Volume 3 no.4 April 1976 Nucleic Acids Research

Reaction of diazoalkanes with 1-substituted 2, 4-dioxopyrimidines. Formation of O^2, N-3 and O^4-alkyl products.

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Received 29 January 1976

ABSTRACT

In non-aqueous solution, diazomethane and diazoethane react with the O^2, O^4 and N-3 sites of uridine, thymidine, 1-methyluracil and 1-methylthymine. Diazoethane has a higher affinity for alkylating oxygens than does diazomethane. The relative ratio of O^2:O^4:N-3 methyl products is 1:2:16 and of ethyl products the ratio is 1:1:2. When the diazoethane reaction is performed in neutral buffered solution, the same proportion of O^2:O^4:N-3 ethyl products is found, but the extent of reaction is very low. O^2-alkylation greatly labilizes the glycosidic bond of thymidine and uridine toward acid hydrolysis. All O^2 and O^4 alkyl 1-substituted 2,4-dioxopyrimidines are dealkylated in weak acid but the O^2 alkyl group is the more stable.

INTRODUCTION

The action of diazomethane on uridine or thymidine and related compounds has been studied since 1934 when Levene and Tipson[1] used diazomethane to prepare 3-methyluridine. In that and several succeeding papers pyrimidine nucleosides were reported to be alkylated on the N-3 only[2-5], regardless of whether the reaction was performed in non-aqueous or aqueous solvents. More recently, using better methods for separation and identification of products, Wong and Fuchs[6] investigated the reaction of ethereal diazomethane with 1-methyluracil in methanol and found 9% of 1,O^4-dimethyluracil as well as the expected 1,3-dimethyluracil (91%). Farmer et al.[7] also reported that thymidine in methanol reacted with ethereal diazomethane to form 5% O^4-methylthymidine in addition to 93% 3-methylthymidine. It thus appeared that O^4-alkylation of pyrimidines by diazomethane occurred to a low extent in methanol solution. O^2-alkylation was not detected[6] although both O^2-methyluridine and O^2-ethyluridine had been synthesized[8,9].

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Abbreviations used: Me, methyl; Et, ethyl; Urd, uridine; Thd, thymidine; Ura, uracil; Thy, thymine.

We have reinvestigated the reaction of uridine, thymidine and 1-methyluracil in methanol solution with diazomethane dissolved in either ether or 1,2-dimethoxyethane and find, in all cases, that both the O^4-methyl and O^2-methyl derivatives are formed (about 15% of total). When diazoethane is used as the alkylating agent the extent of reaction of the ring oxygens is greatly increased (about 50% of total). The increased reactivity of ethylating agents, compared to the analogous methylating agents, toward oxygens of guanosine and cytidine[10,11] as well as phosphate oxygens of nucleotides[12-14] has been found in this laboratory for all alkylating agents studied.

The diazoalkanes have been used for the preparation of the following new derivatives: O^4-ethyluridine; O^2-methylthymidine; O^2-ethylthymidine; O^4-ethylthymidine; 1,O^2-dimethyluracil; 1-methyl, O^2-ethyluracil and 1-methyl, O^2-ethylthymine. Spectral characteristics and chromatographic data are given for these and other alkylated pyrimidines.

Materials and Methods

Materials. Uridine and thymidine were commercial products of the highest purity obtainable. 1-Methyluracil and 1-methylthymine were prepared according to Scannell et al.[15], then isolated and purified by preparative chromatography on Whatman 3MM in 50 n-butanol:30 H_2O:2 acetic acid. O^4-Methyluridine was prepared according to Robins and Naik[16]. 2'(3')-O-alkyl-uridines were synthesized as described earlier[17,18]. Diazoalkanes were prepared immediately before use according to the procedure of Robins et al. (solution A)[19]. Diazoalkanes were generated from methyl- or ethyl-nitrosourea (K&K) and dissolved in 1,2-dimethoxyethane or diethyl ether. All solvents used for the preparation of diazoalkanes as well as the methanol, which was generally the solvent for alkylation, were purchased from Mallinkrodt and were used without further purification.

