9N_3O_5$: C, 50.20; H, 3.45; N, 15.96. Found: C, 50.52; H, 3.67; N, 15.81.

1-Methyl-5-methoxymethyluracil (Vb).

A solution of 0.250 g. (1.60 mmoles) of IIb in 10 ml. of methanol containing 0.1 ml. concentrated hydrochloric acid was refluxed for 30 minutes. The solution was then spin-evaporated *in vacuo* to about 3 ml. and chilled; yield 0.265 g. (97.5%) of white crystals, m.p. 142-143°. Recrystallization from ethyl acetate gave 0.200 g. (73.5%) of analytically pure material, m.p. 146-147°; ν max 3140

(NH); 1710-1650 (C=C, C=O, C=N); 1440 (CH_3); 1105 cm^{-1} (C-O). Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.40; H, 5.90; N, 16.47.

1-Methyl-5-acetoxymethyluracil (VIb).

A magnetically stirred mixture of 0.330 g. (1.90 mmoles) of IIb, 0.156 g. (1.90 mmoles) of sodium acetate in 6.0 ml. of glacial acetic aicd was refluxed for 15 minutes. The hot solution was filtered free of insolubles and spin-evaporated *in vacuo* to a heavy oil. Crystallization from diethyl ether-petroleum ether (b.p. 60-110°) gave 0.330 g. (88.7%) of white leaflets, m.p. 155-157°. Recrystallization from water gave 0.150 g. (40.4%), m.p. 162.5-164°; ν max 3260 (NH); 1740 (C=O acetate); 1720-1600 (C=C, C=N, C=O); 1460 cm^{-1} (CH_3); λ max (water) (*p*H 2) 261 mμ. Tlc using chloroform ethanol (3:1) showed one spot.

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.65; H, 5.18; N, 14.14.

1-Methyl-5-*p*-nitrophenoxymethyluracil (IVb).

To a magnetically stirred suspension of 0.340 g. (1.72 mmoles) of sodium *p*-nitrophenoxide dihydrate in 5 ml. reagent grade acetone, was added 0.300 g. (1.72 mmoles) of IIIb. The suspension was stirred at ambient temperature for 20 minutes, filtered, and washed with four 20 ml. portions of methanol, and dried giving 3.21 g. (67.4%) of light yellow powder, m.p. 242-245° dec. Recrystallization from dioxane gave 2.91 g. (61.2%), m.p. 246-248° dec; ν max 3150 (NH); 3010 (C_6H_4); 1700-1600 (C=C, C=N, C=O); 1460 (CH_3); 1340 (NO_2); 1265, 1110 (C-O); 848 cm^{-1} (*p*-C_6H_4); λ max (water) (*p*H 2) 261, 315 mμ. Tlc using ethyl acetate-dioxane (6:4) showed one spot.

Anal. Calcd. for $C_{12}H_{11}N_3O_5$: C, 51.99; H, 4.00; N, 15.16. Found: C, 52.19; H, 4.25; N, 14.92.

3-Methyl-5-hydroxymethyluracil (IIc).

A solution of 0.255 g. (8.5 mmoles) of paraformaldehyde and 1.0 g. (7.94 mmoles) of 3-methyluracil in 10 ml. of 0.5 *N* sodium hydroxide was kept at 60° for 12 hours. After neutralizing with Dowex 50W-X8, the solution was lyophilized and the residue crystallized from ethyl acetate to give 0.70 g. (56%) of white crystals, m.p. 170-171°; ν max 3400 (OH); 3200 (NH); 1720-1600 (C=C, C=N, C=O); 1080 cm^{-1} (C-O). Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 45.87; H, 4.93; N, 17.70.

3-Methyl-5-methoxymethyluracil (Vc).

A solution of methanol containing 0.468 g. (3.0 mmoles) of IIc and 0.1 ml. of concentrated hydrochloric acid was refluxed for 1 hour, chilled and filtered to give 0.400 g. (80%) of white crystals, m.p. 140-142°. Recrystallization from methanol-diethyl ether gave 0.190 g. (37.3%), m.p. 144-145.5°; ν max 3200 (NH); 1720-1600 (C=C, C=N, C=O); 1460 (CH_3); 1115 (C-O). Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.56; H, 6.02; N, 16.44.

