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Optical Rotatory Dispersion of Nucleic Acid Derivatives. VIII. The Conformation of Pyrimidine Nucleosides in Solution*

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ABSTRACT: The optical rotatory dispersion data for an extended series of substituted uracil, thymine, and cytosine nucleosides are reported. The compounds studied include anomeric pairs, cyclouridines, azapyrimidine nucleosides, pseudouridine, and 2′,3′-unsaturated derivatives. Possible explanations of the sign and magnitude of the Cotton effects in relation to the conformation of these compounds are discussed, and it is concluded that normal pyrimidine β-nucleosides have the *anti* conformation in aqueous solution. A rule predicting the sign of the Cotton effect in pyrimidine furanose nucleosides is proposed. This rule is obeyed by all the compounds for which data are available.

The optical rotatory dispersion (ORD) of nucleic acids, both DNA and RNA, as well as of enzymatically synthesized polynucleotides, has been studied intensively for some time, although accurate results in the region below 300 mμ have only been obtained in the last 3 years, beginning with the work of Yang and Samejima on the four deoxyribonucleotides (Yang and Samejima, 1963) and on DNA and RNA (Samejima and Yang, 1964), and our own work on nucleosides (Ulbricht *et al.*, 1964) (for reviews see Ulbricht, 1964, 1965a). (Solutions of high absorbance can give rotatory artifacts simulating Cotton effects (Urnes and Doty, 1961), but necessity of using dilute solutions in the ultraviolet region still does not seem to be generally appreciated (Lamborg *et al.*, 1965).) These studies have been mainly concerned with the effects of temperature, solvent, and pH, factors known to affect the helical structure of polynucleotides. An interpretation of the ORD curves obtained was not possible until recently, for two reasons: first, because no data were available concerning the monomeric components (nucleosides and nucleotides) of these polymeric substances, and second, because the interaction between adjacent residues in a chain was not understood. Studies of oligo- and polynucleotides by various workers, including Tinoco, Yang, Fasman, Brahms, and their respective co-workers, and the interpretation of the ORD of diadenylic acid in terms of interaction between the two bases (Warshaw *et al.*, 1965) has provided a basis for the understanding of the ORD of these substances (for references, see Michelson *et al.*, 1966; Ulbricht, 1964, 1965a).

In previous papers[1] we reported that the ORD curves of pyrimidine α-nucleosides give negative

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[1] It should be noted that in earlier papers of this series a different numbering system was used for the pyrimidine ring. The atoms referred to in this paper as N-1, N-3, N-4, and C-6 were previously designated N-3, N-1, N-6, and C-4, respectively.

TABLE I: ORD Data of Pyrimidine Nucleosides and Related Compounds.[f]