Chromatographic Systems. Separation of alkyl derivatives was by paper chromatography (Whatman 3MM) and thin layer chromatography using cellulose (Eastman Chromatogram Sheets No. 6065) or silicagel (Eastman Chromatogram Sheets 6060; Merck pre-coated PLG plates 60F-254). The most frequently used solvents were: Solvent I (paper and cellulose sheets) 80 n-butanol:10 ethanol:25 water; solvent II (silicagel) 50 acetone:25 benzene. Chromatographic data obtained with these systems are given in Table 1. Other solvent systems are discussed in the text.

General Alkylation Procedure. The diazoalkane solution was added in 2 or 3 portions, with vigorous agitation, to a 0.08 M methanol suspension of the compound to be alkylated. The volume of diazoalkane solution was about

Table 1. R_F values of alkylated uridine, thymidine, 1-methyluracil and 1-methylthymine*

Compound		Solvent I		Solvent II	
		Methyl	Ethyl	Methyl	Ethyl
Uridine	(0.24)			(0.23)	
O^2-alkyl		0.32	0.49	0.07	0.10
3-alkyl		0.48	0.64	0.52	0.62
O^4-alkyl		0.48	0.64	0.30	0.48
2'(3')-O-alkyl				0.62	0.69
Thymidine	(0.49)			(0.48)	
O^2-alkyl		0.60	0.72	0.20	0.25
3-alkyl		0.72	0.82	0.72	0.78
O^4-alkyl		0.72	0.82	0.40	0.57
1-Methyluracil	(0.49)			(0.59)	
O^2-alkyl		0.59	0.73	0.16	0.25
3-alkyl		0.74	0.83	0.82	0.86
O^4-alkyl		0.74	0.83	0.62	0.75
1-Methylthymine	(0.62)			(0.85)	
O^2-alkyl			0.79		0.65
3-alkyl			0.86		0.93
O^4-alkyl			0.86		0.84
Uracil	(0.42)				
Thymine	(0.49)				

*Chromatographic methods are given in Materials and Methods. Solvent I is n-butanol:ethanol:water (80:10:25 v/v) run on paper. Solvent II is acetone:benzene (2:1) run on deactivated silicagel sheets.

The order, not the R_F values, is reproducible using different batches of silicagel sheets. Figures in parentheses are the R_F values of unmodified compounds.

1.5 times that of the methanol suspension and the diazoalkane was in excess (5-8 fold). The reaction proceeded very quickly and decolorization of the diazoalkane occurred in several seconds. After the addition of the first portion of diazoalkane solution the compound being alkylated was solubilized. After each addition of diazoalkane the reaction mixture was examined by thin layer chromatography for the amount of unreacted starting material (cellulose sheets, Solvent I) in order to avoid a large excess of diazoalkane which could lead to the formation of multialkylated products. When the reaction had proceeded so that little or no unreacted material was present the derivatives were isolated by chromatography.

Methods for the Separation and Characterization of Alkyl Derivatives. Preliminary separation of products from the entire reaction mixture was generally by paper chromatography in Solvent I. The relative movement of derivatives was the same regardless of the compound alkylated. In order of increasing R_F they were: unreacted material, O^2-alkylated, and a mixture of N-3-alkylated and O^4-alkylated (Table 1). The mixture of O^4-alkyl and N-3 alkyl derivatives was well separated by re-chromatography in Solvent II (Table 1). It is worth noting that several other solvent systems using paper or silicagel did not resolve the N-3 and O^4 derivatives.

The possible presence of 2'(3')-O-alkyluridines was checked by chromatography in a borate-containing system (100 n-butanol:13.5 0.8 M boric acid: 0.4 ammonium hydroxide). The R_F values for ribose-alkylated uridines are: 2'(3')-O-MeUrd 0.38, 2'(3')-O-EtUrd 0.53; the mixtures of N-3 and O^4-alkylated uridines (which co-chromatograph with ribose-alkylated uridines in Solvent I) are primarily at the origin but do exhibit some streaking and the furthest R_Fs are: 3-MeUrd + O^4-MeUrd 0.25, 3-EtUrd + O^4-EtUrd 0.33.

For preparative purposes it was possible to separate all reaction products from 0.5 mM ethylated uridine using only silicagel PLC plates in Solvent II. The UV absorbing bands were eluted with methanol, decolorized with charcoal and evaporated to dryness. 3-Ethyluridine, O^2-ethyluridine and O^4-ethyluridine were obtained as glassy, almost colorless solids which were sufficiently pure for PMR analysis.