3-Methyl-5-chloromethyluracil (IIIc).

To a solution of 0.312 g. (2.0 mmoles) of IIc in 15 ml. of diglyme was added 0.25 ml. (3.44 mmoles) of thionyl chloride. After stirring at ambient temperature for 1 hour, filtration gave 0.260 g. (74.6%) of white crystals, m.p. 239.5-241.5°. Recrystallization from acetonitrile gave 0.190 g. (54.4%), m.p. 239.5-241.5°; ν max 3200 (NH); 1700-1600 (C=C, C=N, C=O); 1460 cm^{-1} (CH_3).

Anal. Calcd. for $C_6H_7ClN_2O_2$: C, 41.22; H, 4.01; N, 16.04. Found: C, 41.21; H, 4.00; N, 16.04.

3-Methyl-5-*p*-nitrophenoxymethyluracil (IVc).

To a vigorously stirred suspension of 0.680 g. (3.44 mmoles) of sodium *p*-nitrophenoxide dihydrate in 7 ml. of reagent grade acetone was added 0.600 g. (3.44 mmoles) of IIIc. The suspension was magnetically stirred at ambient temperature for 15 minutes, filtered, washed with three 15 ml. portions of methanol and dried to give 0.400 g. (42.0%), m.p. 213-215°. Crystallization from tetrahydrofuran gave 0.260 g. (27.3%) of white crystals, m.p. 221.5-222.5°; ν max 3200 (NH); 3060 (C_6H_4); 1700-1600 (C=C, C=N, C=O); 1340 (NO_2); 1455 (CH_3); 1110 (C-O); 850 cm^{-1} (*p*-C_6H_4); λ max (water) (*p*H 2) 261, 315 mμ. Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_{12}H_{11}N_3O_5$: C, 51.99; H, 4.00; N, 15.16. Found: C, 52.24; H, 3.99; N, 15.12.

3-Methyl-5-acetoxymethyluracil (VIc).

A solution of 0.208 g. (1.33 mmoles) of IIc and 1.0 ml. of acetic anhydride in 5.0 ml. of glacial acetic acid was kept at 75° for 12 hours. Upon cooling, 1.0 ml. of methanol was added and the solution was spin-evaporated *in vacuo* to dryness. Crystallization from ethyl acetate-petroleum ether (b.p. 60-100°) gave 0.235 g. (89.5%) of white crystals, m.p. 140.5-142.5°. Recrystallization gave 0.165 g. (62.8%), m.p. 144-145°; ν max 3120 (NH); 1740 (C=O acetate); 1720-1600 (C=C, C=N, C=O); 1460 cm^{-1} (CH_3); λ max (water) (*p*H 2) 261 mμ. Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.39; H, 5.20; N, 13.94.

3-Methylthymine (Ic, X = H).

A solution of 0.312 g. (2.0 mmoles) of IIc in 100 ml. of 95% ethanol containing 5 ml. of glacial acetic acid and 0.150 g. of 10% palladium on charcoal was hydrogenated at 2.5 atmospheres for 1 hour. The solution was filtered through celite and evaporated *in vacuo* to give 0.280 g. (100%) of white solid, m.p. 212-214°. Crystallization from ethyl acetate-petroleum ether (b.p. 60-110°) gave 0.200 g. (71.5%) of white crystals, m.p. 216.5-218°; ν max 3160 (NH); 1700-1600 cm^{-1} (C=C, C=N, C=O). Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.54; H, 5.74; N, 19.86.

1,3-Dimethyl-5-hydroxymethyluracil (IId).

