PREPARATION AND STEREOCONFIGURATIONS OF HETERODIMERS OF PYRIMIDINES*

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Summary: Using a novel C-alkylation procedure, four isomers of a heterodimer of a thymine-uracil derivative have been synthesized. With retention of configuration, the stereoconfigurations and the relative rates of alkylation at various sites can be assigned. This knowledge allows the unambiguous identification of the heterodimer isolated from the irradiation of a mixture of thymine and uracil in ice and from the acid hydrolysates of UV-irradiated DNA. By comparing the NMR data gathered from this set of a heterodimer with those sets of uracil and thymine homodimers, the effectiveness and the magnitude of a 1,2-shielding of a neighboring proton by a cis CH_3-group of a cyclobutyl derivative may be suggested. In addition, taking advantage of the comparatively low solubility of the Ba-salt of this thymine-uracil heterodimer, its purification from an admixture of various photodimers was accomplished in a simple manner.

Thus far, heterodimers of pyrimidines (Pyr<>Pyr')[1] have been prepared photochemically by irradiation of a mixture of two pyrimidines in frozen solutions.[2,3] Under these conditions, various isomers of homodimers of each Pyr and of the heterodimer are formed. Not only is there a great deal of difficulty in purifying each isomer but also the quantum yields are invariably low. Although Thy<>Ura has been indicated as a product isolable from the acid hydrolysates of UV-irradiated DNA,[4] chemical understanding of this compound is rather vague. Its stereoconfiguration was assigned first by analogy to Thy<>Thy(c,s)[5] and more recently on the basis of a comparison of NMR spectra.[6]

Our recent discovery[7] of C-alkylation of Pyr<>Pyr with retention of configuration provides an ideal system for the synthesis of heterodimers and for establishing their configurations. Under such mild reaction conditions, we have prepared all four isomers of Me_2Thy<>Me_2Ura from the corresponding Me_2Ura<>Me_2Ura. In Table 1, the specific conditions and results are presented,

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Table 1. The Preparation of $Me_2Thy<>Me_2Ura$ from $Me_2Ura<>Me_2Ura$.

$Me_2Ura<>Me_2Ura$	Ag_2O (mg) [mmole]	CH_3I (ml) [mmole]	5% NaCN (ml)	Recovered (%) [mg]	Product (%) [mg]	
					$Me_2Thy<>Me_2Ura$	$Me_2Thy<>Me_2Thy$
trans - anti	70 [0.3]	0.18 [3.0]	30	48 [27]	23 [14][b,c]	22 [14]
	93 [0.4]	0.24 [4.0]	40	16 [9]	25 [15]	44 [27]
	116 [0.5]	0.30 [5.0]	50	3 [3]	23 [14]	57 [35]
	140 [0.6]	0.36 [6.0]	60	<2 [<1]	17 [10]	63 [39]
trans - syn	70 [0.3]	0.18 [3.0]	30	50 [28]	43 [25][b]	0 [0]
	93 [0.4]	0.24 [4.0]	40	32 [18]	47 [28]	13 [8]
	116 [0.5]	0.30 [5.0]	50	18 [10]	53 [31]	20 [12]
	140 [0.6]	0.36 [6.0]	60	14 [8]	50 [29]	26 [16]
	186 [0.8]	0.48 [8.0]	80	5 [3]	44 [26]	37 [23]
	232 [1.0]	0.60 [10.0]	100	0 [0]	34 [20]	50 [31]
cis - anti	460 [2.0][a]	0.72 [12.0]	200	51 [29]	27 [16][b]	4 [3]
cis - syn	460 [2.0][a]	0.72 [12.0]	200	38 [21]	32 [19][b]	<2 [1]

a. These reactions were carried out in 5 ml of $HCON(CH_3)_2$.