Compound	1st Extremum		2nd Extremum		Amplitude	Ultraviolet
	ϕ	λ (mμ)	ϕ	λ (mμ)	($a \times 10^{-2}$)	λ_{max} (mμ)
Uridine[a] (I)	+4,100	279	−7,600	247	+117	262
Uridine triacetate	+5,600[b]	275	−3,360	248	+90	262
5′-Iodo-5′-deoxy-uridine	+3,420	276	−4,550	249	+80	262
Thymidine[a]	+1,890	282	−7,590	255	+95	267
Thymidine (in DMF)[g]	+11,300	279	−15,800	264	+271	267
α-Thymidine[a] (XII)	−3,660	286	+6,780	252	−104	267
Thymidine 5′-monophosphate diammonium salt[a]	+1,670	280	−5,460	250	+71	267
5-Hydroxymethyl-2′-deoxyuridine	+5,900	279	−2,020	249	+79	266
5-Hydroxymethyl-α-2′-deoxyuridine	−5,680	279	+8,100	249	−138	266
5-Fluoro-2′-deoxy-uridine[a]	+4,350	286	−6,660	253	+110	269
5-Fluoro-α-2′-deoxy-uridine[a]	−6,300	286	+11,550	252	−179	263
Spongouridine (II)	+11,500	275	−17,900	245	+294	263
2-*O*-Ethyl-2′,3′-isopropylideneuridine (XI)	+5,030	272	−17,700	244	+227	248,229
O^2-2′-Cyclouridine (III)	+6,600	253	−20,000	233	+266	250,233
5′-Bromo-5′-deoxy-O^2-2′-cyclouridine	+8,760	256	−17,400	224	+262	247,225
O^2-2′-Cyclouridine 3′,5′-diacetate	+8,640[b]	255	−19,500	223	+281	248,224
	+4,500	260	−30,200	223	+347	
O^2-3′-Cyclouridine (IV)	+960	272	−3,380	242	+43	246
Isopropylidene-O^2-5′-cyclouridine	−14,500	246	+39,400!	212	−539![e]	240
O^2-5′-Cyclothymidine (V)	−2,900	263	+27,000 infl	225	−299	250
			+34,000!	217		
O^6-5′-Cyclo-6-hydroxyuridine (VI)	+23,000	267	−26,000	242	+490	263
5′-*O*-Trityl-2′,3′-dideoxy-2′,3′-didehydrouridine	−6,500	279	+6,120	243	−126	261
2′,3′-Dideoxy-2′,3′-didehydrouridine (VII)	−8,130	275	+10,800	241	−189	261
2′,3′-Dideoxy-2′,3′-didehydrothymine riboside	−5,880	280	+11,250	245	−171	267
6-Azauridine (VIII)	−8,460	275	+11,200	228	−197	263
6-Azauridine triacetate	−10,150[b]	275	+8,740	231	−189	263
Pseudouridine (IX)	−3,100	278	−800	258	−23[c]	262
α-Pseudouridine	−430[d]	278	−1,430	253	~+10	262
Uracil 3-riboside (X)	−3,300	280	+3,900	256	−72	261
1-Methyluracil 3-riboside	−2,640	280	+2,640	270	−53	270
Cytidine[a]	+7,250	285	−7,950	244	+152	271
2′-Cytidylic acid (in 0.15 M KF)	+5,740	286	−14,350	240	+201	271
3′-Cytidylic acid (in 0.15 M KF)	+9,950	286	−10,450	243	+204	271

TABLE I: (*Continued*)

Compound	1st Extremum ϕ	1st Extremum λ (mμ)	2nd Extremum ϕ	2nd Extremum λ (mμ)	Amplitude ($a \times 10^{-2}$)	Ultraviolet λ_{max} (mμ)
5′-Cytidylic acid (in 0.15 M KF)	+4,560	283	−9,120	248	+137	271
3′-Cytidylic acid	+5,100	289	−10,300 to −13,000	tr 250 to 231	∼+165	271
α-3′-Cytidylic acid	−5,900	283	+2,460	254	−84	271
2′-Deoxycytidine hydrochloride[a]	+4,630	290	−6,500	266	+111	280
α-2′-Deoxycytidine	−7,400	283	+13,600	249	−210	271
α-2′-Deoxycytidine (in aqueous 0.01 N HCl)	−1,090	283	+16,400	256	−175	280
Arabinofuranosylcytosine hydrochloride	+11,200[b]	290	−16,600 to −17,100	tr 246 to 230	∼+280	280
5-Hydroxymethyl-2′-deoxycytidine	+9,000	283	−16,200	249	+252	274
6-Hydroxycytidine	+1,180	276	0!	269	+12!	269
6-Hydroxy-2′-deoxycytidine	+4,200	276	0!	253	+42!	268
6-Azacytidine	−5,450	288	+5,450	225	−109[c]	263
α-2′-Deoxy-6-azacytidine	+2,030	270	−780	252	+28[c]	266
2′-Deoxy-6-azacytidine	−4,580	292	0	233	−46	266
Ribopyranosylthymine	+1,060	287	−3,640	260	−47	265
α-Ribopyranosylthymine	−7,450	281	+12,650	248	−201	265
Glucopyranosyl-N^4-acetylcytosine tetraacetate	+13,000[b]	310	−29,600	254	+426	299,250

[a] ORD data extracted from previous publication (see ref 15 of Ulbricht *et al.* (1966)). [b] ORD recorded in methanol. [c] Values quoted are approximate because of the ill-defined nature of the ORD curves. [d] Peak ill defined. [e] Exclamation mark (!) denotes the lowest value recorded (not an extremum). [f] Solvent: water (unlesss otherwise indicated). β-D configuration unless otherwise stated. [g] DMF, dimethylformamide.