The products of alkylation of uridine, thymidine, 1-methyluracil and 1-methylthymine were identified by UV and PMR spectra and on the basis of their chemical behavior. The UV spectral characteristics of O^2-methyluridine[8], O^4-methyluridine[16], O^4-methylthymidine[20] and 1,O^4-dimethyluracil[21] have been published and are in agreement with the same derivatives obtained in this work (Table 2). The new O^2 and O^4 alkyl derivatives exhibit UV spectra analogous to those of the previously described O^2 and O^4 modified pyrimidines (Table 2).

Table 2. Ultraviolet spectral characteristics of O^2 and O^4 alkyl derivatives of uridine, thymidine, 1-methyluracil and 1-methylthymine*

Compound	H_2O		0.1N KOH		0.1N H_2SO_4		10N H_2SO_4	
	λ_{max} nm	λ_{max} nm	λ_{max} nm	λ_{max} nm	λ_{max} nm	λ_{max} nm	λ_{max} nm	λ_{max} nm
O^2-MeUrd**	224,250	212,236	252	237	223,252	212,236	(210),256	232
O^2-EtUrd	226,251	211,236	253	238	224,253	212,236	(210),257	234
O^4-MeUrd***	274	238	275	239	275	237	289	244
O^4-EtUrd	273	237	274	239	274	237	289	244
O^2-MeThd	226,254	215,234	255	237	(223),257	235	(213),263	236
O^2-EtThd	226,255	217,236	256	237	(220),258	236	(215),264	236
O^4-MeThd****	278	240	279	241	279	239	297	239
O^4-EtThd	278	241	279	242	279	241	297	242
1,O^2-diMeUra	224,254	211,238	254	238	(215),256	236	(210),257	232
1-Me,O^2-EtUra	224,255	211,238	255	238	(215),256	236	(210),257	231
1,O^4-diMeUra*****	272	236	272	237	274	236	290	240
1-Me,O^4-EtUra	273	239	273	240	278	241	290	245
1-Me,O^2-EtThy	226,259	214,239	259	239	(215),262	237	(215),264	237
1-Me,O^4-EtThy	278	241	278	241	279	241	299	240

*Spectra were obtained using a Cary Model 15 Recording Spectrophotometer. Numbers in parentheses are shoulders.

**Brown et al.[8] (H_2O) λ_{max} 229,249, λ_{min} 213,238.

***Robins and Naik[16] (H_2O) λ_{max} 274, λ_{min} 235; (0.1N HCl) λ_{max} 271, λ_{min} 235; (0.1N NaOH) λ_{max} 274, λ_{min} 239.

****Lawley et al.[20] (H_2O) λ_{max} 280, λ_{min} 241; (0.1N HCl) λ_{max} 279, λ_{min} 241; (0.1N NaOH) λ_{max} 280, λ_{min} 243.

*****Katritzky & Waring[21] (pH 7) λ_{max} 272; (20N H_2SO_4) λ_{max} 290.

The 60 MH_z PMR data of O^2-, N-3 and O^4-EtUrd and Urd itself for comparison) are collected in Table 3. All alkylated derivatives exhibit signals of methyl [H(β)] and methylene [H(α)] protons (in the case of 3-EtUrd the methylene quartet is superposed with ribose protons) and also lack the signal of the N-H proton which is present in the spectrum of Urd. The signals of methylene protons of O^2-EtUrd and O^4-EtUrd are downfield as compared to the methylene protons of 3-EtUrd. This would be expected for ethyl groups bound to oxygen as compared to nitrogen. The changes of magnetic environment as the result of the lack of a 4-CO carbonyl group in O^4-EtUrd causes the downfield shift of the H-5 proton signal (approx. 0.2 ppm) in this compound in comparison to 3-EtUrd, O^2-EtUrd and unsubstituted uridine. This kind of shift is reported also for O^4-MeUrd[16].