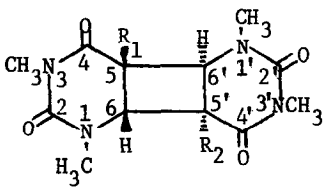
b. All heterodimers were recrystallized from AcOEt. The m.p. of (t,a), (t,s), (c,a) and (c,s) are 176-177, 218-219, 208-209, and 206-207°C, respectively.

c. This compound has an IR spectrum identical with that obtained by Wang (1965).

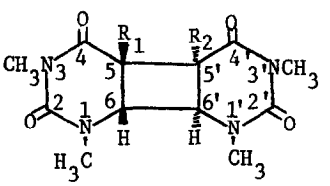
and the following is the general procedure. A mixture of $Me_2Ura<>Me_2Ura$ (56 mg, 0.2 mmole), silver oxide and methyl iodide in dimethylformamide (2 ml) was stirred for 24 hr at room temperature. The mixture was poured into 5% NaCN solution and the product was extracted three times with 50-ml chloroform from this aqueous solution. The combined extract was dried over anhydrous sodium sulfate. After evaporation, the residue was applied on silica gel thin-layer plates and the chromatogram was developed with chloroform:acetone (2:1).

It can be seen (Table 1, column 6) that at low levels of the reagent, $Ag_2O + CH_3I$, the yields of (t,a)- and (t,s)heterodimers were near the highest level. Evidently, these yields were relatively constant as the proportions of the reagent were increased. However, such an increase resulted in nearly proportional increases in the yields of the dimethylated products (Table 1, last column). Thus, there is no apparent advantage for the purpose of preparing heterodimers by increasing the quantity of the reagent. As a matter of fact, the yields were somewhat decreased with the amounts of silver oxide increased to >0.6 mmole (see Table 1).

Our data revealed another aspect that may deserve comment. In comparing the last two columns, the relative reactivities of the four alkylation sites of two trans-isomers may be assessed. As seen in the formulae, the alkylation sites are C(5) and C(5'). The site C(5) (or the first alkylated position) of



trans-anti



trans-syn

both isomers should have comparable reactivities and indeed the extents of alkylation were found to be (23 + 22)% and 43%, respectively at the low level of the reagent. Under the same condition, the succeeding alkylation at C(5') of 22% took place for the (t,a)isomer whereas no $Me_2Thy<>Me_2Thy$ was obtained from the (t,s)isomer. This difference in reactivity is not yet understood,

Table 2: 220 MHz NMR Spectra of Pyr<>Pyr at 25°C. Solvent:$CDCl_3$, Internal Standard:$(CH_3)_4Si$.

Dimer	Chemical Shifts (δ in ppm)				
	N(1)CH_3 [6H,s]	N(3)CH_3 [6H,s]	C(5)CH_3 [s]	C(5)H[b]	C(6)H[b]
trans-anti					
Me_2Ura<>Me_2Ura	3.16(3.13)[a]	3.31(3.30)[a]	-	(3.43)[a] (317.4)[c]	(4.18)[a] (369.1)[c]
Me_2Thy<>Me_2Ura	3.08,3.09	3.26,3.28	1.42[3H]		
Me_2Thy<>Me_2Thy	3.08	3.28	1.41[6H]		
trans-syn					
Me_2Ura<>Me_2Ura	3.09(3.12)[a]	3.27(3.30)[a]	-	(3.72)[a] (326.5)[c]	(3.92)[a] (350.9)[c]
Me_2Thy<>Me_2Ura	3.07,3.10	3.26	1.44[3H]		
Me_2Thy<>Me_2Thy	3.08	3.25	1.40[6H]		
cis-anti					
Me_2Ura<>Me_2Ura	3.14(3.17)[a]	3.16(3.17)[a]	-	(3.98 m)[a] (341.5)[c]	(3.98 m)[a] (371.1)[c]
Me_2Thy<>Me_2Ura	3.10,3.11	3.13,3.14	1.57[3H]		
Me_2Thy<>Me_2Thy	3.10	3.18	1.59[6H]		
cis-syn					
Me_2Ura<>Me_2Ura	3.02(3.10)[a]	3.19(3.22)[a]	-	(3.72,3.8,3.88)[a] (342.0)[c]	(4.06,4.14,4.22)[a] (367.2)[c]
Me_2Thy<>Me_2Ura	2.92,3.07	3.14,3.16	1.67[3H]		
Me_2Thy<>Me_2Thy	3.02	3.16	1.51[6H]		