Cotton effects whereas the pyrimidine β-nucleosides give positive Cotton effects (Ulbricht *et al.*, 1964, 1965). The result provided an explanation for the fact that pyrimidine nucleosides do not obey Hudson's isorotation rules (Emerson and Ulbricht, 1964). In order to find what factors affect the sign and magnitude of the Cotton effects produced by pyrimidine nucleosides, we have now examined the ORD of 50 such compounds, derivatives of the nucleic acid bases uracil, thymine, and cytosine, and their 6-aza analogs. The effect on the ORD curves of varying the substituents, in the glycoside residue or in the pyrimidine ring, could then be determined.

Results

The results obtained are listed in Table I and include relevant data previously reported (Ulbricht *et al.*, 1964, 1965). Among the compounds studied are six pairs of anomeric pyrimidine nucleosides (or nucleotides). In each case the sign of the Cotton effect is as expected (Ulbricht *et al.*, 1964) on the basis of the anomeric configuration at C-1′. Although the anomeric thymidines (Figure 1) have been reported to obey Hudson's rules in dimethylformamide but not in water (Lemieux and Hoffer, 1961), we found that β-thymidine gives a positive Cotton effect in both solvents; this emphasizes the arbitrary nature of $[\alpha]_D$ measurements. The results on the 3′-cytidylic acids (Figure 2) confirm other evidence regarding the structure of α-cytidylic acid, which has been recently isolated from various samples of RNA (Gassen and Witzel, 1965). It should be noted that the measurement of the ORD of a pyrimidine nucleoside provides an extremely simple and sensitive method (for measurements below 300 mμ, only 0.05 mg of compound is

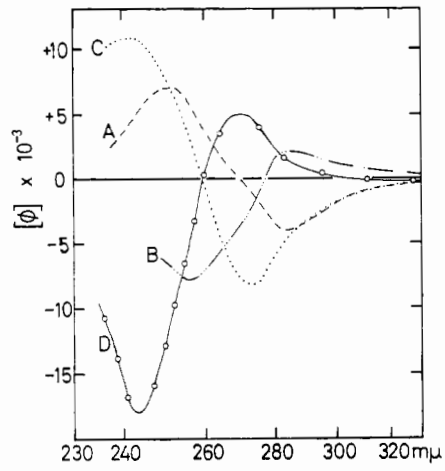


FIGURE 1: ORD curves (in H_2O). (A) (------) α-thymidine (XII); (B) (—···—···—···) β-thymidine; (C) (·····) 2′,3′-dideoxy-2′,3′-didehydro-β-uridine (VII); (D) (—o—o—o—) 2-*O*-ethyl-2′,3-isopropylideneuridine (XI).

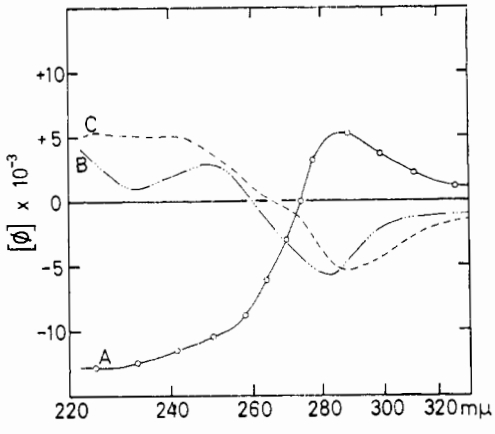


FIGURE 2: ORD curves (in H_2O). (A) (—o—o—o—) β-3′-cytidylic acid; (B) (—···—···—···) α-3′-cytidylic acid; (C) (------) β-6-azacytidine.