Table 3. Chemical shifts (δ, in ppm with respect to TMS internal standard) for the protons of uridine and its ethyl derivatives in d_6-DMSO solution*

Compound	H(β)	H(2',3',4', 5',5'')	H(α)	H(2'OH, 3'OH,5'OH)	H(1')	H(5)	H(6)	H(N-3)
Urd	-	3.4-4.2 m,5	-	4.7-5.4 m,3	5.69 d,1	5.78 d,1	7.91 d,1	11.32 s,1
O^2-EtUrd	1.33 t,3	3.5-4.2 m,5	4.39 q,2	4.8-5.7 m,3	5.72 d,1	5.83 d,1	7.99 d,1	-
3-EtUrd	1.10 t,3	3.5-4.2 m,7		4.7-5.6 m,3	5.87 d,1	5.77 d,1	7.94 d,1	-
O^4-EtUrd	1.27 t,3	3.5-4.1 m,5	4.33 q,2	4.6-5.6 m,3	5.83 d,1	6.02 d,1	8.32 d,1	-

*Spectra obtained using Varian T-60 spectrometer.

In order to distinguish those oxygen alkyl derivatives which are dealkylated in N HCl (100°, 60 min) from N-alkyl derivatives which are stable[11,12,20,22], each alkyl derivative (10-20 absorbancy units) was subjected to acid hydrolysis followed by chromatography in Solvent I. The products of hydrolysis of alkylated thymidines which included bases and nucleosides not separable in Solvent I were chromatographed in a solvent consisting of the upper phase from 60 ethyl acetate:35 water:5 formic acid. The R_F values were:Thd 0.20, Thy 0.33, 3-MeThd 0.40, 3-MeThy 0.51, 3-EtThd 0.80, 3-EtThy 0.89. The products were identified and quantitated on the basis of UV absorbance of eluates.

An additional chemical proof of the identity of O^2-ethyluridine and O^4-ethyluridine is their conversion in methanolic ammonia (37°, 2 days) into isocytidine[8] and cytidine, respectively. The amino compounds were identified by UV spectra.

RESULTS

Both diazomethane and diazoethane are found to react in non-aqueous solution with the N-3, O^2 and O^4 positions of uridine, thymidine and 1-methyl substituted uracil and thymine. No ribose alkylation occurs under these conditions. The extent of reaction of each of the base positions is independent of the pyrimidine alkylated and is solely a function of the reagent (Table 4). When diazomethane is used about 85% of the alkylation is on the N-3, the remaining 15% is about 2/3 O^4-methylation and 1/3 O^2-methylation. Reaction

Table 4. Amount and proportion of products of diazoalkane reaction with 1-substituted 2,4-dioxopyrimidines in methanol*

Pyrimidine	Diazoalkane	% of Total Alkylation** N-3	O^4	O^2	Ratio of products N:O	O^4:O^2
Uridine	methyl	84	11	5	5.5	2.2
Thymidine	methyl	84	10	6	5.3	1.7
1-Methyluracil	methyl	85	11	4	5.7	2.7
Uridine	ethyl***	48	23	26	1.0	0.9
Thymidine	ethyl	55	21	24	1.2	0.9
1-Methyluracil	ethyl	50	29	21	1.0	1.4
1-Methylthymine	ethyl	49	27	24	1.0	1.1

*Reaction conditions and methods for the separation and identification of products are given in Materials and Methods. The extent of reaction varied from 70-100%. Each figure represents the average of 2-3 determinations, generally on two separate reaction mixtures, the carrier for diazoalkanes is ethyl ether or 1,2-dimethoxyethane. Occasionally 1-2% of the total alkyl products was found in an area of the first chromatogram (Solvent I) which was not coincident with the three known products. About 20% methylation, in addition to 80% ethylation, occurred when diazoethane was dissolved in 1,2-dimethoxyethane. The same phenomenon had been observed by Pike et al.[24] when alkylating adenosine under similar conditions. When diazoethane is dissolved in diethyl ether and methylation is impossible, the distribution of ethyl products is essentially the same as when mixed alkylation occurs.

**Expressed as % of absorbancy at λ_{max} in H_2O. In the case of O^4-alkyl derivatives which are not separated from 3-alkyl derivatives in Solvent I, the sample was hydrolyzed in N HCl 100° 1 hr for complete dealkylation of O^4-alkyl derivatives and the amount of this product is expressed as the dealkylated compound. Independent analyses in which N-3 and O^4 derivatives were separated in Solvent II were in good agreement with the acid hydrolysis determination.