[a] The values given in parentheses were reported by D. Elad et al. (1971)[10] and are used for comparison purposes.

[b] Our values are excluded from this Table but are presented in Table 3.

[c] These values were reported by E. Fahr et al. (1972) and were measured with a 90 MHz spectrometer.

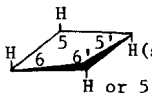
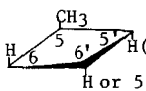
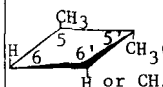
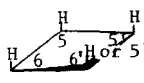
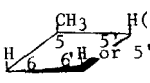
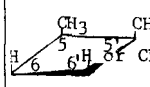
however, it should be noted that each dimethylated product (Me_2Thy<>Me_2Thy) has the same configuration as the corresponding starting material (Me_2Ura<>Me_2Ura). This indicates that the first methylation must take place without any change in the configuration and these heterodimers (Me_2Thy<>Me_2Ura) must retain the same configurations as the respective Me_2Ura<>Me_2Ura.

The knowledge concerning the four heterodimers allowed us to unambiguously establish the structure and stereoconfiguration of the heterodimer obtained from the irradiation of a mixture of Thy and Ura in ice and, consequently, from the acid hydrolysates of UV-irradiated DNA. First, by taking the advantage of the differential solubility of the Ba-salts of dimers, a method was developed to isolate the "pure" ice-heterodimer. It is simply by dissolving the precipitate, obtained from the irradiation of 600 ml of an aqueous solution of Thy(2.5 mmole) and Ura(5.0 mmole) in a frozen state, in 15 ml of a hot 0.1 N $Ba(OH)_2$ solution. This precipitate was collected from the thawed solution by filtration and contained in a mixture of various isomers of Ura<>Ura and Thy<>Thy and also a Thy<>Ura isomer. Only Ba-salt of the latter crystallized out upon standing at room temperature. It is converted to the free Thy<>Ura by acidification of the Ba-salt solution with dil. HCl. Then, upon methylation of this product a compound having m.p., IR and NMR spectra identical with that of Me_2Thy<>Me_2Ura(c,s) was obtained.

In short, four isomers of Me_2Thy<>Me_2Ura were prepared. Because C-methylation under this condition results in retention of configurations, the stereoconfigurations of these isomers can be assigned on the basis of the configurations of isomers of Me_2Ura<>Me_2Ura or Me_2Thy<>Me_2Thy. This, in turn, confirms that Thy<>Ura, isolated as a product from acid hydrolysates of UV-irradiated DNA, have a cis-syn configuration that must be derived from the photoproduct Thy<>Cyt(c,s) by acid-catalyzed deamination.[4]

In addition, some comments should be made concerning the NMR data. Earlier, Fahr et al.[8] studied the 90-MHz spectra of four Me_2Ura<>Me_2Ura and interpreted the four cyclobutyl-proton signals in terms of an AA'BB' system.

Table 3: The Chemical Shifts (δ in ppm) of 220 MHz NMR Spectral Signals of C(5)H and C(6)H of Pyr<>Pyr at 25°C
Solvent: $CDCl_3$, Internal Standard: $(CH_3)_4Si$.