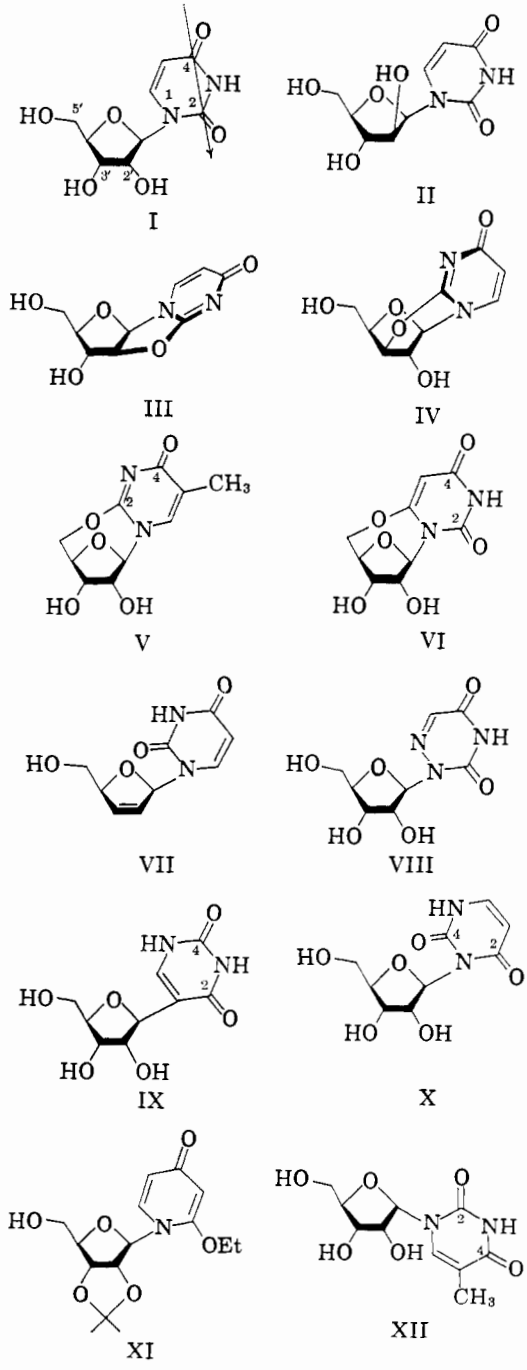
required) for determining the anomeric configuration.[2] This is of particular importance, not only because the synthesis of deoxyribosides from deoxyribosyl halides inevitably produces a mixture of the α and β anomers (Ulbricht, 1965b), but also because recently developed methods of synthesis lead to anomeric mixtures of nucleosides from other sugars (Ulbricht, 1965a,b). (This method of determining the anomeric configuration applies equally well to purine nucleosides where, however, the signs of the Cotton effects are reversed (Ulbricht *et al.*, 1964; Emerson *et al.*, 1966).)

[2] The apparent exceptions to which attention has been drawn by Walton *et al.* (1966) are covered by the rule formulated later in this paper.

Since the stereochemistry at C-1′ is the same in α-D as in β-L derivatives, it is to be expected that β-L-pyrimidine nucleosides will be found to give negative Cotton effects; we have not had any such compounds available for study.

Variation of the substituents in the 3′ and 5′ positions appears to have little effect on the amplitudes of the ORD curves; compare, for example, uridine (I) (Figure 3 and Chart I) with uridine triacetate and

CHART I



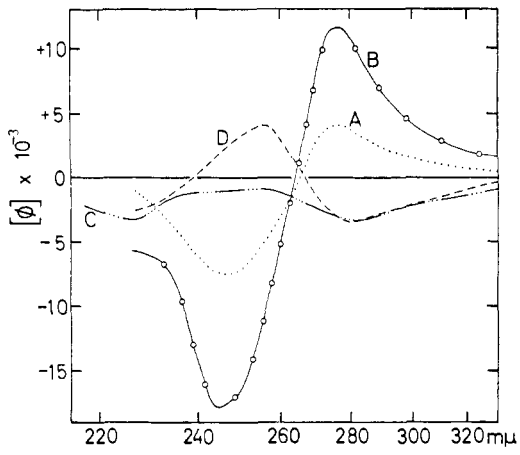


FIGURE 3: ORD curves (in H_2O). (A) (·····) β-uridine (I); (B) (—o—o—o—) β-spongouridine (II); (C) (—···—···—) β-pseudouridine (IX); (D) (------) β-uracil-3-riboside (X).