***Uridine reacted with ethereal diazoethane in aqueous solution buffered at pH 6.5-7.0 to a much lesser extent (~ 5%). All three ethyl derivatives were identified and were found in the same proportion as in non-aqueous solution.

with diazoethane greatly increases the amount of O^4 and O^2 substitution and in all cases, only half the total ethylation is on the N-3, the other half divided almost equally between O^2 and O^4 substitution. Thus the N:O ratio for methylation is about 5.5 and for ethylation it is about 1. In the same way if we express the preference of O-alkylation using the ratio O^4:O^2, then for methylation O^4:O^2 = 2.2 and for ethylation the ratio is 1.1 (Table 4).

In addition to the data in Table 4, we find that O^2-MeUrd and O^4-MeUrd are obtained when uridine is alkylated not only in methanol or 1,2-dimethoxyethane solution but also in dimethyl sulfoxide, dimethyl formamide and tertiary butanol. No differences in products were noted when diazoalkanes are dissolved in ethyl ether or 1,2-dimethoxyethane. These results indicate that we are not observing an artifact due to a catalyst present in the solvent. (The catalytic action of $SnCl_2$ in the diazomethane alkylation of carbohydrate hydroxyls was discovered accidentally using non-distilled methanol from metal containers[23]). If however diazoethane is dissolved in 1,2-dimethoxyethane we observe that some methylation occurs as also noted by Pike et al.[24]

Alkylation of the O^2, N-3 or O^4 positions in 1-substituted 2,4-diketopyrimidines introduces characteristic changes in UV-spectra. The nature of changes introduced by substitution of the alkyl group in a particular position is essentially the same in all compounds investigated (i.e. Urd, Thd, 1-MeUrd and 1-MeThy) and the spectra of homologous methyl and ethyl derivatives are virtually identical (Table 2). The comparison of UV-spectra in water of alkylated derivatives with the parent compounds shows that O^2-alkylation causes a significant blue shift (8-14 nm) of the λ_{max} and the appearance of a new short wavelength maximum at about 225 nm, whereas O^4-alkylated derivatives exhibit a red shift of the λ_{max} (5-13 nm). The position of the λ_{max} of commonly known N-3 alkyl derivatives is essentially unchanged.

Due to the lack of a dissociable N-3 proton after alkylation, the spectra of all derivatives are the same in H_2O and in 0.1 N KOH with the exception of a small (1-2 nm) red shift in the λ_{max} in the case of both N and O alkylated uridines and thymidines (Table 2). This shift is caused by the dissociation of proton(s) from the carbohydrate moiety and it is not observed in spectra of alkyl derivatives of 1-MeUra and 1-MeThy (Table 2)[25].

Characteristic changes are observed when neutral and acidic spectra are compared. The pK_a of protonation of 1,O^4-diMeUra is reported as +0.65, and the λ_{max} of its protonated form as 290 nm versus 272 nm for the neutral form[21]. The same kind of shift in λ_{max} (about 20 nm) accompanied by about twice the increase of the ε_{max} is observed in 10 N H_2SO_4 for all O^4-alkyl derivatives reported here (Fig. 1). (Full protonation is not necessarily reached in 10 N H_2SO_4). N-3, O^4-Ethylene-1-methyluracilium methanesulphonate, which represents the fixed cation form, exhibits a λ_{max} 290 nm in MeCN and 288 in 0.1 N HCl[26]. This, and the analogous behavior of 1-substituted cytosines, indicates that the protonation site of 1,O^4-di-substituted uracils is nitrogen N-3.