To a vigorously stirred mixture of 4.26 g. (30 mmoles) of IIa and 9.65 g. (70 mmoles) of anhydrous potassium carbonate was added 9.95 g. (70 mmoles) of methyl iodide. After stirring 12 hours at ambient temperature, the solution was chilled and neutralized with 80% aqueous acetic acid. Evaporation *in vacuo* to dryness gave a white residue which was extracted with 8 x 50 ml. portions of chloroform. The combined extracts were dried over magnesium sulfate and evaporated *in vacuo* to give 4.5 g. (88%) of white powder, m.p. 136-138°. A small portion was twice recrystallized from acetone to give the analytical sample; ν max 3450 (OH); 1715-1680 (C=C, C=O); 1470 (CH_3); 1080 cm^{-1} (C-O). Tlc using chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.67; H, 6.03; N, 16.67.

1,3-Dimethyl-5-methoxymethyluracil (Vd).

A solution of 15 ml. of methanol containing 0.610 g. (3.6 mmoles) of IId and 0.1 ml. of concentrated hydrochloric acid was

refluxed for 0.5 hours, chilled and filtered; yield, 0.490 g. (74%) of white crystals, m.p. 126-129°. Recrystallization from methanol gave the analytical sample, m.p. 129.5-130.5°; ν max 1710-1650 (C=C, C=O); 1470 (CH_3); 1105, 1085 cm^{-1} (C-O). Tlc using ethyl acetate showed one spot.

Anal. Calcd. for $C_8H_{12}N_2O_3$: C, 52.17; H, 6.57; N, 15.21. Found: C, 52.34; H, 6.76; N, 15.18.

1,3-Dimethyl-5-chloromethyluracil (IIId).

A solution of 0.340 g. (2.0 mmoles) of IId and 0.25 ml. (3.5 mmoles) of thionyl chloride in 5 ml. of diglyme was allowed to stand at ambient temperature for 2 hours. After chilling, the mixture was filtered to give 0.286 g. (76%) of white crystals, m.p. 147-148°. A portion was recrystallized from acetonitrile to give the analytical sample, m.p. 148-149°; ν max 1710-1640 (C=C, C=O); 1470 cm^{-1} (CH_3).

Anal. Calcd. for $C_7H_9ClN_2O_2$: C, 44.58; H, 4.80; N, 14.86. Found: C, 44.72; H, 4.70; N, 14.99.

1,3-Dimethyl-5-*p*-nitrophenoxymethyluracil (IVd).

To a solution of 0.188 g. (1.0 mmole) of IIId and 0.149 g. (1.0 mmole) of sodium iodide in 5 ml. of dimethylformamide was added 0.161 g. (1.0 mmole) of sodium *p*-nitrophenoxide. The suspension was stirred at ambient temperature for 6 hours followed by addition of 100 ml. water to precipitate 0.195 g. (67%) of crude product, m.p. 165-167°. Crystallization from acetonitrile-ethanol gave 0.145 g. (50%) of white crystals, m.p. 171.5-173°; ν max 1715-1600 (C=C, C=O); 1470 (CH_3); 1350 (NO_2); 1115 (C-O); 852 cm^{-1} (*p*-C_6H_4); λ max (water) (*p*H 1) 272, 315 mμ. Tlc using ethyl acetate-petroleum ether (b.p. 60-110°) showed one spot.

Anal. Calcd. for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.89; H, 4.59; N, 14.48.

1,3-Dimethyl-5-acetoxymethyluracil (Vld).

A solution of 0.170 g. (1.0 mmole) of IId in 2.0 ml. of acetic anhydride and 6.0 ml. of acetic acid was kept at 70° for 12 hours. The solution was then evaporated to dryness *in vacuo*. Crystallization from toluene-petroleum ether (b.p. 30-60°) gave 0.162 g. (76.4%) of white crystals, m.p. 97-99°. Recrystallization gave 0.131 g. (61.7%) of analytically pure material, m.p. 99.5-100.5°; ν max 1720 (C=O acetate); 1700-1620 (C=C, C=O); 1450 cm^{-1} (CH_3); λ max (water) (*p*H 2) 271 mμ. Tlc using ethyl acetate petroleum ether (b.p. 60-110°) 3:1 showed one spot.

Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.79; H, 5.73; N, 13.02.

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