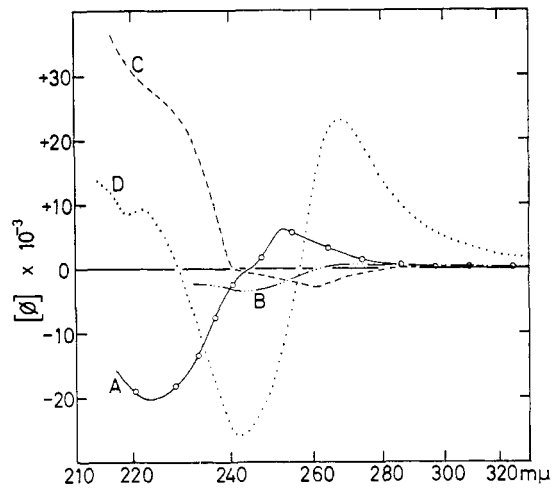


FIGURE 4: ORD curves (in H_2O). (A) (—o—o—o—) O^2-2′-cyclouridine (III); (B) (—···—···—) O^2-3′-cyclouridine (IV); (C) (------) O^2-5′-cyclouridine (V); (D) (·····) O^6-5′-cyclo-6-hydroxyuridine (VI).

5′-iodo-5′-deoxyuridine. Previous results (Ulbricht *et al.*, 1964) have shown that whether the sugar moiety is ribose or 2-deoxyribose makes no major difference to the ORD. Comparison of 2′ epimers shows that much larger amplitudes are obtained in arabinosides, *e.g.*, spongouridine (II, Figure 3) and β-arabinofuranosylcytosine, than in the corresponding ribosides (uridide and cytidine). A similar effec has been observed in lyxosides; β-lyxosides have larger amplitudes than the corresponding ribosides (T. Nishimura, private communication, 1965).

O^2-2′-Cyclouridine (III, Figure 4), which is an anhydroarabinoside, also gives a positive Cotton effect of much higher amplitude than uridine, as do its derivatives 5′-bromo-5′-deoxy-O^2-2′-cyclouridine and O^2-2′-cyclouridine 3′,5′-diacetate. O^2-3′-Cyclouridine (IV, Figure 4) gives a small positive Cotton effect. In the case of the O^2-5′-cyclouridine derivatives (*e.g.*, the thymidine derivative V, Figure 4) the sign of the Cotton effect is negative, the amplitudes are very large, and no second extremum could be reached, although the inflection at about 230 mμ may correspond to an extremum. O^6-5′-Cyclo-6-hydroxyuridine (VI, Figure 4) shows a very large positive Cotton effect. The uracil nucleoside derivatives containing a 2′,3′-double bond in the furanose ring, *e.g.*, 2′,3′-dideoxy-2′,3′-didehydro-β-uridine (VII, Figure 1), give negative Cotton effects.

With 6-azauridines (VIII) and 6-azacytidines (Figure 2) the sign of the Cotton effect is reversed, *i.e.*, those having the β configuration at C-1′ give negative Cotton effects. Triacetylation of the ribose group of 6-azauridine does not alter the amplitude significantly. The deoxyazacytidines, although giving the same sign of Cotton effect as the corresponding azacytidines, give curves of much smaller amplitude. Both α- and β-pseudouridines (β = IX, Figure 3) give ill-defined ORD curves; however, it would seem that the α compound gives a positive and the β compound a negative Cotton effect. The two compounds in which the glycoside residue is attached at N-3 instead of N-1, *e.g.*, uracil-3-riboside (X, Figure 3), also give negative Cotton effects.

Discussion

To rationalize these ORD results, it seems reasonable to assume that the variation in both sign and magnitude of the Cotton effect is due to the changing conformation of the planar pyrimidine ring with reference to the sugar ring, *i.e.*, the position of the chromophore relative to the asymmetric centers in the sugar. Other workers have also pointed out that correlation of the sign of the Cotton effect to the absolute configuration at the nearest asymmetric center requires that the rotameric composition about single bonds within the chromophore moiety be more or less fixed (Briggs and Djerassi, 1965). In most of the simple pyrimidine nucleosides studied, rotation about the glycosidic (N^1-$C^{1'}$) bond is theoretically possible, but in practice is restricted by steric factors (see below). Information about the change in ORD as the relative positions of the pyrimidine and ribose rings are changed is provided by the cyclouridines in which the chromophore is fixed, by direct bonding to the sugar ring. A comparison of the ORD curves of cyclouridines and those uridines where rotation about the glycosidic bond is less restricted should give important information regarding the conformations adopted by these latter compounds, and we have offered an interpretation on this basis (Ulbricht *et al.*, 1966). However, the fact that the ultraviolet absorption of the cyclo- and non-cyclouridines is somewhat different (see Table I) must be taken into account.