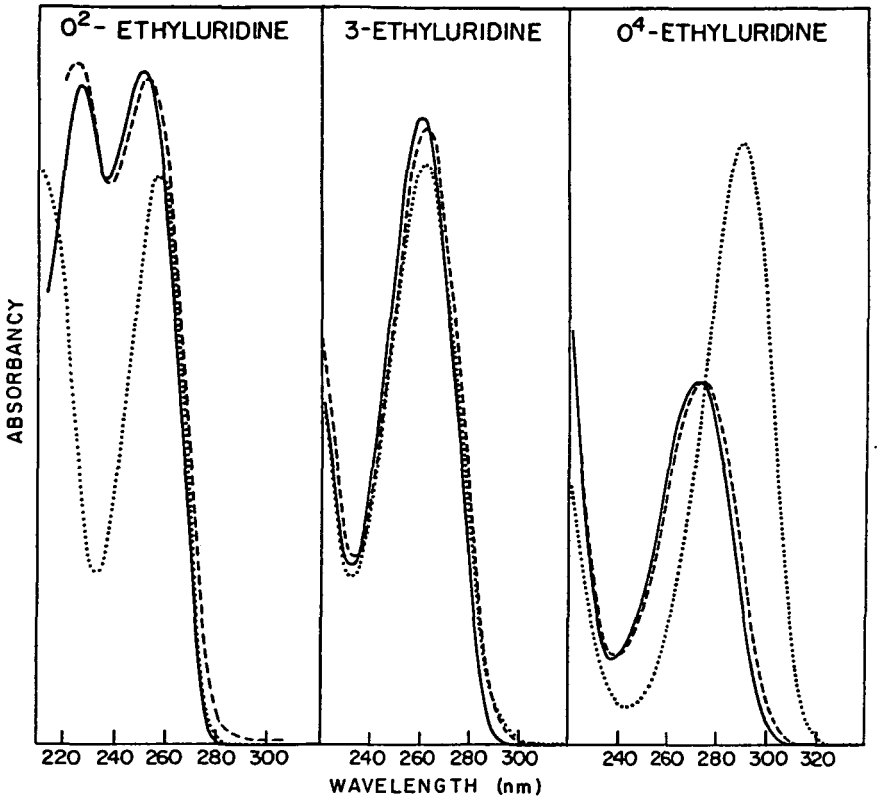

Fig. 1. UV absorption spectra of ethyluridines in H_2O (——), 0.1 N KOH (----) and 10 N H_2SO_4 (●●●). The λ_{max} and λ_{min} of these and other alkyl derivatives are in Table 2.

The acidic spectra of 1,O^2-di-substituted derivatives (in 10 N H_2SO_4) exhibit a red shift (3-9 nm) of long wavelength λ_{max} with accompanying decrease of ε_{max} (about 15%). Very characteristic is the decrease of ε_{min} (about 70%) and change of short wavelength maximum into a shoulder with an accompanying blue shift (10-15 nm). Due to the very low pK_a of protonation of 1,3-di-substituted derivatives (pK_a = -3.25 for 1,3-diMeUra[21]) there are only slight changes between the spectra in H_2O and 10 N H_2SO_4.

Spectral data are collected in Table 2, and in Fig. 1 the spectra of ethylated uridines are shown as representative of all alkyl derivatives described here. Due to the acid and alkali lability of O-alkyl derivatives their stability was checked in 0.1 N KOH and 0.1 and 10 N H_2SO_4 and it was found that the spectra were unchanged during the time necessary to record the spectra.

The stability of alkyl groups toward acid hydrolysis was used to distinguish O^2 and O^4 alkylpyrimidines from N-alkyl derivatives. All of the O-alkyl derivatives are completely de-alkylated after treatment with N HCl at 100° for 60 min. However, a comparison of the stability of O^4 and O^2 alkyl groups indicated that the O^2 substituent is more stable. Whereas O^4-methyluridine is 90% converted to uridine in 0.01 N HCl after 21 hr at room temperature, it takes 48 hr at 37° in the same acid concentration for comparable reaction of O^2-methyluridine.

During N HCl (100°, 60 min) hydrolysis of thymidine and its alkyl derivatives, a further observation was made that in addition to de-alkylation, some breakage of the glycosidic bond occurred. Thymidine itself is hydrolyzed to thymine (33%), a fact earlier noted by Pfizner and Moffatt[27] who reported the half life of thymidine as 56 min under similar conditions. The glycosidic bonds of 3-ethylthymidine and O^4-ethylthymidine are hydrolyzed to the same extent as thymidine (30-35%). In contrast, O^2-alkylation of thymidine greatly destabilizes the glycosidic linkage and 96% of O^2-ethylthymidine is converted to thymine. This destabilization resulting from O^2-alkylation is also observed with O^2-alkyluridine. O^2-Ethyluridine gives after hydrolysis 10% uracil (as well as uridine), while no base is found after hydrolysis of uridine, 3-alkyluridine or O^4-alkyluridine. The N-1 methyl bond in 1-methyl, O^2-ethylthymine and 1-methyl-O^2-ethyluracil is stable when hydrolyzed in N HCl.