	I; Me_2Ura<>Me_2Ura	II; Me_2Thy<>Me_2Ura	III; Me_2Thy<>Me_2Thy	$\Delta\delta_{I:II}$	$\Delta\delta_{I:III}$
trans-dimer					
anti	C(5,5')H 3.54[2H] C(6,6')H 4.12[2H]	C(5')H 3.44[1H,q,4.5,10.5][a] C(6)H 3.66[1H,d,4.5] C(6')H 4.12[1H,d,10.5]	- C(6,5')H 3.67[2H,s]	0.10 0.46 0.00	0.45
syn	C(5,5')H 3.73[2H] C(6,6')H 3.88[2H]	C(5')H 3.66[1H,d,10] C(6)H 3.49[1H,d,6.5] C(6')H 3.78[1H,q,10,6.5]	- C(6,5')H 3.66[2H,s]	0.07 0.39 0.10	0.22
cis-dimer					
anti	C(5,5')H 3.77[2H] C(6,6')H 4.11[2H]	Complex ABC pattern at 3.56∿3.76[3H]	- C(6,5')H 3.29[2H,s]		0.82
syn	C(5,5')H 3.78[2H] C(6,6')H 4.08[2H]	C(5')H 3.25[1H,d,7.5] C(6)H 3.68[1H,d,4.5] C(6')H 4.06[1H,q,7.5,4.5]	- C(6,6')H 3.78[2H,s]	0.53 0.40 0.02	0.30

[a] Coupling constants (J) are given in Hz.

A simple two-line pattern was displayed in the 220-MHz spectra because of the lower resolution resulting in the disappearance of any splitting with coupling constants J<4 Hz. In the four $Me_2Thy<>Me_2Ura$, the (c,a)isomer yielded a complex ABC pattern[9] but the other three isomers displayed an AMX pattern in their NMR spectra (Table 3, column 3). This difference may be rationalized in terms of the CH_3-shielding effect. As can be seen, the C(6)H signals shifted upfield ($\Delta\delta$, column 5) as much as 0.48, 0.39, and 0.40 for (t,a)-, (t,s)-, and (c,s)-$Me_2Thy<>Me_2Ura$ as compared with those of the corresponding $Me_2Ura<>Me_2Ura$. Clearly, effective 1,2-shielding takes place with a neighboring proton cis to the CH_3-group. In an analogous position, C(5')H of $Me_2Thy<>Me_2Ura$(c,s) exhibits a substantial $\Delta\delta$ of 0.53. On the other hand, much less significant 1,2- or 1,3-shielding effects ($\Delta\delta$ <0.10) were observed with a trans-proton or a C(6')H, respectively (see below). On account of these differences, one would expect the chemical shifts of C(6)H and C(6')H of $Me_2Thy<>Me_2Ura$(c,a) shifted upfield to the proximity of the C(5')H. Thus, chemical shifts of the three protons become close so that $\nu_{C(6)H} - \nu_{C(6')H}$ and $\nu_{C(6')H} - \nu_{C(5')H}$ are of comparable magnitude, consequently, an ABC system is exhibited. Otherwise, an eight-line AMX pattern would normally be expected.

This reasoning may be extended to include our consideration of $Me_2Thy<>Me_2Thy$ (Table 3, columns 4 and 6). The expected $\Delta\delta$ of 0.45 was observed for the (t,a)isomer. However, the magnitude of the $\Delta\delta$(0.82) for the (c,a)isomer is approximately twice that normally observed. This is probably because each of the two equivalent protons is shielded by both neighboring cis CH_3-groups. Reduced $\Delta\delta$'s (0.22 and 0.30) were observed for the two syn isomers and are probably due to a change in the net shielding effect. One possibility is the steric interaction of the two neighboring CH_3-groups which is nonexistent in the anti isomers with the two CH_3-groups at the 1,3-positions of the cyclobutyl rings. Such an interaction may affect the orientation of the two pyrimidine

moieties resulting in a slight decrease in the $\Delta\delta$'s. It may be interesting to examine our supposition not only with pyrimidine dimers but also cyclobutyl derivatives in general.

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