It seems unlikely that hydrogen bonding can play an important role in determining the conformation

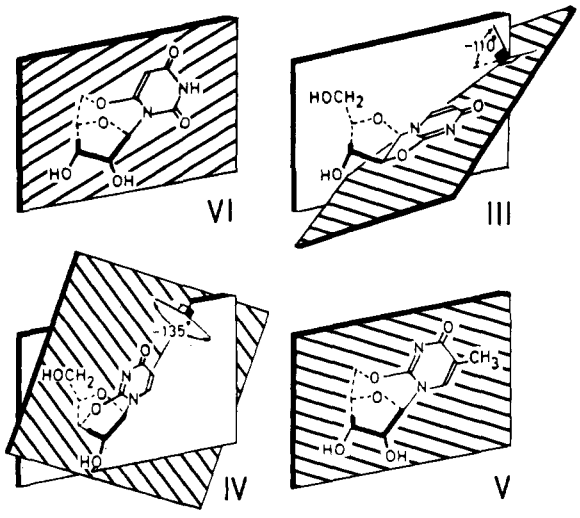


FIGURE 5: Stereochemical drawings of the cyclonucleosides III–VI. The plane of the chromophore is shaded, and the reference plane is unshaded. Thick lines are in front of the planes, broken lines behind them, and plain lines are in the reference plane.

of the pyrimidine ring relative to the sugar ring, first because most ORD curves were recorded in water, and second because the removal of potential hydrogen donors in the sugar ring makes little difference to the sign and amplitude of the Cotton effects recorded; compare, for example, uridine and uridine triacetate or 6-azauridine and its triacetate. One may assume, therefore, that steric considerations are the major factors which determine the conformations adopted. A study of models indicates that in uracil, thymine, and cytosine nucleosides the proximity of the carbonyl group in position 2 of the pyrimidine ring to the groups on C-2′ and C-5′ of the sugar interferes with free rotation about the glycosidic bond; hence, the pyrimidine ring, although not rigidly held, has a preferred conformation such that the oxygen atom on C-2 is directed away from the furanose ring (*e.g.*, β-uridine (I)). The effect of inversion of the hydroxy group on C-2′ of the furanose ring in a β-riboside (giving, *e.g.*, spongouridine (II) Figure 3) would be to hold the pyrimidine ring in a more rigid conformation thereby increasing the magnitude of the Cotton effect, as was found. It is interesting to note that 2-*O*-ethyl-2′,3′-isopropylideneuridine (XI), which has a chromophore resembling that of the cyclouridines rather than uridine, gives a Cotton effect of the same sign as the simpler noncyclouridines but with a much larger amplitude, and in this case there is virtually no free rotation about the glycosidic bond and the *O*-ethyl group must be directed away from the furanose ring. (The presence of an isopropylidene group may affect the magnitude of the Cotton effect in this compound.) The O^2-2′-cyclo derivatives (*e.g.*, III) which may be regarded as anhydroarabinosides give Cotton effects having the same sign as the noncyclouridines, and the magnitude is close to that found in arabinosides (*cf.* II and III).

TABLE II: Stereochemical Data.