DISCUSSION

The differences between our results and those of other investigators who did not find O^2-alkylation of uridine or thymidine may be due to several reasons. First, the diazomethane reaction was primarily studied and in this case O-alkylation (particularly O^2-methylation) might be overlooked due to the relatively low extent (approx. 5%) in comparison to N-methylation. Another reason may be the ineffective chromatographic methods employed. Due to the lability of O-alkyl derivatives, only neutral solvents can be used and a single solvent system is not sufficient to separate all products. Generally however, products were isolated by crystallization, rather than by examining entire reaction mixtures. None of these reasons appear to apply to the work of Wong and Fuchs[6] who failed to find 1,O^2-dimethyluracil which we find as a clearly detectable product of the reaction of 1-methyluracil with diazomethane.

Using multiple methods of separation and testing all fractions at all stages for O-alkylation with acid volatility as a criterion, we find O^2-MeUrd and O^4-MeUrd as products of the diazomethane reaction in a variety of solvents

including methanol, dimethylsulfoxide, dimethylformamide, 1,2-dimethoxyethane and tertiary butanol. O^2- and O^4-Alkyl products were also formed when 1,2-dimethoxyethane as well as ethyl ether were used as carriers of diazoalkanes. These results indicate that the formation of O^2- and O^4-alkyl derivatives of 1-substituted uracil and thymine is not due to a contaminant in the reaction mixture which could act as a catalyst, but is due to the innate properties of the reaction. Furthermore in water buffered so that the pH is 6.5-7.0, the same products are found. Although the extent of reaction is very low, the proportion of O^2,O^4 and N-3 ethyl derivatives is the same as in the non-aqueous reaction.

Wong and Fuchs[6], based on the lack of 1,O^2-dimethyluracil among the methylation products of 1-methyluracil in their reaction, suggest that the charge in the anion derived from 1-methyluracil (in the initial step of reaction with diazomethane) is distributed only along the N-3$\overset{--}{—}$C$\overset{--}{—}O^4$ bond system. The data presented here shows that all three nucleophilic centers: O^2, N-3 and O^4 are available for alkylation. It is therefore more likely that the distribution of the charge is along the $O^2\overset{---}{—}C\overset{---}{—}$N-3$\overset{---}{—}C\overset{---}{—}O^4$ bond system. This is also supported by spectroscopic data[28].

According to Gompper's theory[29], the course of reaction of the alkyldiazonium cation with the anion derived from 1-substituted uracil or thymine is determined by factors influencing the capability of the alkyldiazonium ion to react more closely to the S_N2 scheme (N-alkylation preferred) or S_N1 scheme (O-alkylation preferred). The major factor in the work reported here is the nature of the alkyl group where we find a significant decrease of the N-alkyl:O-alkyl ratio for diazoethane ($\sim$ 1) compared to diazomethane ($\sim$ 5.5). It can also be observed that the O^2nucleophilic center is more sensitive toward S_N1 attack than the O^4 center (O^4-Me:O^2-Me = $\sim$ 2; O^4-Et:O^2-Et = $\sim$ 1). This dependence of the specificity of reaction on the nature of the alkylating group has been repeatedly found in our previous work on the reactions, in aqueous neutral solution, of nucleotides and nucleic acids with a series of alkylating agents of biological significance[10-14,30,31]. It should be noted that the reactions of nucleosides and nucleic acids are not necessarily the same. In a detailed study of the action of dialkylsulfates, alkylalkanesulfonates, and alkylnitrosoureas on neutral, aqueous solutions of uridine and thymidine, no detectable O-alkylation of either the base or carbohydrate moiety was found[14]. On the other hand the same alkylating agents acting on poly U under comparable conditions gives both base and ribose O-alkyl products (Kuśmierek and Singer, in preparation).

ACKNOWLEDGEMENTS

The authors are indebted to Professor H. Fraenkel-Conrat for his interest and advice throughout this investigation. The present address of Dr. J. T. Kuśmierek is the Institute of Biochemistry and Biophysics, Academy of Sciences, Warsaw, Poland. This work was supported by Grant CA 12316 from the National Cancer Institute, Bethesda, Maryland USA.

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