Compd	Angle of Pyrimidine Ring with Ref Plane (deg)	$a \times 10^{-2}$
VI	0	+490
III	~110	+266
IV	~135	+43
V	180	−299

Two comparisons are of particular interest. O^6-5′-Cyclo-6-hydroxyuridine (VI) has a chromophore very similar to that of uridine and also gives a positive Cotton effect. Its fixed conformation is like that proposed for uridine, and it is noteworthy that the Cotton effect of this cyclonucleoside (VI) is the largest of any in this series, which supports the hypothesis that it is this conformation which is associated with a positive Cotton effect. On the other hand, it is the O^2-5′-cyclouridines (*e.g.*, V), which have the opposite conformation (the pyrimidine ring in uridine has been rotated 180° about the glycosidic bond), that give *negative* Cotton effects (see Figure 4). This cannot be attributed to the change in the chromophore because the O^2-2′-cyclouridines have a similar chromophore but still have a large positive Cotton effect. O^2-3′-Cyclouridine, whose conformation is intermediate between the O^2-2′ and O^2-5′ compounds, has a *small* positive Cotton effect.

An attempt to illustrate the relative stereochemistry of III–VI more clearly is shown in Figure 5. If the plane in which the pyrimidine ring lies in VI is taken as the reference plane, the angle that the pyrimidine ring makes with this reference plane in III–VI varies from 0 to 180° (see Table II), and as it does so, the positive Cotton effect is reduced in amplitude and finally becomes negative.[3] (The hypothetical reference plane is chosen for convenience, as it demonstrates the point most simply; it is related to the real plane of the ribose ring.)

A classification of spectral bands in pyrimidines has been presented by Clark and Tinoco (1965).[4]

[3] It has been pointed out to us (H. Witzel, personal communication, 1966) that the alternative conformation of the seven-membered ring in this series of compounds would permit hydrogen bonding between the C^2=O and 2′-OH groups in VI, and that the angle between the planes in III and IV would then be 45 and 90°, respectively.

[4] See also Berthod *et al.* (1966). These authors also mention the B_{1u} band (approximately 230 mμ), which is not always visible in the ultraviolet absorption spectrum. Some of the Cotton effects observed by us may be owing to transitions of both B_{2u} and B_{1u} bands, with the extrema overlapping (see inflections in curves of Figure 4). This would not invalidate our argument about configurational assignments, because the first extremum observed is almost certainly only owing to the B_{2u} band.

They concluded that the B_{2u} band in uracil is polarized along the line joining the two keto groups (see I), in agreement with the experimental result for the 260-mμ band in the nucleoside analog 1-methylthymine (Stewart and Jensen, 1964). We suggest that the sign of the Cotton effect in uracil, thymine, and cytosine pentofuranosides can be predicted on the basis of the following rule. The sign of the Cotton effect will be positive if: (1) the nucleoside has a preferred conformation due to restricted rotation about the glycosidic bond and (2) a line from the $C^4{=}O$ (or $C^4{-}NH_2$) group passing through the $C^2{=}O$ group passes from above to below the plane of the furanose ring, provided that the chromophore is not twisted to such an extent that the line passes through $C^{5'}$. ("Above" is defined as the same side of the furanose ring as $C^{5'}$.)

Of the compounds discussed in detail above, I–IV and VI fulfill the conditions of the rule and give positive Cotton effects. In V the line passes through $C^{5'}$ and the Cotton effect is negative. Models show that in the 2′,3′-unsaturated compounds (*e.g.* (VII)) there is no restricted rotation about the glycosidic bond (the hydrogen atom on C-2′ is in the plane of the furanose ring); the Cotton effects are negative. In the N^1-α anomers (*e.g.*, α-thymidine (XII)) and in β-pseudouridine (IX) the line passes from *below* to *above* the plane of the furanose ring; the Cotton effects are negative. In the N^3-ribosides (*e.g.*, X), there is no preferred conformation, since both keto groups are in the "*ortho*" position to the glycosidic nitrogen atom; the Cotton effects are negative. Thus, the results obtained with the 26 uracil and thymine pentofuranosides are in agreement. The 11 cytosine pentofuranosides also obey the rule. Since we originally suggested, on the basis of our preliminary results, that the sign and magnitude of the Cotton effect in pyrimidine nucleosides could be related to their conformation (Ulbricht *et al.*, 1965), Fric *et al.* (1966), who do not refer to our paper, have reported results which are in agreement with ours. In a preliminary statement of the rule for predicting the sign of the Cotton effect in pyrimidine furanose nucleosides (Ulbricht *et al.*, 1966) we noted that their compounds also obey the rule. (In purine nucleosides, rotation about the glycosidic band is much freer, and the Cotton effects are smaller (Emerson *et al.*, 1966). It may be possible to suggest a similar rule for these compounds.)

We have not studied sufficient pyranosides to be able to draw any conclusions about these compounds. The three pyranosides we have measured (listed at the bottom of Table I) obey the rule, as do five of the six pyranosides measured by Fric *et al.* (1966). The exception was α-D-glucopyranosylthymine, which has a *positive* Cotton effect; this was attributed to the more flexible conformation of the sugar residue in this compound. In this connection it is interesting to note that nuclear magnetic resonance (nmr) studies have revealed that the sugar ring in the α- and β-2′-deoxyribopyranosides of thymine exists in different conformations in the two anomers (T. L. V. Ulbricht, unpublished results). Comparison of the ORD of such a pair of anomers clearly becomes much more difficult.

That negative Cotton effects are given by the 2′,3′-unsaturated compounds was first reported by Ruyle *et al.* (1965). The absence of steric factors restricting rotation may not be the only reason for the different behavior of these derivatives. Although our results show that the anomeric configuration at C-1′ is of predominant importance, the replacement of two asymmetric centers (C-2′ and C-3′) by a double bond, *i.e.*, the introduction of a new π-electron system α,β to C-1′ and homoconjugated with the pyrimidine chromophore, certainly influences the optical rotatory behavior. Our proposed rule does not attempt to cover compounds containing additional chromophores, and it is interesting that a 2′,3′-thionocarbonate gives a *negative* Cotton effect of large amplitude (Ruyle *et al.*, 1965). It is known that interaction between two chromophores in the same molecule can lead to Cotton effects of much higher amplitude (Moscowitz *et al.*, 1962).

The direction of polarization of the B_{2u} band in 6-azapyrimidines is not known. It is unlikely that there is any steric factor which could produce a conformation for azauridine significantly different from that shown (VIII), which is similar to that of uridine (I). Hydrogen bonding cannot be the cause of the reversal of the sign of the Cotton effect, since azauridine and its triacetate give very similar curves. If our interpretation of the other results is correct, it would suggest that the direction of polarization of the B_{2u} band in azauracil is not the same as in uracil.

We conclude that the pyrimidine β-nucleosides which are normal constituents of nucleic acids have the *anti* conformation in aqueous solution (as shown for uridine (I)). This is the conformation found in DNA, in a dinucleoside phosphate (Shefter *et al.*, 1964), and in crystalline nucleosides (Sundaralingam and Jensen, 1965). In making quantitative comparisons between the rotations of polynucleotides and their monomeric components it should be remembered that when the *anti* conformation is fixed, as it is in a stacked polynucleotide, the amplitude of the Cotton effect is significantly increased.

Experimental Section

β-Uridine, β-thymidine, β-thymidine 5′-monophosphate diammonium salt, β-6-azauridine, β-6-azauridine triacetate, β-cytidine, β-2′-deoxycytidine hydrochloride, and β-6-azacytidine were commercial samples. Their purity was checked by paper chromatography and ultraviolet spectroscopy.

β-Uridine triacetate (Brown *et al.*, 1956), α-2′-deoxycytidine (Fox *et al.*, 1961), and β-D-glucopyranosyl-N^4-acetylcytosine tetraacetate (Ulbricht and Rogers, 1965) were prepared as described elsewhere. The remaining compounds were obtained as gifts.

ORD measurements were made with the Bellingham

and Stanley–Bendix-Ericsson recording spectropolarimeter "Polarmatic '62" at 18–22°. Concentrations varied from $c = 0.1$ to 0.004, the solutions used always having an absorbance well below 2, to minimize any possible artifacts. All curves were run in triplicate, and curves or parts of curves which were not reproducible were discarded. The useful (nonstray) light transmission of the spectropolarimeter was continuously monitored during readings, and 20% transmission was taken as the minimum acceptable value. The base line was recorded before measuring each compound. Little baseline shift was observed during a particular series of readings, but was observed as between different runs on the instrument, and it is for this reason that we have always quoted results in terms of amplitudes, as we have found that amplitudes are reproducible to ±10%.

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